

Environment-related life-history trait variations of the red-bellied piranha *Pygocentrus nattereri* in two river basins of the Bolivian Amazon

F. DUPONCHELLE*†, F. LINO‡, N. HUBERT‡§, J. PANFILI||,
J.-F. RENNO‡¶, E. BARAS¶, J. P. TORRICO‡,
R. DUGUE*¶ AND J. NUÑEZ‡¶

*IRD UR 081 / ULRA, Universidad Mayor San Simón, Cochabamba, Bolivia,
‡IRD UR 081 / IBMB, Universidad Mayor San Andrés, Campus Cota Cota, La Paz,
Bolivia and ||IRD UR 070. Laboratoire ÉCOLAG, UMR 5119, Université Montpellier 2,
cc 093, Place E. Bataillon, 34095 Montpellier Cedex 5, France

(Received 9 October 2006, Accepted 17 May 2007)

Life-history traits of *Pygocentrus nattereri* were compared in two populations inhabiting connected tributaries of the upper Madera River: the white water Mamoré River and the clear water Iténez River. As white waters provide better trophic conditions than clear waters, the size at maturity, fecundity, reproductive effort, condition and growth of *P. nattereri* should be greater in the more productive white water river (Mamoré) than in the less-productive clear water river (Iténez). Breeding periods were highly seasonal and similar in both rivers and under strong influence of photoperiod. Oocyte size-frequency distributions, together with the frequent occurrence of recovering females indicated that an individual female spawns at least twice during the breeding season. As predicted, fish of the Mamoré were significantly larger at maturity and had higher fecundity and condition factor values than those of the Iténez. Fish from both rivers matured as yearlings. The higher growth potential of females was better expressed in the Mamoré than in the Iténez, where growth differences between sexes were weak. Females had a significantly better growth in the Mamoré than in the Iténez. The observed life-history traits associations were consistent with the hypothesis of better trophic conditions in the Mamoré. In addition, previous genetic analyses evidenced that the colonization of the two basins is recent and that extant populations have very similar genetic backgrounds. This suggests that the observed variations in life-history traits of *P. nattereri* are not related to historical factors (genetic drift) between two phylogeographically distinct lineages, but rather due to the contrasting environmental conditions in the white and clear waters.

© 2007 The Authors

Journal compilation © 2007 The Fisheries Society of the British Isles

Key words: breeding; growth; Iténez; Mamoré; Serrasalminae; white/clear waters.

†Author to whom correspondence should be addressed at present address: IRD, UR 175, GAMET, BP 5095, 34196 Montpellier, France. Tel.: +33 4 67 16 64 01; fax: +33 4 67 16 64 40; email: fabrice.duponchelle@ird.fr

¶Present address: IRD, UR 175, GAMET, BP 5095, 34196 Montpellier, France.

§Present address: Département de Biologie, Pavillon Charles-Eugène Marchand, Université Laval, Sainte-Foy, Québec, G1K 7P4 Canada.

INTRODUCTION

The influence of white, black or clear water (Sioli, 1984) systems on Amazonian fish distribution and abundance is discussed in the literature (Goulding, 1980; Lowe-McConnell, 1987; Saint-Paul *et al.*, 2000). By contrast, the influence of the different types of waters on fish life histories has received little attention. For those fish species inhabiting both white and clear (or black) waters, the associated differences in chemical composition, production and community patterns might be expected to induce variations in reproductive and growth characteristics. The nutrient-rich white waters are more productive and harbour more biomass than black and clear waters (Lowe-McConnell, 1987; Junk *et al.*, 1989; Junk, 1997; Saint-Paul *et al.*, 2000). The cost of reproduction is one of the basic postulates of life-history theory (Stearns, 1992; Wootton, 1998). This postulate assumes that finite resources of an organism must be allocated to maintenance, growth and reproduction and that any surplus allocation to one of these compartments will have negative consequences ('cost') for the others. Theoretical (Gadgil & Bossert, 1970) and empirical studies have emphasized the positive correlation between food availability and reproductive output (Wootton, 1998). Variations in food availability can induce phenotypic changes in fecundity, growth rate, age and size at maturity in fishes (Scott, 1962; Bagenal, 1969; Wootton, 1973; Hislop *et al.*, 1978; Reznick, 1983, 1990; Stearns & Crandall, 1984; Townshend & Wootton, 1984).

The red-bellied piranha *Pygocentrus nattereri* Kner (Pisces Serrasalmidae) is one such species widely distributed across tropical South America. It has been reported in all major tributaries of the Amazon (Solimões, Madeira, Negro, Tapajos, Xingu and Tocantins), as well as in the São Francisco, Orinoco, Paraguay-Parana and Del Plata Rivers (Géry, 1964; Lauzane & Loubens, 1985; Lowe-McConnell, 1987; Lauzane *et al.*, 1990; Fink, 1993; Machado-Allison & Fink, 1995; Hubert & Renno, 2006). It frequently occurs in the white water rivers draining the Andes, and in the clear waters from the Brazilian and Guyana shields, but it is rare in black waters (Fink, 1993; Jégu, 2003). As most other sharp-teethed piranhas, *P. nattereri* is reputedly a voracious predator (Pauly, 1994). Several studies, however, confirmed that its diet primarily comprised fishes, but also arthropods, molluscs, other invertebrates and plant material (Bonetto *et al.*, 1967; Nico & Taphorn, 1988; Winemiller, 1989; Winemiller & Kelso-Winemiller, 1993; Pauly, 1994). Furthermore, *P. nattereri*, as several other species of piranha, preys upon sick and injured fishes and scavenges over cadavers of fishes and other vertebrates, thereby playing an important ecological function as 'cleaning and health squads' (Goulding, 1980; Sazima & Guimares, 1987; Schulte, 1988; Sazima & Machado, 1990; Pauly, 1994). *Pygocentrus nattereri* also represents a major source of animal protein for human communities throughout its range. It is also sought by fish hobbyists worldwide. Despite the ecological and economical importance of this species, however, its life-history strategies (in natural environments) have received little attention. Except for scant information on the seasonality of breeding and size at first sexual maturity (British Guiana, Lowe-McConnell, 1964; Mamoré River, Bolivia, Loubens & Aquim, 1986) or breeding behaviour (Paraguay River basin, Brazil, Uetanabaro *et al.*, 1993), most knowledge on the reproductive biology of this species

originates from aquarium specimens (Pauly, 1994). In particular, it is currently unknown whether the life-history strategies of *P. nattereri* vary between the types of water (*i.e.* white, clear and black; Sioli, 1984), which are known to govern the structure of fish communities in the Amazon basin (Goulding, 1980; Lowe-McConnell, 1987; Saint-Paul *et al.*, 2000).

In the present study, reproductive (*e.g.* breeding season, first sexual maturity and fecundity) and growth characteristics of *P. nattereri* were compared in two connected rivers of the Bolivian Amazon: a white water river (Mamoré) and a clear water river (Iténez). The prediction that better trophic conditions in the Mamoré River would lead to differences in life-history traits of *P. nattereri*, such as larger size at maturity or reproductive effort and better condition factor and growth in the Mamoré River than in the Iténez River, was tested.

MATERIALS AND METHODS

STUDY AREA AND ENVIRONMENTAL DATA

Fish were sampled in the Mamoré (Isiboro-Sécure River, from 14°49' to 16°20' S) and Iténez River basins (San Martín and San Joaquín Rivers, from 13°9' to 14°8' S). The Iténez is a tributary of the Mamoré, which joins the Yata and Beni Rivers to form the Madera River (Fig. 1). There is no physical (natural or man-made) obstacle, and thus no major barrier to gene flow between the Iténez and the Mamoré Rivers.

The Mamoré River is a typical white water environment (Sioli, 1984). Its waters, which mainly originate from the Andes, are characterized by a neutral or basic pH, a high mineral content and large amounts of suspended sediments (Corbin *et al.*, 1988; Guyot *et al.*, 1991). The Iténez is a clear water system (Sioli, 1984). Its transparent waters, which drain the Brazilian shield, are characterized by acidic to neutral pH, low nutrient and suspended sediment contents (Roche & Fernandez-Jauregui, 1988; Navarro & Maldonado, 2002).

Environmental data were obtained at the nearest possible locations from the study sites, usually in the cities and Magdalena (on the San Martín River) and Trinidad, for the Iténez and Mamoré River basins, respectively (Fig. 1). Data on rainfall patterns were provided by the Administración y Servicios de Aeropuertos Nacionales (ASAANA) weather service (Trinidad, Bolivia). Day lengths were calculated from the times of sunset and sunrise obtained from the Bureau des Longitudes (<http://www.bdl.fr/>). Information on water level for the Mamoré (Puerto Varrador) was provided by IRD-HYBAM (Institut de Recherche pour le Développement-Hydrologies des Bassins Amazoniens) and SENAMHI (Servicio Nacional de Meteorología e Hidrología, Bolivia); for the Iténez (Piedras negras) it was provided by IRD-HYBAM and ANA (Agência Nacional de Águas, Brazil).

FISH SAMPLING

Fish were captured in the mainstream and in permanently connected lagoons (usually old river arms), mainly with hook and line, but also with gillnets (50 m in length, 2 m in height, lifted every 6 h). Hook and line were used during the day and crepuscular hours in most available habitats, whereas gillnets were used both day and night. Because the width of the river or lagoons in the sample sites sometimes exceeded several hundred metres, gillnets were always set along the banks, for the sake of homogeneity. Sampling effort varied between sample sites because the main objective was collecting a minimum of 50 fish per sampling site per sampling occasion.

Fish were collected between July 2001 and March 2005. For logistic reasons, both river basins could not be sampled every month. Whenever possible, each basin was

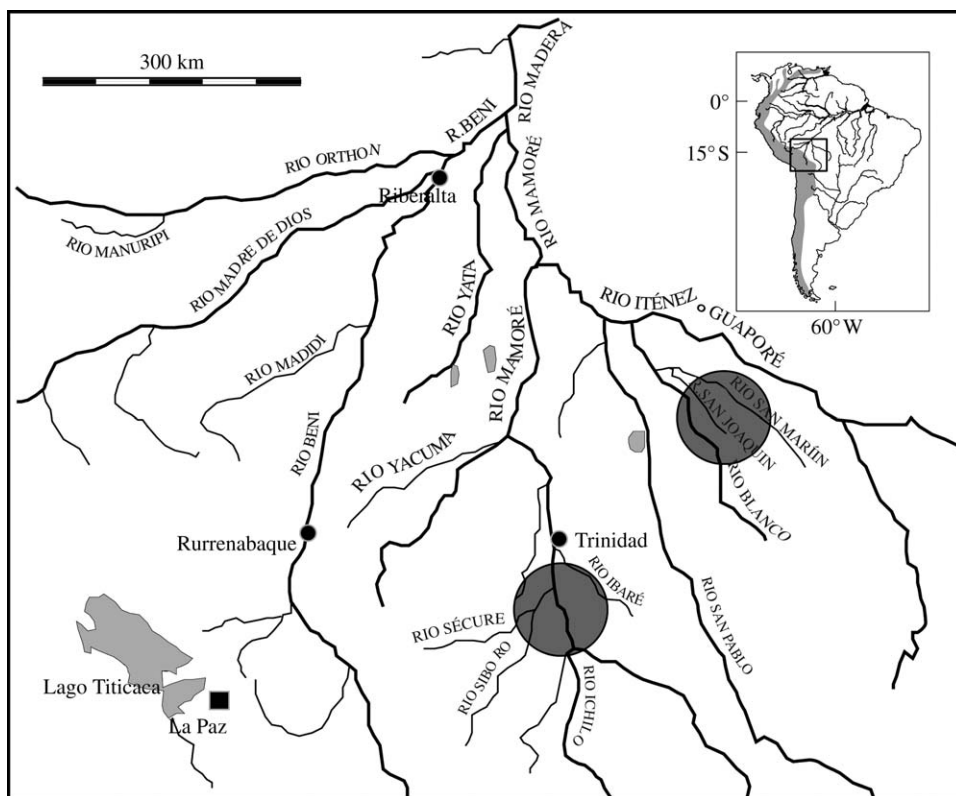


FIG. 1. Study area and sampling locations (●) in the Bolivian Amazon.

sampled alternatively every 2 months (bi-monthly). The Bolivian Amazon, however, is characterized by one of the largest floodplains of the Amazon (Guyot *et al.*, 1999), which makes fish sampling particularly difficult during high waters owing to a dilution effect. On several occasions, very few or no specimens of *P. nattereri* could be caught during a field trip. Henceforth, for a more accurate estimation of an annual cycle, data pooled over the sampling period were used for the determination of the breeding season and the sizes at first maturity. Similar numbers of specimens were captured at each breeding season in the two river basins, so it is believed that between-river differences are not skewed by a particular year during the sampling period. Estimates of fecundity for the two rivers were carried out using fish caught only during the breeding season 2001–2002.

BIOLOGICAL SAMPLE ANALYSIS

Live fish (essentially those captured with hook and line) were rapidly killed by an overdose of anaesthetic (phenoxy-ethanol). The standard length (L_S) and body mass (M) of each fish were measured to the nearest 1 mm and nearest 1 g, respectively. The largest otoliths (the asterisci) were extracted, washed in clear water, dried and stored in referenced envelopes for later reading. Gonads were weighed (M_G) to the nearest 0.1 g and macroscopically checked for maturity stage. On each fish, a portion of gonad was preserved in Bouin's fluid for later histological analyses. Preserved gonads were further processed under classical histological methods with haematoxylin and eosin staining for confirmation of maturity stages.

The breeding season was estimated from the monthly proportions of the females' gonadal maturation stages. The maturity scale of gonadal maturation stages used was adapted from Loubens *et al.* (1984) and Loubens & Aquim (1986). For females, the stages of gonadal maturation were: stage 1, immature; stage 2, early recrudescence; stage 3, early vitellogenesis; stage 4, advanced vitellogenesis (characteristic of females that are soon to spawn); stage 5, gonads with ovulated oocytes (ovules) and stage 6, post spawn. Stage 6–2 describes gonads of resting females and stage 6–3 of recovering females. Stage 6–3 is characterized by a few atretic follicles still visible, some remaining empty spaces and a new batch of vitellogenic oocytes developing, indicating that females are developing a second batch of eggs after laying the first. Gonads in advanced vitellogenesis (stage 4) were preserved in 5% formalin for later determination of fecundity and oocyte size. For males, stage 1 corresponds to immature fish, stage 2 to maturing or resting individuals and stage 3 to ripe fish.

The size at first sexual maturity (L_{S50}) is defined as the L_S at which 50% of the individuals are at an advanced maturation stage during the breeding season (*i.e.* at least stage 3 for females and stage 2 for males). The L_{S50} is estimated by fitting the percentage of mature individuals (%N) per 10 mm L_S intervals to a logistic regression function weighted by the total number of individuals in each size class: $\%N = \{1 + e^{[-a(L_{SC} - L_{S50})]}\}^{-1}$, where L_{SC} = central value of each size class and a = a constant. Gonads of fish within the size range of the L_{S50} were verified in histology for better accuracy in the determination of immature *v.* resting mature fish.

Batch fecundity (*i.e.* the number of oocytes laid per spawning event) and oocyte size were estimated for each mature (*i.e.* stage 4) female from three ovarian fragments (mean \pm S.D. 0.25 ± 0.00 g each). For each fragment, the oocytes were separated under a binocular microscope and photographed with a digital camera. The number and size of oocytes (minimum and maximum diameters, D_{\min} and D_{\max} , respectively) were determined with the NIH Image software. For each oocyte, the mean arithmetic diameter ($\sqrt{D_{\min}}$ and D_{\max}) was calculated. Data from different ovarian fragments were first tested for consistency then pooled for calculating fecundity by reference to the mass of the gonads (M_G). Each ovarian fragment was analysed individually, and when the three counts were not significantly different they were pooled for calculating fecundity by reference to M_G .

The condition factor (K) was calculated as: $K = ML_S^{-3}$.

Age and growth characteristics were determined from otolith examination. The asterisci of 580 fish were analysed both on transverse and sagittal sections stained with toluidine blue (1%) after acid etching (EDTA 5%), following the method described for other Serrasalminae [*Colossoma macropomum* (Cuvier), Loubens & Panfili (1997) and *Piaractus brachipomus* (Cuvier), Loubens & Panfili (2001)]. Stained marks, which correspond to translucent seasonal increments according to Loubens & Panfili (1997), were interpreted and counted along the otolith dorso-ventral axis. The otolith edge was ranked as stained, opaque or undetermined in order to validate the timing of mark deposition when calculating the monthly percentage of stained edges. After the validation stage, stained marks were counted to estimate the age of the fish. The last stained mark was not counted if it was on the otolith margin, and thus in formation. The individual age in months was then calculated taking into account the mean hatching date for the populations (determined during the reproductive study), the date of capture and the number of stained marks.

STATISTICAL ANALYSES

For both rivers, logistic regression models provided an estimate of $L_{S50} \pm$ S.D. Therefore, sizes at maturity between sexes or rivers were compared using *t*-tests (d.f. calculated from the number of size classes). ANCOVA was used to compare linear regressions of fecundity and M for fish of both rivers. Comparisons of mean oocyte diameters between fish of both rivers were carried out using *t*-tests. The values of K were compared with ANOVA using river, sex and month as factors.

The von Bertalanffy growth function (VBGF) was calculated using a non-linear estimation (quasi-Newton method) following the equation: $L = L_{\infty} \{1 - e^{-K_b(t - t_0)}\}$, where L is the mean L_S at age t , L_{∞} is the asymptotic L_S , K_b the growth coefficient and t_0 the age at length 0.

The age at first sexual maturity (A_{50}) was calculated from the VBGF as follows: $A_{50} = \{-\ln[1 - (L_{S50}L_{\infty}^{-1})]K_b^{-1}\} + t_0$.

The growth parameters of the VBGF were compared using the likelihood ratio test (Tomassone *et al.*, 1993) and applying the weighted sum of squares of Kimura (1980). For k populations, the likelihood ratio test S_{ML} was compared with χ^2 using 3 d.f. (three parameters): $S_{ML} = \sum_{i=1}^k n_i [\ln(S_c^2) - \ln(S_k^2)]$, where n_i is the number of individuals of the k th population, S_c^2 is the residual variance of the pooled model (for all populations) and S_k^2 is the residual variance of the models of the k populations. The same likelihood ratio test was used for pair-wise comparisons of the growth models.

RESULTS

Over the study period, 1063 red-bellied piranha were captured (Table I). The sex ratio was significantly skewed in favour of females in both basins (1.81:1 for the Mamoré, χ^2 , d.f. = 1, $P < 0.001$; 1.47:1 for the Iténez, χ^2 , d.f. = 1, $P < 0.001$). The mean L_S were significantly greater in the Mamoré than in the Iténez, both for males and females (Mann-Whitney rank sum test, $n = 666$, $P < 0.001$ for females; $n = 397$, $P < 0.001$ for males) as were the mean M (Mann-Whitney rank sum test, $n = 666$, $P < 0.001$ for females; $n = 397$, $P < 0.001$ for males). In both basins, females were significantly larger than males ($n = 681$, $P < 0.05$ in the Mamoré; $n = 377$, $P < 0.01$ in the Iténez).

TABLE I. Standard length (L_S), body mass (M), age (A_{50}) and L_S at first sexual maturity (L_{S50}), and von Bertalanffy growth parameters (L_{∞} , K_b , t_0) of *Pigocentrus nattereri* in the Mamoré and Iténez River basins

	Mamoré		Iténez	
	Females	Males	Females	Males
N	439	242	227	155
L_S range (mm)	90–280	84–250	94–247	94–250
Mean \pm S.D. L_S (mm)	184 \pm 42	178 \pm 35	173 \pm 36	161 \pm 36
M range (g)	33–1300	23–900	40–780	35–640
Mean \pm S.D. M (g)	343 \pm 229	298 \pm 164	257 \pm 157	212 \pm 138
Mean \pm S.D. L_{S50} (mm)	140.5 \pm 1.2	114.0 \pm 0.4	129.4 \pm 0.0	108.8 \pm 0.5
A_{50} (months)	12.8	7.4	8.8	4.7
n	194	120	106	69
Mean \pm S.E. L_{∞} (mm)	236.17 \pm 2.23	221.09 \pm 2.89	223.91 \pm 4.78	238.93 \pm 9.17
Mean \pm K_b	0.67 \pm 0.06	0.60 \pm 0.07	0.59 \pm 0.09	0.40 \pm 0.07
t_0 (year)	-0.28	-0.59	-0.74	-1.15
r^2	0.86	0.88	0.82	0.88

N , total number of individuals used; n , number of individuals used in growth analyses; r^2 , determination coefficient for the VBGF.

BREEDING SEASON

Over the sampling period, gonad maturation started in July to August in both rivers [Fig. 2(a)]. The first maturing females (stage 3) were observed with the first rains, just before the beginning of the increased rainfall levels [Fig. 2(b)]. This period also coincided with increasing day-length [Fig. 2(c)]. Recovering females (stages 6–3) were only observed from November to January in the Mamoré and from November to February in the Iténez.

The actual breeding season started in August to September in the Mamoré and apparently 1 month later in the Iténez [Fig. 2(c)], with the occurrence of the first ripe (stage 4 and 5) gonads. It peaked between November and December in both river basins, and declined rapidly thereafter, with <10% of the females in reproductive condition in February and March, and none later in the season. The end of the breeding season in March was also confirmed by the absence of maturing and recovering (stages 3 and 6–3) females at these months [Fig. 2(a)].

In both rivers, the breeding cycle was not synchronized with water level: breeding started during the dry season, it peaked at the start of the flood period and ended before water levels were maximal [Fig. 2(b), (c)]. By contrast, the beginning of the breeding season coincided with the first rainfalls, which started slightly earlier in the Mamoré than in the Iténez. It also coincided with the time of the year when day-length was increasing. The strong decline in the proportion of females in reproductive condition throughout January and February took place during periods, where rainfalls were still abundant and water level was rising. By contrast, it coincided with the time of the year when day-length started decreasing. All three environmental variables were significantly correlated with the breeding cycle, in phase in the case of photoperiod (cross correlation, $r = 0.762$ in the Mamoré and $r = 0.707$ in the Iténez), with 1 month time lag for the rainfalls ($r = 0.750$ in the Mamoré and $r = 0.720$ in the Iténez) and with a 4 months time lag for the water level ($r = 0.684$ in the Mamoré and $r = 0.583$ in the Iténez).

SIZE AND AGE AT FIRST SEXUAL MATURITY

In both river basins, females attained sexual maturity at a significantly larger L_{50} than males (t -test, d.f. = 32, $P < 0.001$; d.f. = 29, $P < 0.001$ for the Mamoré and Iténez, respectively; Fig. 3 and Table I). The L_{50} of females was higher in the Mamoré than in the Iténez (d.f. = 32, $P < 0.001$). Similarly, males attained sexual maturity at a slightly larger size in the Mamoré than in the Iténez, although the difference was weak (d.f. = 33, $P < 0.05$).

In both river basins, female and male red-bellied piranhas matured as yearlings (actually close to 12 months for females in the Mamoré), and in both rivers, males became mature a few months before females. Red-bellied piranhas become mature at a younger age in the Iténez than in the Mamoré (Table I), but this difference probably has a limited impact since males and females become mature as yearlings and can participate in the next reproductive season. Furthermore, it is probable that the age at first maturity was underestimated for the males from the Iténez, because of the small sample size, which resulted in a high t_0 value.

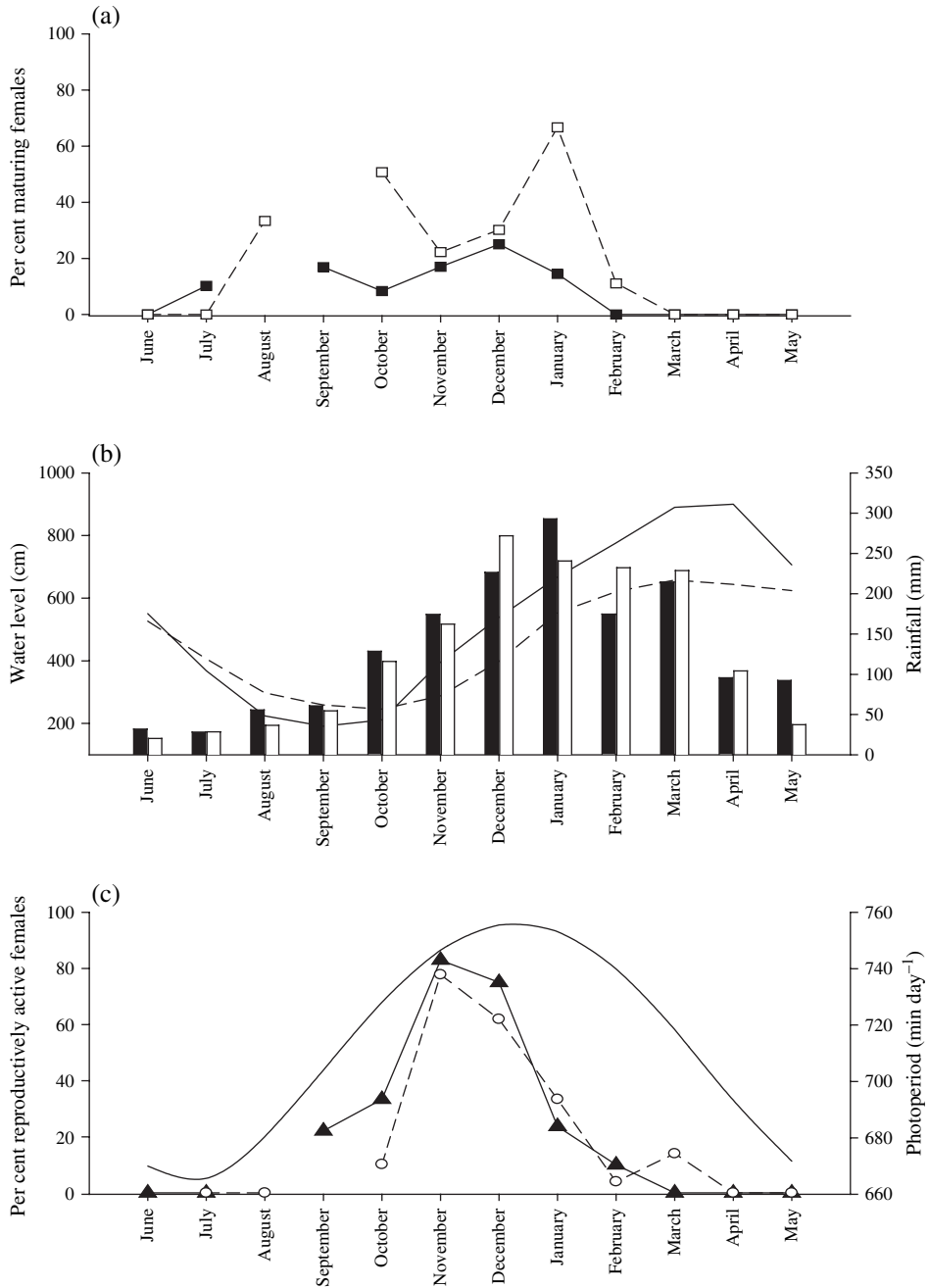


FIG. 2. Monthly (a) percentages of maturing (stage 3) and recovering (stage 6–3) *Pigocentrus nattereri* females in the Mamoré (■) and Iténez (□) Rivers, (b) Mamoré rainfall (■) and water level (—) and Iténez rainfall (□) and water level (---) and (c) percentages of reproductively active females in the Mamoré (▲) and Iténez (○) Rivers in relation to photoperiod (—). Reproductively active females corresponded to the gonadal maturation stages 4, 5, 6 and 6–3.

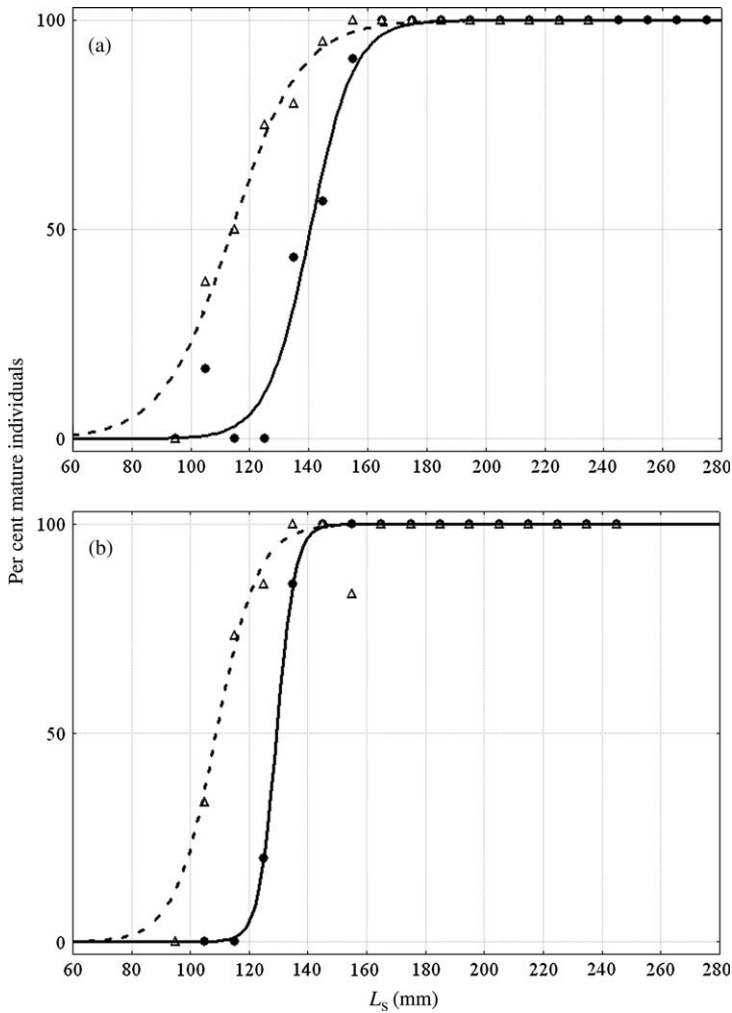


FIG. 3. Standard length at first sexual maturity (L_{S50}) for males (Δ) and females (\bullet) of *P. nattereri* in (a) Mamoré and (b) Iténez River basins.

FECUNDITY AND OOCYTE SIZE

In both river basins, the frequency distributions of oocyte sizes in ripe (stage 4 or 5 or pre-egg-laying) females were bimodal (Fig. 4). The first mode consisted of oocytes <1.4 mm in diameter in both rivers, and the second one of oocytes with diameters ranging from 1.5 to 2.3 mm in the Mamoré, and from 1.5 to 2.2 mm in the Iténez. Only oocytes belonging to the second mode were considered for comparisons of fecundity and oocyte size between basins. These distributions, together with the frequent occurrence of recovering (stage 6–3) females indicated that all or some female red-bellied piranha spawn at least twice during the breeding season in both basins.

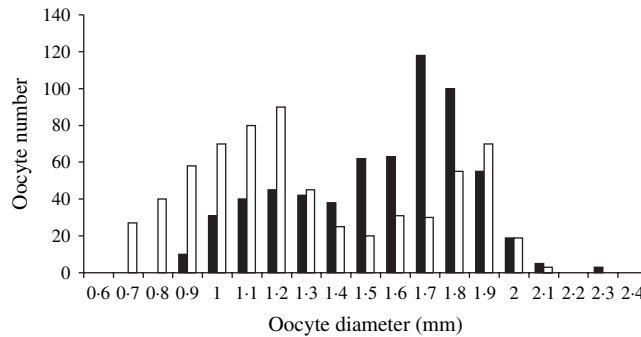


FIG. 4. Size frequency distribution of oocytes in ripe females (stage 4 or 5) from the Mamoré (■) and Iténez (□) River basins.

In both river basins, the batch fecundity of *P. nattereri* was positively correlated with M (Fig. 5). In the Mamoré, batch fecundities ranged from 6056 to 35 676 oocytes for females of 258 and 657 g, respectively. In the Iténez, they ranged from 3551 to 21 213 oocytes for females of 126 g and 518 g, respectively. The comparison between linear regression models of fecundity and M confirmed that the batch fecundity of *P. nattereri* was higher in the Mamoré than in the Iténez (ANCOVA on ln-transformed data, d.f. = 3, 32, $P < 0.001$). For example, a 500 g female would lay *c.* 18 874 eggs in the Mamoré and 13 562 eggs in the Iténez. The size of oocytes, however, did not differ between basins (Mann–Whitney rank sum test, $n = 29$, $P > 0.05$; mean \pm s.d. 1.62 ± 0.07 and 1.65 ± 0.05 (mm), in the Iténez and Mamoré, respectively).

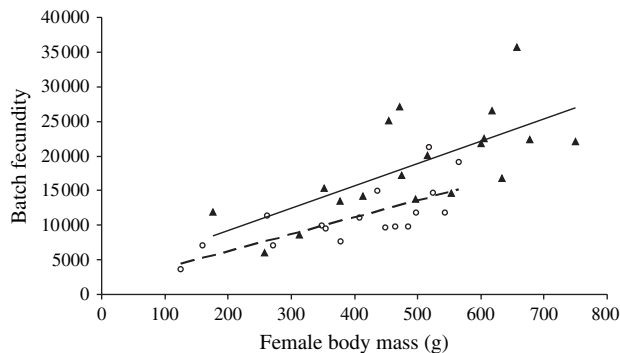


FIG. 5. Relationships between batch fecundity and female body mass of *Pigoceus nattereri* in the Mamoré (▲) and Iténez (○) River basins. The curves were fitted by Mamoré: $y = 32.260x + 2743.548$ ($r^2 = 0.472$, $P < 0.001$) and Iténez: $y = 24.342x + 1390.952$ ($r^2 = 0.536$, $P < 0.001$).

CONDITION, AGE AND GROWTH

The analysis of K indicated significant variations between sexes (ANOVA, d.f. = 11, $P < 0.01$) and months (d.f. = 11, $P < 0.001$) within rivers. The most

important difference, however, was observed between rivers, where fish from the Mamoré had significantly higher mean K than fish from the Itenez (d.f. = 1, $P < 0.001$) during the rising and high water period from November to June (mean \pm s.e. females: 4.71 ± 0.03 and 4.41 ± 0.04 for the Mamoré and Itenez, respectively; males: 4.82 ± 0.05 and 4.32 ± 0.06 for the Mamoré and Itenez, respectively). During the low-water period, K values were similar in both rivers (females: 4.47 ± 0.04 and 4.46 ± 0.05 for the Mamoré and Itenez, respectively; males: 4.63 ± 0.04 and 4.61 ± 0.05 for the Mamoré and Itenez, respectively).

About 20% of the 580 otoliths under scrutiny were impossible to interpret and were excluded from the analysis. Size-at-age relationships were estimated from the alternation of translucent (stained) and opaque zones on the otoliths in 314 fish from the Mamoré (194 females and 120 males) and in 169 fish from the Itenez (106 females and 63 males). The first stained mark after the core was usually large, composite, less coloured and its limits were not well defined [Fig. 6(a)]. After the first two or three stained marks, the relative thickness of the opaque and stained zones progressively became more regular.

The analysis of monthly percentages of stained (translucent) otolith edges validated the formation of a single translucent mark per year. The pattern was similar in the Itenez and the Mamoré, so only pooled data are presented in this study [Fig. 6(b)]. The translucent zone mainly formed during the dry and the early flooding periods, between August and November. The opaque zone formed essentially during the periods of rising and high waters, between

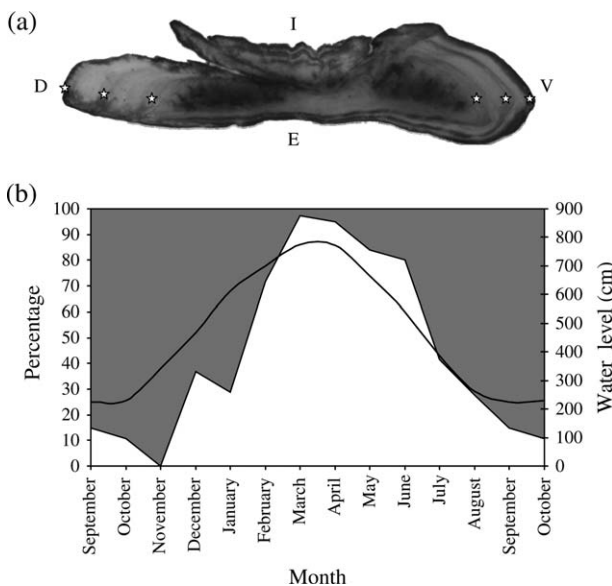


FIG. 6. (a) Otolith (asteriscus) stained transverse section of *Pygocentrus nattereri* showing two stained marks (☆) and another one forming on the edge. D, dorsal face; E, external face; I, internal face; V, ventral face. (b) Monthly percentages of translucent (■, stained) and opaque (□, opaque) otolith edges in relation to water level (—).

December and July. Seasonal growth variation was inferred from the relative widths of the two seasonal increments on the otoliths, which had approximately equal formation times [Fig. 6(b)]. Opaque zones were much wider than translucent ones, indicating a higher growth rate [Fig. 6(a)]. The formation of the translucent zone coincided with a period of reduced growth and was considered as an annulus, whereas the opaque zone corresponded to faster growth during more favourable conditions. The peak period for the annulus, during November, corresponded with the height of the breeding season and was selected as the mean hatching date in both rivers.

The growth curves of each sex in the two river basins were estimated with the von Bertalanffy growth function (VBGF; Fig. 7 and Table I). In both rivers, females grew significantly faster than males, but the between-sex growth dimorphism was greater in the Mamoré than in the Iténez (maximum likelihood test S_{ML} , d.f. = 3, $P < 0.001$ and S_{ML} , d.f. = 3, $P < 0.05$, respectively). Females grew faster in the Mamoré than in the Iténez (S_{ML} , d.f. = 3, $P < 0.001$), while the growth of males did not differ between rivers (S_{ML} , d.f. = 3, $P > 0.05$). The size variance was greater during the first 2 years of life than at older ages. The life span of *P. nattereri* was slightly longer in the Mamoré than in the Iténez for both sexes (9 v. 8 years for females and 8 v. 7 years for males). In both rivers, L_{∞} were smaller than the maximum observed lengths, indicating low levels of exploitation for this species.

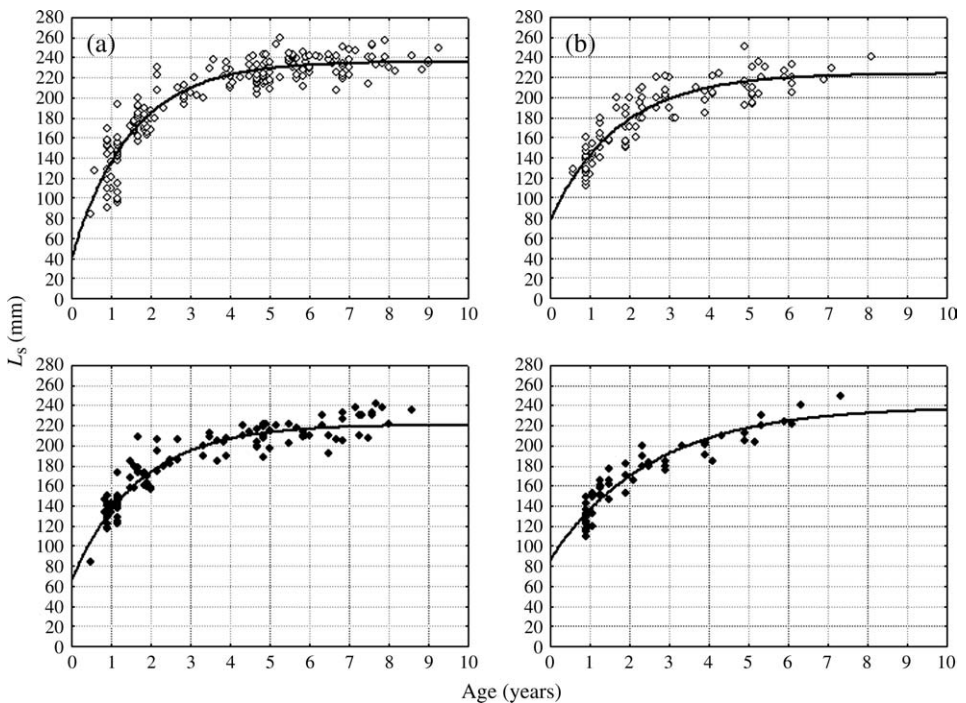


FIG. 7. von Bertalanffy growth curves for *Pygocentrus nattereri* females (◇) and males (◆) in the (a) Mamoré (white waters) and (b) Iténez (clear waters) basins.

DISCUSSION

SEX RATIO

In both basins, the sex ratio was skewed in favour of females over the sampling period. Given the variety of habitats, times of day (or night) and the fact that the sampling was carried out over several years in two river basins, a sampling bias seems unlikely. Female-biased sex ratios were also reported for 10 out of 16 species studied by Loubens & Aquim (1986) in the Mamoré River, including *P. nattereri* and other characids (Loubens & Aquim, 1986), so this phenomenon is not infrequent. There were *c.* 150 small specimens, however, whose sex could not be determined in the field because of small size or state of preservation in gillnets; these were probably males because immature testes are more difficult to identify or spot than ovaries. This might account for part of the biased sex ratio.

BREEDING SEASON

Fish reproduction in the Amazon basin is often seasonal and frequently interpreted in relation to hydrological and rainfall dynamics (Lowe-McConnell, 1964, 1987; Kramer, 1978; Goulding, 1980, 1981), particularly in characids (Goulding, 1980; Vazzoler & Menezes, 1992). The breeding season of *P. nattereri* in the Bolivian Amazon was strongly seasonal: it extended over 5–6 months but peaked over no more than 2 months, in November and December. These results confirmed previous preliminary observations in the Mamoré (Loubens & Aquim, 1986) and in the Paraná Rivers (Vazzoler & Menezes, 1992). In the Bolivian Amazon, the breeding period of *P. nattereri* was desynchronized with water level and coincided better with the rainfall and photoperiod cycles. A set of environmental stimuli may be more reliable in cueing reproduction than a single stimulus, helping to ensure that the proper conditions are met for maximizing reproductive success (Bromage *et al.*, 2001). The red-bellied piranhas are known to lay their eggs on newly submerged vegetation (Uetanabaro *et al.*, 1993), so spawning under rising waters, as already observed for other piranha species (Vazzoler & Menezes, 1992; Ruffino & Isaac, 1995), was expected. The availability of submerged vegetation during early rising waters might be the ultimate cue that triggers spawning. Rainfall is a precursor of rising waters, so it is not surprising either that the maturing period starts with the first rainfall, either through perception of the sound generated by the rain drops or the physico-chemical changes occurring in the water by dilution of allochthonous material washed out from the riverbanks (Lowe-McConnell, 1979; Wootton, 1998). Rainfalls, however, can be sudden and their pattern can strongly vary between years, so relying on rainfalls only might be a risky strategy. Variations of day-length are more consistent between years, so they might be a more reliable cue (or an additional one) for inducing sexual maturation, even though they might prove insufficient as a proximate cue for spawning. This may account for why red-bellied piranha started maturing during the period of increasing day-length and eventually spawned during early rising waters in both rivers. Interestingly, the height of the breeding period occurred

exactly at the same months in the two rivers (November and December), when photoperiod was maximum. The reason why the breeding intensity of *P. nattereri* decreased from January onwards cannot be accounted for by changes in rainfall and water level either, since rainfall was still abundant and water level was rising or stable, thereby giving red-bellied piranha access to numerous potential spawning sites on submerged vegetation. By contrast, it is possible that red-bellied piranha respond to decreasing day length, which has been demonstrated to inhibit gonadal maturation in several species, such as the European cyprinid *Barbus barbus* (L.) (Poncin, 1988). In temperate regions, this mechanism has been interpreted as an energy conservation mechanism, which enables spawners to save energy because offspring hatched beyond a certain date would not be capable growing to the size that would enable them to survive the first harsh season of life, *i.e.* winter (Poncin, 1988; Baras, 1996; Baras & Philippart, 1999). Even though there is no major decline in water temperature in the tropics, the dry season can be compared to winter in the temperate regions, because receding waters force fishes to immigrate from the plentiful floodplain into the main river, where food density is lower and predation risk is greater. As a corollary, the issue of attaining a cut-off size before returning to the main river, and thus to be hatched soon enough and grow fast enough, might prove important in the tropics as well. This might favour the selection of a mechanism that would inhibit sexual maturation under decreasing day length. An additional factor which might favour the selection of such a mechanism is the possible importance of having the capability to reproduce as yearlings. Even though red-bellied piranha grow at a fast rate during the first year of their life, a delay of a few months in spawning may suffice to compromise the survival of the young or their capacity to spawn during the next season, which in both cases decrease the parent's fitness. Reproducing early in the season also allows for multiple spawning events within a season.

The present results suggest that photoperiod may be a key environmental factor determining the periodicity of reproduction in natural populations of red-bellied piranha, as already observed in the Nile tilapia *Oreochromis niloticus* (L.) (Duponchelle *et al.*, 1999). This is consistent with the idea that seasonally changing pattern of day-length is responsible for the 'cueing and timing of reproduction in the majority of fish' (Bromage *et al.*, 2001). Nonetheless, in the absence of experimental confirmation, it cannot be ascertained that decreasing day-length is indeed triggering the cessation of spawning: it might be possible that females are already spawned out by that time.

SIZE AND AGE AT MATURITY

Published data on the size at first maturity in piranhas are scarce (Vazzoler & Menezes, 1992; Lamas & Godinho, 1996), particularly in *P. nattereri*. The only available information comes from the studies of Lowe-McConnell (1964) in the Rupununi River, British Guyana (160 mm L_S), and from Loubens & Aquim (1986) in the Mamoré River, who reported preliminary estimations between 150 and 180 mm L_S . The sizes at first maturity observed during this study were slightly smaller, ranging from 130 to 140 mm for females and 109 to 114 mm for males. The difference with Loubens and Aquim's (1986) results might be

a reflection of the sampling periods. These authors carried out their study during a period of particularly high and prolonged floods, whereas this study took place under relatively low floods (Loubens *et al.*, 1992; Hamilton *et al.*, 2004). High and prolonged floods provide better trophic conditions, essentially because fishes can take advantage of the floodplain during longer periods (Lowe-McConnell, 1964, 1987; Junk *et al.*, 1989; Junk, 1997). This might explain the larger sizes at first maturity observed by Loubens & Aquim (1986), as size at maturity may vary according to resource availability, directly or indirectly through growth rate variations (Reznick, 1983, 1990; Stearns & Crandall, 1984; Stearns & Koella, 1986; Stearns, 1992; Wootton, 1998).

In both basins, males and females reached sexual maturity during their first year or very early in their second (between 7 and 13 months). The calculated differences in age at maturity between the fish of the two rivers or between sexes of the same river were more theoretical than practical. Indeed, as fish of both basins had a similar, restricted breeding season, with a peak breeding activity in November-December (which can be taken as a mean hatching date) and that they reached maturity within a year, they all effectively reproduced for the first time at approximately the same age during the breeding season following their hatching. The red-bellied piranhas reproduce as yearling in both rivers, but at larger sizes in the Mamoré, which allows for a higher fecundity at first reproduction. The larger size at first maturity observed in the Mamoré during this study mainly reflect differences in growth rates, which is consistent with the hypothesis of more favourable trophic conditions in the Mamoré.

FECUNDITY AND OOCYTE SIZE

The presence of oocytes at different developmental stages in ripe females of *P. nattereri* suggests that they can spawn at least twice during the breeding period. Similar ovarian characteristics were reported and multiple spawning was suspected for another piranha, *Serrasalmus spilopleura* Kner in the Itumbiara Reservoir, Brazil (Lamas & Godinho, 1996). This observation further supports the view that it might be important for piranhas to spawn as early as possible during the rising waters, because it allows time for a second spawn during favourable conditions for offspring survival. Those offspring produced from early spawning may be more likely to grow to maturity by the next reproductive season.

Before this study, information on the fecundity of *P. nattereri* was restricted to captive specimens, which laid <5000 eggs per spawning (Pauly, 1994). These values compare to the smallest females (126 g and 141 mm L_S) with ripe gonads observed during this study, suggesting constraints on egg production under captive conditions. The common trade-off between egg size and number in fishes (Smith & Fretwell, 1974; Elgar, 1990) was not observed for *P. nattereri*. Although oocyte sizes were nearly identical in the two rivers, red-bellied piranha females produced significantly more oocytes per spawning event in the Mamoré than in the Itenez, indicating that the amount of energy allocated per spawning event is higher in the Mamoré than in the Itenez. The frequency of spawning events is not known, so it is difficult to determine whether higher fecundity in the Mamoré is compensated for by shorter between-spawn intervals

in the Itenez. Between-spawn interval and fecundity, however, are dependent on foraging efficiency and therefore dependent on food availability (Reznick & Yang, 1993). In the Itenez, where food abundance is low, shorter between-spawn intervals are unlikely. Hence, it seems reasonable to assume that the higher fecundity of females in the Mamoré represents a higher overall reproductive effort, owing to better trophic conditions in this river.

AGE AND GROWTH

In the subfamily Serrasalminae, females generally attain a larger size than males, as exemplified by *C. macropomum* and *P. brachypomus* (Araujo-Lima & Goulding, 1997; Loubens & Panfili, 1997, 2001). This study indicates that this pattern of between-sex growth dimorphism also applies to *P. nattereri*, thereby supporting preliminary observations in the Mamoré River by Loubens & Aquim (1986). The largest fish captured during this study (280 mm L_S) were slightly larger than those observed in British Guyana (260 mm L_S ; Lowe-McConnell, 1964) or in the Tocantins River (250 mm L_S ; Dos Santos *et al.*, 1984) and in the Pantanal, Mato Grosso (240 mm L_S , Sazima & Machado, 1990). During this study, red-bellied piranhas were larger but also were proportionally heavier for a given L_S in the Mamoré than in the Itenez, supporting the hypothesis of more favourable trophic conditions in the Mamoré.

The monthly percentage of translucent edges on the otoliths validated the formation of one annulus per year. The otolith opaque zone formed during the period of fast growth under high waters, when fishes have access to the plentiful floodplain in the savannah and forest (Lowe-McConnell, 1964, 1987; Junk *et al.*, 1989; Junk, 1997). The translucent (stainable) mark corresponds to a period of slow growth during the drought and the early flooding season. This alternation of periods of fast and slow growth is widespread not only among characids (*C. macropomum* and *P. brachypomus*; Loubens & Panfili, 1997, 2001) and pimelodid catfishes [*Pseudoplatystoma fasciatum* (L.) and *Pseudoplatystoma tigrinum* (Valenciennes); Loubens & Panfili, 2000] in the Bolivian Amazon but also in the Pantanal, Brazil [*Pseudoplatystoma corruscans* (Spix & Agassiz); Mateus & Petrere, 2004; *Sorubim lima* (Bloch & Schneider), Penha *et al.*, 2004].

Studies on growth of *P. nattereri*, or other piranha species, in natural environments do not seem to have been carried out so far. The only available information comes from Pauly (1994), who used published length at estimated age for *P. nattereri* in the British Guyana (Lowe-McConnell, 1964). Although Pauly's (1994) results ($L_\infty = 260$ mm, $K = 0.893$ and $t_0 = -0.05$) were estimated from a very weak data set, the rapid growth reported was confirmed by the present study. Females in the Mamoré could reach 170 mm (L_S) in 1 year, which is comparable to Pauly's (1994) results (160 mm L_S) and observations on *Pygocentrus cariba* (Humboldt) (165 mm at *c.* 1 year; Nico & Taphorn, 1986). As suggested earlier, rapid growth in the first year may help maximize egg production during the first spawning season.

Size variation at a given age in *P. nattereri* was largest during the first 2 years of life. This was particularly evident in specimens from the Mamoré, for which analyses were based on larger samples. At least three explanations might

account for this. First, this early size dispersion suggests there were several within-year cohorts (more than one spawning event per female), with size differences progressively becoming less conspicuous with age. Second, there could be a slight bias in the estimation of younger stages owing to the extended breeding period of the species. The peak breeding period (November) was chosen as the mean hatching date, but hatchings could probably occur as early as September and as late as February, accounting for 2 to 3 months 'error' (plus or minus) in the age estimation of younger individuals. Third, depending on where and when the young hatched in the flooded area, the encountered trophic conditions may vary and account for important growth differences among individuals during the first year.

In both rivers, females grew larger and faster than males, but the growth difference between females and males was more important in the Mamoré than in the Iténez. Females in the Mamoré also grew larger and faster than females in the Iténez, whereas males did not differ significantly. These differences can be largely accounted for by the difference between the trophic conditions in the two rivers. Female red-bellied piranhas have a greater growth potential than their male counterparts, which is a common feature in the Serrasalminae (Araujo-Lima & Goulding, 1997; Loubens & Panfili, 1997, 2001). On the other hand, it is widely acknowledged that reproduction is much more energetically costly in females than in males among species with a restricted degree of parental care (Wootton, 1998). In situations where food is abundant, females can probably find enough food so as to compensate for this greater energetic investment and eventually grow faster than males. This situation might apply to the Mamoré River. By contrast, if food resources are scarcer, females might have greater difficulties to pay the reproductive debt and grow faster than males, as might be the case in the Iténez River. Males, which have a less demanding reproductive effort, are less affected than females in their growth rates, so between-river differences might be less conspicuous for males than females, as was the case during this study.

LIFE-HISTORY TRAITS AND THE TROPHIC CONDITION HYPOTHESIS

The starting hypothesis in this study was that *P. nattereri* would encounter more favourable trophic conditions in the white waters of the Mamoré than in the clear waters of the Iténez and that these contrasted environmental conditions would induce differences in life-history traits. Differences in size at first sexual maturity, fecundity and growth of *P. nattereri* between the two rivers were indeed observed as expected. Alternative explanations, however, might also be invoked, such as differences in size-selective mortality ratios between the two rivers. Life-history theories, supported by several empirical studies, predict that a low adult to juvenile mortality ratio in a population would lead to an increased size at maturity at an earlier age and decreased reproductive effort (Gadgil & Bossert, 1970; Law, 1979; Michod, 1979; Reznick *et al.*, 1990; Stearns, 1992). A lower adult to juvenile mortality ratio in the Mamoré, due to different predator sizes or fishing pressures than in the Iténez, might then account for the larger size at maturity observed in the Mamoré. There

is no indication that difference in fishing pressure exist between the two rivers for this species (pers. obs.) and comparative analyses of the red-bellied piranha's predator size and abundance or size-mortality schedules have never been carried out in Bolivian Rivers. The larger sizes at maturity observed in the Mamoré, however, were not accompanied by earlier age at maturity as predicted by the size-selective mortality theory. The theory also predicts a decreased reproductive effort in case of low adult to juvenile mortality ratio, whereas the available evidence rather suggested a higher investment per spawning event in the Mamoré. These results together with the significantly better condition factors observed in the Mamoré throughout the study period strongly suggest that the differences in trophic conditions between the white and clear waters are likely to account for a large part of the observed life-history variations between the *P. nattereri* populations from the Mamoré and Iténez Rivers.

On the other hand, if the two geographical populations (Mamoré and Iténez) had limited gene flow for a sufficient period, the observed variations in life-history traits might also be explained by genetic divergences between the two populations through the action of stochastic events (genetic drift and accumulated mutations). Recent phylogenetic analysis, however, revealed a common and very recent evolutionary history of *P. nattereri* in the Bolivian Amazon Rivers with a low level of nucleotide diversity (unresolved phylogeographic tree) and high haplotype sharing among rivers, resulting either from ancestral polymorphism or the persistence of current migrations between the rivers (Hubert, 2005; Hubert *et al.*, 2007). These genetic results suggest that the observed variation in life-history traits are not due to genetic divergence through stochastic events, but rather to contrasted environmental conditions between the white and clear waters resulting in adaptive phenotypic plasticity or genetic selection (through differential selective pressure). Adaptive response of life-history traits to contrasted environmental conditions may be achieved either by phenotypic plasticity or genetic selection, depending on time scales involved (Giesel, 1976; Caswell, 1983; Partridge & Harvey, 1988; Stearns, 1992). Discriminating between phenotypic plasticity and genetic selection in the observed life-history variation of *P. nattereri* would require the comparison of individuals from the two geographical populations kept under identical experimental conditions or mutually transplanted (Mann *et al.*, 1984; Reznick *et al.*, 1990; Belk, 1995; Duponchelle *et al.*, 1998).

This research was supported by Institut de Recherche pour le Développement (IRD, France) in collaboration with the Instituto de Biología Molecular y Biotecnología – Universidad Mayor San Andrés (IBMB-UMSA) La Paz Bolivia, the Unidad de Limnología y Recursos Acuáticos – Universidad Mayor San Simon (ULRA-UMSS) Cochabamba Bolivia. Special thanks go to H. Muñoz, F. Carvajal, L. Cordova, J. Camacho and A. Inturias for field help and to P. Vauchel (UR HYBAM-IRD), SENAMHI (Servicio Nacional de Meteorología e Hidrología), Bolivia and ANA (Agência Nacional de Aguas), Brazil for providing water level data. We also wish to thank two anonymous referees for their useful comments. We acknowledge F. Laloe (IRD France) and F. Guilhaumon (Université Montpellier 2, France) for their assistance with statistical analyses and J.-D. Durand (IRD France) and P. Berrebi (Université Montpellier 2, France) for their useful comments on an earlier version of the manuscript.

References

- Araujo-Lima, C. A. R. M. & Goulding, M. (1997). *So Fruitful a Fish. Ecology, Conservation and Aquaculture of the Amazon's Tambaqui*. New York, NY: Columbia University Press.
- Bagenal, T. B. (1969). The relationship between food supply and fecundity in brown trout *Salmo trutta* L. *Journal of Fish Biology* **1**, 167–182.
- Baras, E. (1996). Commentaire à l'hypothèse de l'éternel retour de Cury (1994): proposition d'un mécanisme fonctionnel dynamique. *Canadian Journal of Fisheries and Aquatic Sciences* **53**, 681–684.
- Baras, E. & Philippart, J. C. (1999). Adaptive and evolutionary significance of a reproductive thermal threshold in *Barbus barbus*. *Journal of Fish Biology* **55**, 354–375.
- Belk, M. C. (1995). Variation in growth and age at maturity in bluegill sunfish: genetic or environmental effects? *Journal of Fish Biology* **47**, 237–247.
- Bonetto, A., Pignalberi, C. & Cordoviola, E. (1967). Las "palometas" o "piranhas" de las lagunas del Paraná medio. *Acta Zoologica Lilloana* **23**, 45–65.
- Bromage, N., Porter, M. & Randall, C. (2001). The environmental regulation of maturation in farmed finfish with special reference to the role of photoperiod and melatonin. *Aquaculture* **197**, 63–98.
- Caswell, H. (1983). Phenotypic plasticity in life-history traits: demographic effects and evolutionary consequences. *American Zoologist* **23**, 35–46.
- Corbin, D., Guyot, J.-L., Calle, H. & Quintanilla, J. (1988). Datos físico-químicos de los medios acuáticos de la zona del Mamoré central, region de Trinidad, Amazonia Boliviana. *Informe ORSTOM (BOL), NO. 8*. La Paz: ORSTOM.
- Dos Santos, G. M., Jégu, M. & de Merona, B. (1984). *Catálogo de peixes comerciais do Baixo Rio Tocantins*. Manaus: Electronorte/INPA.
- Duponchelle, F., Pouyaud, L. & Legendre, M. (1998). Evidence of environmental effects on reproductive characteristics in Nile tilapia (*Oreochromis niloticus*) populations from man-made lakes of Côte d'Ivoire. *Aquatic Living Resources* **11**, 137–144.
- Duponchelle, F., Cecchi, P., Corbin, D., Nunez, J. & Legendre, M. (1999). Spawning season variations of female Nile tilapia, *Oreochromis niloticus*, populations from man-made lakes of Côte d'Ivoire. *Environmental Biology of Fishes* **56**, 377–389.
- Elgar, M. A. (1990). Evolutionary compromise between a few large and many small eggs: comparative evidence in teleost fish. *Oikos* **59**, 283–287.
- Fink, W. (1993). Revision of the piranha genus *Pygocentrus* (Teleostei, Characiformes). *Copeia* **1993**, 665–687.
- Gadgil, M. & Bossert, W. H. (1970). Life historical consequences of natural selection. *American Naturalist* **104**, 1–2.
- Géry, J. (1964). Contributions à l'étude des poissons Characoïdes – 27: Systématique et évolution de quelques piranhas (*Serrasalmus*). *Vie et Milieu* **XIV**, 597–617.
- Giesel, J. T. (1976). Reproductive strategies as adaptations to life in temporally heterogeneous environments. *Annual Review of Ecology and Systematics* **7**, 57–79.
- Goulding, M. (1980). *The Fishes and the Forest. Explorations in Amazonian Natural History*. Berkeley, CA: University of California Press.
- Goulding, M. (1981). *Man and Fisheries on an Amazon Frontier*. Boston and London: Junk Publishers.
- Guyot, J.-L., Corbin, D., Quintanilla, J. & Calle, H. (1991). Hydrochimie des lacs dans la région de Trinidad (Amazonie bolivienne). Influence d'un fleuve andin: le Rio Mamoré. *Revue d'Hydrobiologie Tropicale* **24**, 3–12.
- Guyot, J.-L., Jouanneau, J.-M. & Wasson, J.-G. (1999). Characterisation of the river bed and suspended sediments in the Rio Madeira drainage basin (Bolivian Amazonia). *Journal of South American Sciences* **12**, 401–410.
- Hamilton, S. K., Sippel, S. J. & Melack, J. M. (2004). Seasonal inundation patterns in two large savanna floodplains of South America: the Llanos de Moxos (Bolivia) and the Llanos del Orinoco (Venezuela and Colombia). *Hydrological Processes* **18**, 2103–2116.

- Hislop, J. R. G., Robb, A. P. & Gauld, J. A. (1978). Observations of feeding level on growth and reproduction in haddock, *Melanogrammus aeglefinus* (L.) in captivity. *Journal of Fish Biology* **13**, 85–98.
- Hubert, N. (2005). Evolution de l'ichtyofaune en Amérique du sud: biogéographie des Characiformes et phylogéographie des piranhas *Serrasalmus* Lacépède, 1803 et *Pygocentrus* Müller et Troschel, 1844. PhD Thesis, Muséum National d'Histoire Naturelle, Paris.
- Hubert, N. & Renno, J.-F. (2006). Historical biogeography of South American freshwater fishes. *Journal of Biogeography* **33**, 1414–1436.
- Hubert, N., Duponchelle, F., Nuñez, J., Paugy, D., Garcia-Davila, C. & Renno, J. (2007). Phylogeography of the piranhas genera *Serrasalmus* and *Pygocentrus*: implications for the diversification of the Neotropical Ichthyofauna. *Molecular Ecology* **16**, 2115–2136.
- Jégu, M. (2003). Serrasalminae. In *Check List of Freshwater Fishes of South and Central America* (Reis, R. E., Kullander, S. O. & Ferraris, C. J., eds), pp. 182–196. Porto Alegre: Edipucrs.
- Junk, W. J. (1997). *The Central Amazon Floodplains. Ecology of Pulsing System*. Berlin: Springer.
- Junk, W. J., Bayley, P. B. & Sparks, J. S. (1989). The flood pulse concept in river floodplain systems. In *International Large River Symposium* (Dodge, D. P., ed.). *Canadian Special Publication of Fisheries and Aquatic Sciences* **106**, 110–127.
- Kimura, D. K. (1980). Likelihood methods for the von Bertalanffy growth curve. *Fishery Bulletin* **77**, 765–776.
- Kramer, D. L. (1978). Reproductive seasonality in the fishes of a tropical stream. *Ecology* **59**, 976–985.
- Lamas, I. R. & Godinho, A. L. (1996). Reproduction in the piranha *Serrasalmus spilopleura*, a neotropical fish with an unusual pattern of sexual maturity. *Environmental Biology of Fishes* **45**, 161–168.
- Lauzane, L. & Loubens, G. (1985). *Peces del Rio Mamoré*. Paris: Editions de l'ORSTOM.
- Lauzane, L., Loubens, G. & Le Guennec, B. (1990). Pesca y biología pesquera en el Mamoré medio (region de Trinidad, Bolivia). *Interciencia* **15**, 452–460.
- Law, R. (1979). Optimal life histories under age-specific predation. *American Naturalist* **114**, 399–417.
- Loubens, G. & Aquim, J. L. (1986). Sexualidad y reproducción de los principales peces de la cuenca del Río Mamoré, Béni, Bolivia. *Informe científico N° 5*. Trinidad, Bolivia: ORSTOM-UTB-BENI.
- Loubens, G. & Panfili, J. (1997). Biologie de *Colossoma macropomum* (Teleostei: Serrasalmidae) dans le bassin du Mamoré (Amazonie bolivienne). *Ichthyological Exploration of Freshwaters* **8**, 1–22.
- Loubens, G. & Panfili, J. (2000). Biologie de *Pseudoplatystoma fasciatum* et *P. tigrinum* (Teleostei: Pimelodidae) dans le bassin du Mamoré. *Ichthyological Exploration of Freshwaters* **11**, 13–34.
- Loubens, G. & Panfili, J. (2001). Biologie de *Piaractus brachipomus* (Teleostei: Serrasalmidae) dans le bassin du Mamoré (Amazonie bolivienne). *Ichthyological Exploration of Freshwaters* **12**, 51–64.
- Loubens, G., Aquim, J. L. & Robles, E. (1984). Primeras observaciones sobre la sexualidad y la reproducción de las principales especies de peces de la region de Trinidad, Béni, Bolivia. *Informe científico N° 1*. Trinidad, Bolivia: ORSTOM-UTB-BENI.
- Loubens, G., Lauzane, L. & Le Guennec, B. (1992). Les milieux aquatiques de la region de Trinidad (Béni, Amazonie bolivienne). *Revue d'Hydrobiologie Tropicale* **25**, 3–21.
- Lowe-McConnell, R. H. (1964). The fishes of the Rupununi savanna district of British Guiana, South America. Part 1. Ecological groupings of fish species and effects of the seasonal cycle on the fish. *Journal of the Linnean Society (Zoology)* **45**, 103–144.
- Lowe-McConnell, R. H. (1979). Ecological aspects of seasonality in fishes of tropical waters. *Symposium of the Zoological Society of London* **44**, 219–241.

- Lowe-McConnell, R. H. (1987). *Ecological Studies in Tropical Fish Communities*. Cambridge: Cambridge University Press.
- Machado-Allison, A. & Fink, W. (1995). Sinopsis de las especies de la subfamilia Serrasalminae presentes en la cuenca del Orinoco. Claves, Diagnósis e Ilustraciones. Caracas-Venezuela: LITO COLOR R.I., C.A.
- Mann, R. H. K., Mills, C. A. & Crisp, T. (1984). Geographical variation in the life-history tactics of some species of freshwater fish. In *Fish Reproduction: Strategies and Tactics* (Potts, G. W. & Wootton, R. J., eds), pp. 171–186. London: Academic Press.
- Mateus, L. A. F. & Petrere, M. J. (2004). Age, growth and yield per recruit analysis of the pintado *Pseudoplatystoma corruscans* (Agassiz 1829) in the Cuiaba River basin, Pantanal Matogrossense, Brazil. *Brazilian Journal of Biology* **64**, 257–264.
- Michod, R. E. (1979). Evolution of life histories in response to age mortality factors. *American Naturalist* **113**, 531–550.
- Navarro, G. & Maldonado, M. (2002). *Geografía Ecológica de Bolivia: vegetación y ambientes acuáticos*. Cochabamba, Bolivia: Centro de Ecología Simon I, Patiño – Departamento de Difusión.
- Nico, L. G. & Taphorn, D. C. (1986). Those bitin' fish from South America. *Tropical Fish Hobbyist* **34**, 24–27, 30–34, 40–41, 56–57.
- Nico, L. G. & Taphorn, D. C. (1988). Food habits of piranhas in the low llanos of Venezuela. *Biotropica* **20**, 311–321.
- Partridge, L. & Harvey, P. H. (1988). The ecological context of life history evolution. *Science* **241**, 1449–1455.
- Pauly, D. (1994). Quantitative analysis of published data on the growth, metabolism, food consumption, and related features of the red-bellied piranha, *Serrasalmus nattereri* (Characidae). *Environmental Biology of Fishes* **41**, 423–437.
- Penha, J. M. F., Mateus, L. A. F. & Barbieri, G. (2004). Age and growth of the duck-bill catfish (*Sorubim cf. lima*) in the Pantanal. *Brazilian Journal of Biology* **64**, 125–134.
- Poncin, P. (1988). Le contrôle environnemental et hormonal de la reproduction du barbeau, *Barbus barbus* (L.), et du chevaine, *Leuciscus cephalus* (L.) (Pisces Cyprinidae), en captivité. *Cahiers d'Ethologie appliquée* **8**, 173–330.
- Reznick, D. A. (1983). The structure of guppy life histories: the trade-off between growth and reproduction. *Ecology* **64**, 862–873.
- Reznick, D. A. (1990). Plasticity in age and size at maturity in male guppies (*Poecilia reticulata*): an experimental evaluation of alternative models of development. *Journal of Evolutionary Biology* **3**, 185–203.
- Reznick, D. & Yang, A. P. (1993). The influence of fluctuating resources on life history: patterns of allocation and plasticity in female guppies. *Ecology* **74**, 2011–2019.
- Reznick, D. A., Briga, H. & Endler, J. A. (1990). Experimentally induced life history evolution in a natural population. *Nature* **346**, 357–359.
- Roche, M. A. & Fernandez-Jauregui, C. (1988). Water resources, salinity and salt yields of the rivers of the Bolivian Amazon. *Journal of Hydrology* **101**, 305–331.
- Ruffino, M. & Isaac, V. (1995). Reproductive strategies and biological parameters of several Brazilian Amazon fish species. *NAGA. The ICLARM Quarterly* **18**, 41–45.
- Saint-Paul, U., Zuanon, J., Villacorta Correa, M. A., Garcia, M., Noemi Fabre, N., Berger, U. & Junk, W. J. (2000). Fish communities in central white- and black-water floodplains. *Environmental Biology of Fishes* **57**, 235–250.
- Sazima, I. & Guimares, S. A. (1987). Scavenging on human corpses as a source for stories about man-eating piranhas. *Environmental Biology of Fishes* **20**, 75–77.
- Sazima, I. & Machado, F. A. (1990). Underwater observation of piranhas in western Brazil. *Environmental Biology of Fishes* **28**, 17–31.
- Schulte, W. (1988). *Piranhas in the Aquarium*. Neptune City, NJ: T.F.H. Publications.
- Scott, D. P. (1962). Effects of food quantity on fecundity of rainbow trout, *Salmo gairdneri*. *Journal of the Fisheries Research Board Canada* **19**, 715–731.
- Sioli, H. (1984). The Amazon and its main affluents: hydrography, morphology of the river courses, and river types. In *The Amazon: Limnology and Landscape Ecology*

- of a Mighty Tropical River and its Basin* (Sioli, H., ed.), pp. 127–165. Dordrecht: Dr W. Junk Publishers.
- Smith, C. C. & Fretwell, S. D. (1974). The optimal balance between size and number of offspring. *American Naturalist* **108**, 499–506.
- Stearns, S. C. (1992). *The Evolution of Life Histories*. Oxford: Oxford University Press.
- Stearns, S. C. & Crandall, R. E. (1984). Plasticity for age and size at sexual maturity: a life history response to unavoidable stress. In *Fish Reproduction: Strategies and Tactics* (Potts, G. W. & Wootton, R. J., eds), pp. 13–33. London: Academic Press.
- Stearns, S. C. & Koella, J. C. (1986). The evolution of phenotypic plasticity in life history traits: predictions of reaction norms for age and size at maturity. *Evolution* **40**, 893–913.
- Tomassone, R., Dervin, C. & Masson, J. P. (1993). *Biométrie: modélisation de phénomènes biologiques*. Paris: Masson.
- Townshend, T. J. & Wootton, R. J. (1984). Effects of food supply on the reproduction of the convict cichlid, *Cichlasoma nigrofasciatum*. *Journal of Fish Biology* **24**, 91–104.
- Uetanabaro, M., Wang, T. & Abe, A. S. (1993). Breeding behaviour of the red-bellied piranha, *Pygocentrus nattereri*, in nature. *Environmental Biology of Fishes* **38**, 369–371.
- Vazzoler, A. E. d. M. & Menezes, N. A. (1992). Síntese de conhecimentos sobre o comportamento reprodutivo dos Characiformes da América do Sul (Teleostei, Ostariophysi). *Revista Brasileira de Biologia* **52**, 627–640.
- Winemiller, K. O. (1989). Ontogenetic diet shifts and resource partitioning among piscivorous fishes in the Venezuelan llanos. *Environmental Biology of Fishes* **26**, 177–199.
- Winemiller, K. O. & Kelso-Winemiller, L. C. (1993). Predatory response of piranhas to alternative prey. *National Geographic Research & Exploration* **9**, 344–357.
- Wootton, R. J. (1973). The effect of size of food ration on egg production in the female three-spined stickleback, *Gasterosteus aculeatus* L. *Journal of Fish Biology* **5**, 89–96.
- Wootton, R. J. (1998). *The Ecology of Teleost Fishes*, 2nd edn. Dordrecht: Kluwer Academic Publisher.