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Induced breeding and larval rearing of Surubí, *Pseudoplatystoma fasciatum* (Linnaeus, 1766), from the Bolivian Amazon

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Abstract

Brooders of Surubí (*Pseudoplatystoma fasciatum*) were caught in the Ichillo River (Bolivian Amazon) and adapted to captivity conditions for 1 year in the facilities of the experimental aquaculture station of 'El Prado' (Santa Cruz de la Sierra) under natural temperature and photoperiod conditions. Induced reproduction was obtained by means of Ovaprim® (Syndel, Canada) injections and artificial fertilization. Sperm and ova were obtained by gentle stripping of male and female brooders. Fertilized eggs were incubated in 60 L Zug jars. A mean hatching rate of 73.7 ± 19.0% was obtained after 24 h at 26.5 °C. For larval rearing, several protocols were tested with different settings of photoperiod, light intensity, food type and period of distribution, and stocking density. The best survival rates were obtained with *Artemia* nauplii feeding in total darkness. A high level of aggressiveness between larvae and precocious appearance of jumpers was observed, but these can be controlled with appropriate rearing conditions.

Keywords: *Pseudoplatystoma fasciatum*, larval rearing, photoperiod, induced spawning, Bolivian Amazon

Introduction

The fishing pressure keeps increasing in the Amazon region (Almeida, McGrath & Ruffino, 2001; Ruffino 2004, 2005) despite reports of over-exploitation of some of the most important species (Reinert & Winter 2002; Petrere, Barthem, Córdoba & Gómez 2004). In this context, the development of aquaculture is a viable alternative to capture fisheries for providing a sustainable source of proteins for local fishermen communities and populations of the developing cities in the Amazon region. Currently, the freshwater marketable fish in this area come from inland capture (667 000 MT for 2005) and from aquaculture (246 000 MT) (FAO 2005; Ruffino 2005). In some instances, the necessity for fast-producing systems has encouraged the introduction of 'ready-to-use' alien species, essentially the tilapias, carps and African catfish (Welcomme 1988), but at the cost of environmental issues pertaining to biodiversity. The culture of economically important Amazonian species must be considered in order to provide an alternative to alien species introductions in the Amazon basin. The Bolivian Amazon represents a great potential for fish culture, especially in its northern and central parts, where high temperatures and good water quality are available all year round. One of the limiting factors of fish culture development is the lack of sustained fingerling production for the important commercial species. Within the catfish family Pimelodidae, three species, *Pseudoplatystoma fasciatum*, *P. tigrinum* and *P. coruscans*, share very similar characteristics and constitute important commercial fish resources in the Amazon and adjacent regions (Almeida 2006). *Pseudoplatystoma fasciatum* and

P. tigrinum occur throughout the Amazon basin, and in the Bolivian Amazon (Loubens & Panfili 2000), whereas *P. coruscans* is distributed in the Rio Sao Francisco and Río de la Plata Basins (Barthem & Goulding 1997; Tavares 1997). Recently, the genus *Pseudoplatystoma* has been subdivided into eight species, three long recognized ones and five new species: *P. punctifer* (Castelnau), *P. reticulatum* Eigenmann & Eigenmann, *P. orinocoense* n. sp., *P. metaense* n. sp. and *P. magdaleniatum* n. (Buitrago–Suárez & Burr 2007). According to the geographic distribution of the described species, the species studied here would be *P. punctifer*. Nevertheless, in this paper, we will continue to use the former name, *P. fasciatum*, for the sake of consistency with previous studies on the same species in the region. These Pimelodidae are among the most appreciated and expensive catfish species in the fish markets of South America. Given the increasing demand for these species, several attempts of artificial reproduction have been undertaken with *P. fasciatum* (Kossowski & Madrid 1985; Rodriguez 1996; Padilla Pérez, Alcántara Bocanegra & Ismiño Orbe 2001; Gervásio Leonardo, Romagosa, Borella & Batlouni 2004) and *P. coruscans* (Sato, Cardoso, Sallum & Godinho 1997). However, all these studies reported low larval survival rates, and so fingerling mass production is not a common reality for these species yet. Furthermore, these first experiments pointed out the high level of aggressiveness and cannibalism among larvae of *P. fasciatum* (Kossowski & Madrid 1985; Kossowski & Madrid 1991; Kossowski 1996).

The aggressive behaviour of most catfish larval stages (Britz & Pienaar 1992; Appelbaum & Kamler 2000; Giri, Sahoo, Sahu, Sahu, Mohanty, Mukhopadhyay & Ayyappan 2002) is one of the main factors affecting larval mortality (Qin & Fast 1996; Appelbaum & Kamler 2000). Cannibalism is frequent among cultured fish larvae, especially in piscivorous species, which develop a wide gape and oral teeth at a precocious age (Baras & Jobling 2002). Among the factors that govern the impact of cannibalism in fish, size heterogeneity is probably the most striking one (Qin & Fast 1996; Kestemont, Jourdan, Houbart, Melard, Paspatis, Fontaine, Cuvier, Kentouri & Baras 2003; Hseu, Huang & Chu 2007; Mandiki, Babiak, Krol, Rasolo & Kestemont 2007). Other factors that modulate cannibalism, either directly or indirectly through an increase in size heterogeneity, include the feeding environment, rearing density, water temperature, light regime and intensity (Boeuf & Le Bail 1999; Appelbaum & Kamler 2000; Kestemont *et al.* 2003). The amount and quality (or type) of food and its availability have an obvious influence on larval survival and cannibalism because a lack of satiety or a nutritional deficiency exacerbates this natural behaviour (Qin & Fast 1996). If the feeding period and meal frequency can influence larvae survival, it has also been reported in several species that light phase duration can directly influence cannibalistic behaviour (Appelbaum & Kamler 2000).

The present study aimed at providing (1) more reliable bases for the hormonally induced reproduction of *P. fasciatum*, (2) a better characterization of the effect of biotic and abiotic factors in larval intensive rearing conditions and (3) the identification of appropriate food and feeding regimes for maximizing larval survival and growth. A methodology was developed for induced breeding of *P. fasciatum* and three different food sources for first feeding of larvae: *Artemia* nauplii, decapsulated *Artemia* cysts and earthen pond zooplankton were compared. As *P. fasciatum* is a nocturnal animal, feeding mostly during the night, we investigated in parallel the influence of three important environmental factors (light regime and intensity, stocking density and feeding period) on the survival, growth and size heterogeneity of larvae.

Material and methods

Fish husbandry

Fifty fish from 2 to 12 kg were captured with nets and hook and line by local fishermen in 2002 in the Ichilo river and its tributaries (Fig. 1) near Puerto Villaroel (16°52′S; 64°46′W). The fish were transported in aerated tanks to the facilities of the 'El Prado' research station, about 150 km south of their capture place, tagged individually with PIT-tags and transferred to 2000 m^2 outdoor earthen ponds for acclimatization.

During the following year, Surubí brooders were reared at a low stocking density (one fish per 40 m^2) and fed with live fish (*Prochilodus nigricans*, *Aequidens* sp.) pig and beef liver and commercial pelleted feed.

Male and female selection

During the reproductive season (from November to March), sexual maturity of each broodfish was checked every month after anaesthesia in 2-phenoxy ethanol (0.4 mL L^{-1}) to minimize trauma to the fish and operators (Surubí possess strong pectoral spines). For males, the emission of sperm following

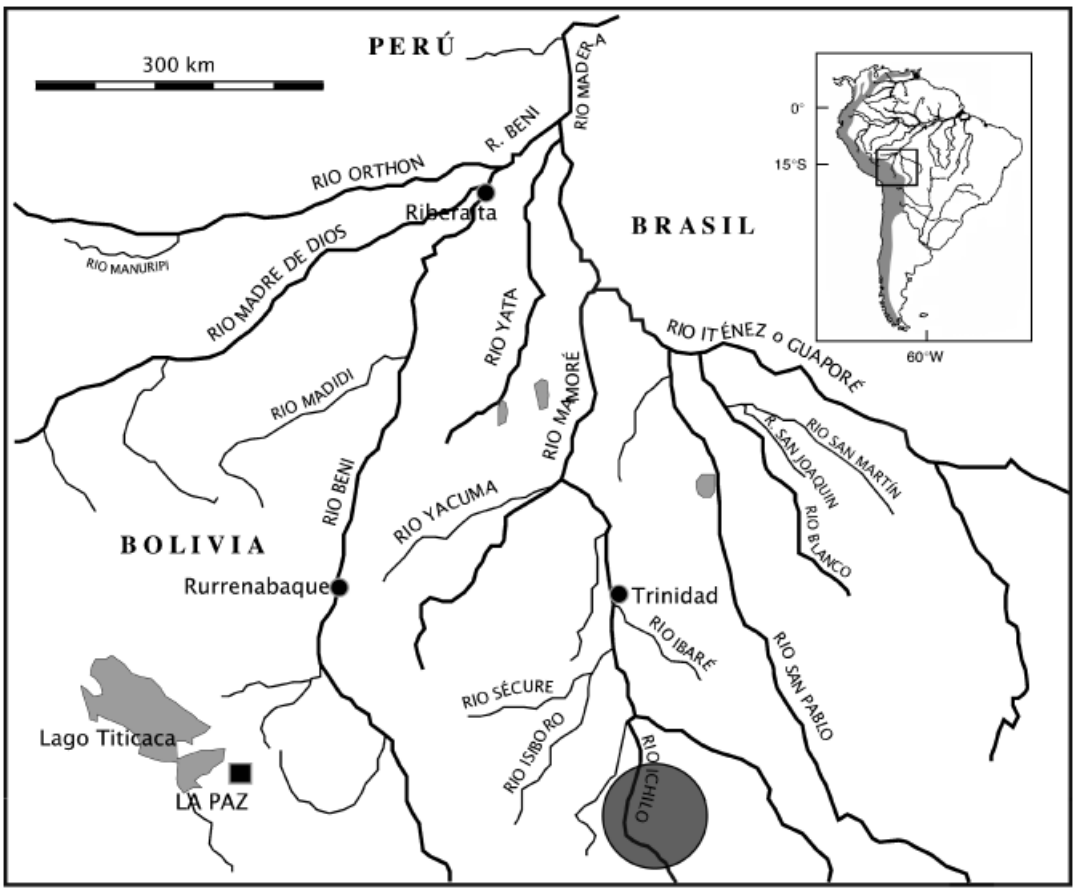

Figure 1 Schematic map of the capture area of *Pseudoplatystoma fasciatum* breeding stock in the Bolivian Amazon. The Grey circular area corresponds approximately to the fishing area in the Río Ichilo basin.

gentle abdominal pressure was verified. The maturity of females was determined by examination of follicle samples taken by an intra-ovarian biopsy.

Ovarian samples were placed in physiological saline and observed under the stereomicroscope (magnification × 20) to determine the proportion of atretic oocytes. Thereafter, saline solution was removed and Serra's solution was added to examine the position of the germinal vesicle. A subsample (containing at least 50 oocytes) of each biopsy was preserved for 24 h in physiological saline with 2% formalin, and photographed under the stereomicroscope. Oocyte diameter distributions were determined from digital photographs, using the IMAGE J (NIH) software package. For each female, the mean oocyte diameter and its coefficient of variation were calculated.

Only males giving sperm following a gentle abdominal massage were injected with Ovaprim® (Syndel, Qualicum Beach, Canada).

Hormonal treatment, artificial fertilization and incubation

Females were injected intraperitoneally with Ovaprim at a total dose of 0.5 mL kg^{-1} body weight, administered as two injections; a priming one at 10% of the total dose and, 12 h later, a resolving one at 90% of the total dose. Females were individually examined by a gentle abdominal massage starting 6 h after the second injection, and then every hour until ovulation. When ovulated eggs were present, the female was stripped after drying the papilla with a paper towel and ova were colleted in dry plastic recipients. Males were treated with 50% of the total female dose in a single injection given at the same time as the female priming injection. The sperm was collected in clean dry syringes before the first female check in order to fertilize ovulated eggs straight after their collection. Sperm fractions were stored on ice in clean dry test tubes. One sample of each collected sperm

fraction was tested for motility before being used for artificial fertilization. The routine test consisted in observation under a microscope (magnification × 400) of sperm activation after a 1:100 dilution in distilled water.

Batches of ovulated eggs (100 g) were gently mixed together with 5 mL of 0.154 M NaCl and 200 μL of milt for 60 s, and then 30 mL of distilled water was added with constant gentle stirring for another 60 s. Fertilized eggs were rinsed three times with 100 mL of water from the incubation circuit, and then transferred into 60 L Zug cylindro-conic jars in a recirculating and thermoregulated (26.5 ± 2.0 °C) water system. In the rest of the manuscript, we refer to fish age by reference to the moment of hatching (i.e. hours or days post hatch, hph and dph respectively), which took place 24 h after fertilization. At 24 hph, embryos were collected, counted by a volumetric method and distributed in 60 L tanks in a flow-through ($60\,L\,h^{-1}$) thermoregulated recirculating water system (mean temperature of 26.5 ± 2.0 °C). Food distribution started generally at 4 dph.

Effects of food type on the growth and survival of larvae

Three different food types were tested: decapsulated cysts of brine shrimp, brine shrimp nauplii and natural zooplankton. Natural zooplankton (mainly *Moina* and copepods) was provided by continuous water pumping from a plankton-enriched earthen pond in the vicinity of the experimental tanks. Water was filtered through a 200 μm mesh in order to prevent the entrance of insect larvae into the experimental tanks. All diets were distributed in excess (controlling the presence of live food 30 min after distribution) five times a day (8:00, 10:00, 12:00, 14:00 and 6:00 hours). Food comparison trials were performed using 100 full sibling Surubí larvae aged 4 dph. Larvae were counted and distributed in nine circular 60 L tanks (three replications per food type) connected to a ($60\,L\,h^{-1}$) water recirculating system (for *Artemia* cysts and nauplii treatments) or flow-through pond water at $60\,L\,h^{-1}$ (natural zooplankton treatment). The ammonia and nitrite concentrations were monitored during the experiment and they never exceeded 0.3 and 0.05 $mg\,L^{-1}$ of $N–NH_4$ and $N–NO_2$ respectively. The mean temperature was maintained nearly constant at 27.0 ± 1.2 °C during the experiment and so was dissolved oxygen (between 8 and 9 $mg\,L^{-1}$) by continuous aeration. The experiment was conducted under a natural photoperiod: 12 h of light and 12 h of darkness (12L:12D). Three controls were performed at 15, 28 and 35 dph to determine the survival rates; standard length was measured only at 28 dph by image analysis.

Effects of light intensity on the growth and survival of larvae

In its natural environment, the Surubí, *P. fasciatum*, is known as a nocturnal feeding fish (negative phototaxis). We observed that larvae became photophobic very rapidly after yolk sac absorption when they acquired swimming capabilities. For this reason, we tested three levels of light intensity during larval rearing (< 0.01, 1 and 10 lx), corresponding to dark, shaded light, and low light respectively, under a 12L:12D photoperiod regime (except for the 24 h dark treatment). No light intensity brighter than 10 lx was tested in order to avoid additional stress due to excessive light intensity.

Twelve sets of 1900 larvae aged 2 dph were distributed in twelve 60-L tanks (three treatments with four replicates). The average temperature in the tanks was 28.5 ± 1.0 °C. All tanks received the same amount of food in slight excess (*Artemia* nauplii), distributed six times daily (3:00, 7:00, 11:00, 15:00, 19:00 and 21:00 hours), with control half an hour after each distribution to make sure that the number of preys available was in excess. At 9 dph, larvae were sampled for survival and size determination using image analysis.

Effects of stocking density on the growth and survival of larvae

Three stocking densities were evaluated using (almost) logarithmic steps between densities (i.e. 10, 30, 100 larvae L^{-1}). Two light regimes (12L:12D and 0L:24D) were tested for each stocking density, in order to evaluate the interaction between the two environmental variables (six treatments, three replications per treatment combination). Fish were fed five times a day (8:00, 10:00, 12:00, 14:00 and 16:00 hours) in slight excess with newly hatched *Artemia* nauplii. Water temperature was maintained at 26.7 ± 1.0 °C.

Effect of photoperiod and food distribution schedule on the growth and survival of larvae

Three light regimes were evaluated: 0L:24D; 12L:12D and 24L:0D. Artemia *nauplii* were distributed in

slight excess six times a day, over a period of 24 or 12 h. In the latter case, food was delivered during the period corresponding to the photophase. All tanks received the same amount of food daily. Water temperature was maintained at 26.6 ± 0.6 °C.

A complete factorial design was used for the two variables (feeding with two modalities and photoperiod with three levels) using 18 experimental tanks. Each photoperiod group consisted of a line of six 60 L tanks covered with an opaque black plastic curtain. The 12L:12D and 24L:0D groups were equipped with day-light-type bulbs positioned 1.5 m above the tanks, giving a light intensity of 10 lx at the water surface. Each combination of photoperiod and feeding period was run 8 days in triplicate with 6000 2 dph larvae per tank, equivalent to a stocking density of 100 larvae L^{-1}. At 10 dph, survivors were counted and the standard length was determined by image analysis of digital photographs of a sub-sample of 30 larvae for each of the 18 experimental tanks.

Statistical analysis

Data were processed using either ANOVA, Factorial ANOVA or design analysis functions. When a treatment effect was significant, and when the interaction between factors was not significant, Bonferroni's *post-hoc* tests were performed. Null hypotheses were rejected at $P < 0.05$.

Results

Female and male selection and induction of final oocyte maturation and spermiation

Only females exhibiting a unimodal distribution with a modal oocyte diameter between 0.8 and 0.9 mm (Fig. 2), a high percentage of translucent oocytes (> 70%) with a migrating germinal vesicle and a low oocyte diameter variability (CV% < 15%) were chosen for inducing oocyte maturation. For males, if sperm was contaminated with urine during the stripping step, motile spermatozoa were observed even after dilution in 9‰ NaCl solution. Such urine contaminated sperm rapidly lost its fertilizing ability and was discarded.

Under these conditions, we observed 23 ovulation responses on a total of 31 inductions (74.2%) from 19 females (five females induced two, three or four times during the study). On average, eggs were collected at 230.0 ± 32.6 degrees-hours (d-h) at 27.6 ± 1.7 °C, which corresponded approximately to 8.5 h of latency after the second ovaprim injection. Latency was significantly and negatively correlated to water temperature ($R^2 = 0.49$; $P < 0.001$), Fig. 3a. The observed fecundity showed a positive correlation ($R^2 = 0.75$) with female body weight (Fig. 3b), with a slope of 147 000 ovules kg^{-1}. The mean fertilization and hatching rates reached 84.0 ± 20.8% and 73.7 ± 19.0% respectively (Table 1).

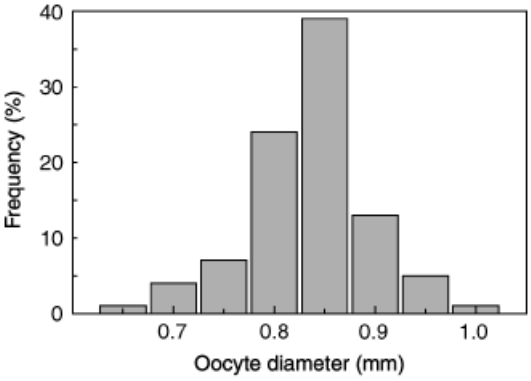

Figure 2 Typical unimodal size-frequency distribution of oocytes from a ripe female of *Pseudoplatystoma fasciatum* sampled by ovarian cannulation. Oocyte diameter measurements were performed using IMAGE J software package (here, oocyte diameter : 0.81 mm and CV% = 7.7%, $n = 94$).

Effect of food type on larval survival

The effect of food type (*Artemia* nauplii, decapsulated *Artemia* cysts and natural zooplankton) was determined 15, 28 and 35 days post hatching (Fig. 4). There was a significant effect of food type (one way ANOVA, $F_{2,24} = 5.01$, $P = 0.015$). Survival rates at 15 dph were high with all food types: 91% for *Artemia* nauplii, 73% and 65% for natural zooplankton and decapsulated *Artemia* cysts respectively. Individual comparisons among food types using Bonferroni's *post-hoc* tests at 15, 28 and 35 dph are indicated in Fig. 4. Overall, *Artemia* nauplii gave the best survival rates at each sampling time and survival remained almost constant and over 55% at 28 and 35 dph. Decapsulated *Artemia* cysts gave significantly lower survival rates when compared with nauplii at every sampling time, declining below 50% at 28 dph and below 40% at 35 dph. In the natural zooplankton treatment the survival rates were similar to those with *Artemia* nauplii at 15 dph, but decreased sharply afterwards down to 2% at 28 dph. At 28 dph, fish receiving

Artemia nauplii were significantly larger (mean sizes of 24.44 vs. 18.8 mm SL; $t = 11.85$; $P < 0.0001$; d.f. = 309) and more homogenous in size than those fed with decapsulated cysts (CVs of 16.60% vs. 23.24% respectively). With both food types, the size frequency distributions (Fig. 5) comprised jumpers, which were almost twice as large as the mean size of survivors, and certainly large enough to cannibalize the smallest individuals in their respective tanks. The rare survivors among the fish raised in green water were essentially large individuals, and thus probably cannibals, by reference to the very low survival rates in these groups at 28 dph to 0% at 35 dph.

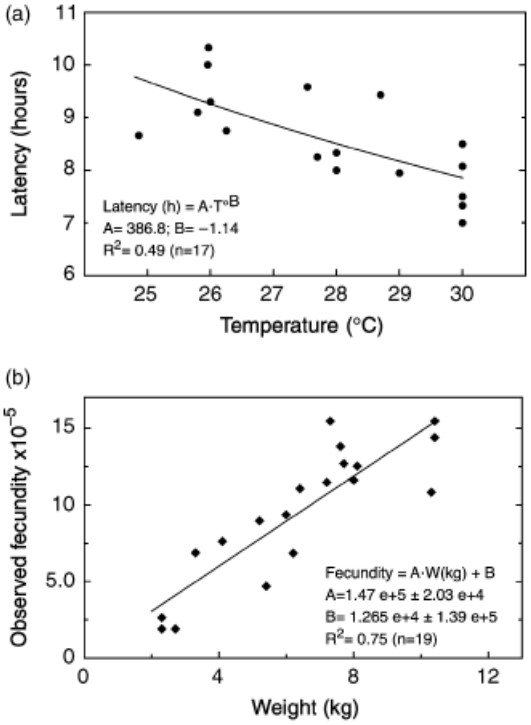

Figure 3 Latency period to ovulation after the second ovaprim injection as a function of temperature (a), and number of ova collected by stripping after ovulation (fecundity) as a function of female body weight (b) in *Pseudoplatystoma fasciatum*. In Fig. 3a, the data presented are restricted to females for which ova of good quality could be obtained (fertilization rates > 70%).

Effect of light intensity on growth and survival

The reduction of light intensity had a positive significant effect on growth ($F_{2,6} = 39.38$, $P < 0.0001$) and survival ($F_{2,6} = 16.06$, $P = 0.0039$) (Fig. 6). Indeed, the survival and size of larvae of Surubí at 9 dph were inversely proportional to light intensity. Survival was significantly higher in the dark than in the other two treatments, but did not differ significantly between low and shaded light. Larvae were significantly larger in the shaded light and dark treatments than in low light. Furthermore, the larvae raised under 10 lx were more heterogeneous and comprised 'jumpers', in contrast to those raised under permanent darkness (CV = 11.37% and 9.1% respectively).

Effect of photoperiod and density on growth and survival

Three different densities have been tested (600, 1800 and 6000 larvae per 60 L tank) corresponding to 10, 30 and 100 larvae L^{-1}, with two different photoregimes: one corresponding to natural photoperiod (12L:12D) and the other (0L:24D) corresponding to complete darkness (Fig. 7a). Survival rates at 12 dph were significantly higher in complete darkness than under a natural photoperiod ($F_{2,14} = 21.22$, $P = 0.004$), whereas density had no significant effect on survival ($F_{2,14} = 0.06$, $P = 0.92$).

Photoperiod had no significant effect on the mean length of larvae ($F_{2,14} = 3.63$, $P = 0.077$), whereas den-

Table 1 Synthesis of the results obtained following the application of ovaprim treatment to females of *Pseudoplatystoma fasciatum* from the Bolivian Amazon

	Ovulation latency (degree – hours)	Ovule weight (mg)	Female weight (kg)	Relative fecundity (ovules kg^{-1} bw)	Fertilization rate 10 h post fertilization (%)	Hatching rate (%)
Minimum	132.23	0.535	2.3	14 937.1	19.12	40.76
Maximum	271.45	0.66	10.6	38 9251.1	99.35	92.96
Mean	230	0.59	6.8	143 523	83.97	73.68
SD	32.6	0.02	2.6	84 186	20.58	19.01

Values ($n = 23$) represent minimum, maximum, mean and standard deviation (SD).

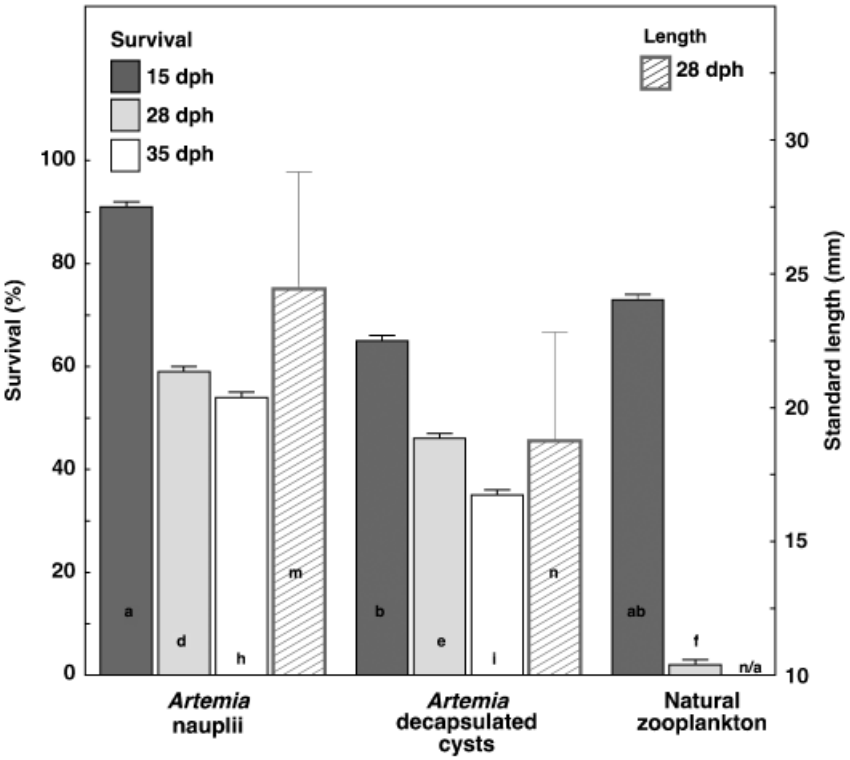

Figure 4 Survival rates of surubí larvae aged 15, 28 and 35 days post hatch (dph), subjected to three different feed types: *Artemia salina* nauplii, decapsulated *Artemia salina* cysts and natural zooplankton from an earthen pond. Values represent the mean of three replicates ± standard deviation. Different letters indicate significant differences at $P < 0.05$ among food types at each sampling time: 15, 28 and 35 days post hatching (one-way ANOVA and Bonferroni's *post hoc* tests).

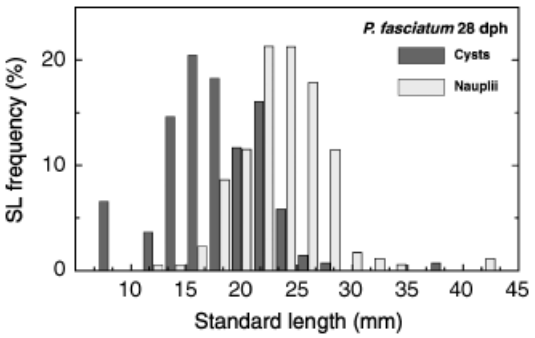

Figure 5 Standard length (SL) Frequency of 28 dph Surubí larvae fed with *Artemia* nauplii or decapsulated cyts. Means ± standard deviation.

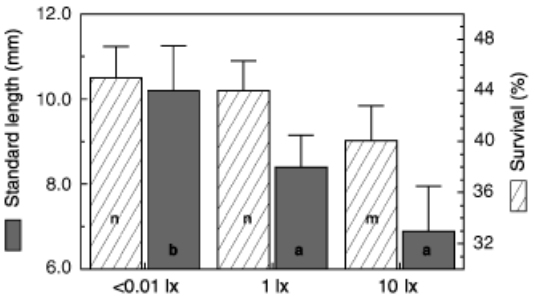

Figure 6 Standard length (left *y* axis) and survival rates (right *y*-axis) of *Pseudoplatystoma fasciatum* larvae reared from 2 to 9 dph under three different light intensities. Values represent mean of triplicates ± standard deviation. Bars not sharing a common script (a, b or m, n) differ significantly at $P < 0.05$ (Bonferroni's *post hoc* tests).

sity did have a significant effect ($F_{2,14} = 4.48$, $P = 0.032$) (Fig. 7b). Larvae were significantly longer at low density (10 larvae L^{-1}) than at high density (100 larvae L^{-1}). At intermediate density (30 larvae L^{-1}), fish size was intermediate but not significantly different from those at low and high densities.

Effect of photoperiod and feeding regime on survival

Two feeding regimes were tested (the same amount of food distributed over 12 or 24 h, from 4 to 10 dph) in combination with three photoperiods (24D:0L, 12D:12L and 0D:24L). Survival data (Fig. 8) were analysed using an experimental design analysis procedure. The design comprised two factors: a photoperiod with three levels (0, 12, 24 h of dark) and a feeding regime with two levels (12 and 24 h).

Both the photoperiod ($F_{2,11} = 6.66$, $P = 0.035$) and the feeding regime ($F_{2,11} = 12.80$, $P = 0.0095$) had a significant effect on survival (Fig. 8). No significant interaction was detected between photoperiod and

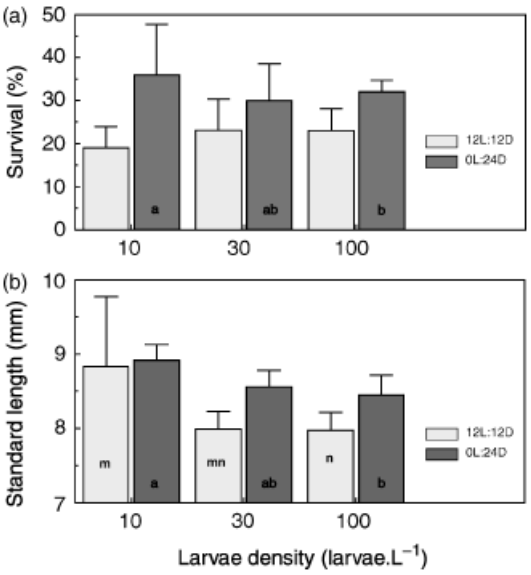

Figure 7 Effects of Photoperiod and stocking density on the survival rate (a) and length (b) of *Pseudoplatystoma fasciatum* larvae. Values are the means of triplicates ± standard deviation.

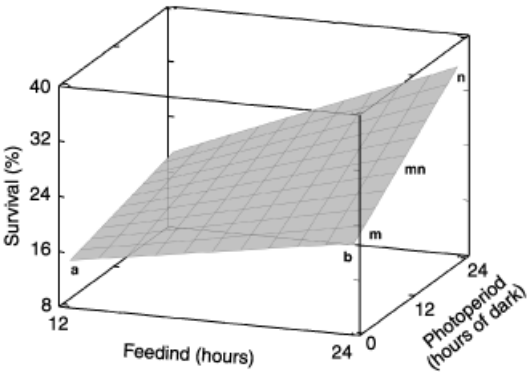

Figure 8 Effects of photoperiod (0L:24D; 12L:12D; 24L:0D) and feeding distribution (over 12:00 or 24:00 hours) on the survival of *Pseudoplatystoma fasciatum* larvae. Response surface model established using a complete factorial design (3 × 2) run on triplicates for each combination. Design analysis was performed using multifactorial ANOVA.

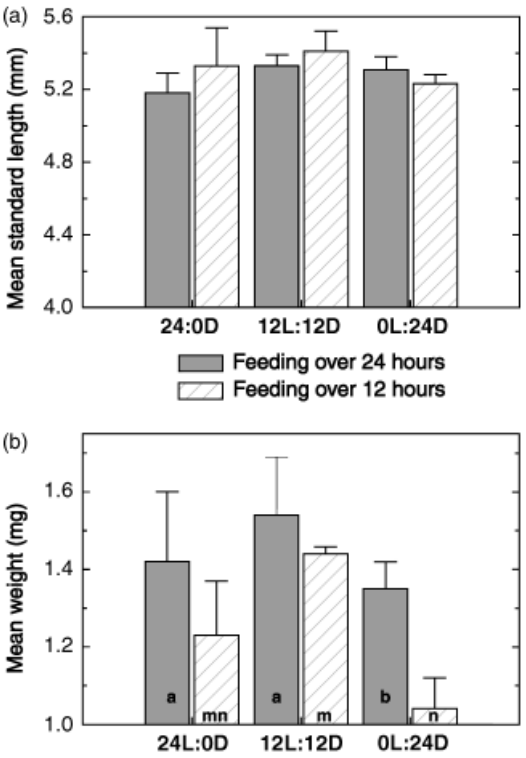

Figure 9 Effects of photoperiod (0L:24D; 12L:12D; 24L:0D) and feeding distribution (over 12:00 or 24:00 hours) on standard length (a) and weight (b) of *Pseudoplatystoma fasciatum* larvae. Bars not sharing a common script (a, b or m, n) differ significantly at $P < 0.05$ (Bonferroni's *post hoc* tests).

feeding regime ($P = 0.183$). Maximum survival (38%) was obtained with the 24-h dark and 24 h feeding regime combination, while lower survival (15%) corresponded to larvae reared under the permanent illumination and 12-h feeding regime combination. Neither photoperiod ($F_{2,12} = 2.52$, $P = 0.12$) nor feeding regime ($F_{2,12} = 0.13$, $P = 0.72$) had a significant effect on standard length (Fig. 9a), whereas both factors had a significant effect on mean weight ($F_{2,12} = 9.05$, $P = 0.004$ for the photoperiod and $F_{2,12} = 12.44$, $P = 0.004$ for the feeding regime) (Fig. 9b). There was no interaction between the two factors ($P = 0.35$). The highest mean weights were observed in the 24-h feeding regime, although the difference was significant only in complete darkness (0L:24D, $P = 0.004$). However, for both feeding regimes, the highest mean weights were observed under a natural photoperiod (12L:12D) and the lowest in complete darkness (0L:24D). The mean body weights were similar for larvae raised under natural photoperiod and permanent illumination (24L:0D), but differed significantly from those maintained in permanent darkness.

Discussion

Female and male selection and induction of final oocyte maturation and spermiation

In this study, we describe a reliable method to induce final oocyte maturation in the Surubí, *P. fasciatum*,

using ovaprim at the recommended dosage (0.5 mL kg^{-1}). This result further supports the finding that the combination of LH-RHa and an anti-dopamine factor, here domperidone, is a potent inductor of final oocyte maturation in a broad range of fish species (Lin, van der Kraak, Liang, Peng, Li, Lu, Zhou, Chang & Peter 1986; Peter, Lin & Van der Kraak 1988) and particularly in catfish species (De Leeuw, Goos, Richter & Eding 1985; Goos, Joy, De Leeuw, Van Oordt, Van Delft & Gielen 1987; Richter, Eding, Goos, De Leeuw, Scott & Van Oordt 1987; Legendre, Linhart & Billard 1996; Alok, Krishnan, Talwar & Garg 1998). One of the advantages over carp pituitary extract (CPE), which is frequently used in fish farms, is that the biological activity of ovaprim or other LH-RHa preparations is standardized and constant if the products are stored properly. It is also most likely that a part of the success in inducing the final maturation of females emerged from the determination of objective criteria for female selection. These criteria were based on the direct observation of oocytes obtained by an intra-ovarian biopsy as already reported for this species (Gervásio Leonardo *et al.* 2004) and for other cultured African and Asian catfish (Gilles, Dugué & Slembrouck 2001; Slembrouck, Komarudin, Maskur & Legendre 2004). We observed that high fertilization and hatching rates were associated with high proportions of translucent oocytes with migrating germinal vesicle and low coefficients of variation of the modal diameter of postvitellogenic oocytes. Once female selection is achieved, another important factor is the time of latency, which has to be considered very carefully because it determines high fertilization and hatching rates (results not shown). We recommend using the number of d-h rather than the elapsed time, because the d-h calculation integrates all temperature variations during the maturation process and is thus more accurate. We observed in different induction trials that in overripe females ($>$ 250 d-h), stripping was generally very easy, but fertilization and hatching rates were very low. By contrast, stripping before 180 d-h was generally unproductive. Nevertheless, we exceptionally obtained ovulated eggs as early as 130 d-h for early-responding females and as late as 270 d-h for late-responding females (see Table 1). In this study, we attained an overall 74.2% proportion of ovulation response ($n = 31$ inductions). This methodology enabled the production of large numbers of viable larvae per female. The collection of sperm from males did not require hormonal induction, but it is recommended to inject males with 50% of the female total dose in order to increase milt volume and facilitate its collection. Attention has to be focused on the sperm collection procedure to avoid urine contamination, which may activate spermatozoa motility. It is preferable to collect the sperm in separate fractions and to test each fraction separately before its use for artificial fertilization.

Effect of Food type on larval survival

Artemia nauplii were shown to be adequate for feeding Surubi larvae as soon as they start exogenous feeding. Larvae fed with decapsulated cysts of *Artemia* survived almost equally well, but their growth was slower, possibly because inert cysts are less attractive than live prey, leading to a lower ingestion, or because they are less digestible than nauplii, especially for a species that starts feeding at a relatively small size (ca. 4.5 mm SL). Decapsulated cyts and *Artemia* nauplii are now widely used for a large variety of cultured species. Nevertheless, other more affordable alternatives can be used to initiate larvae feeding on live prey until weaning. Previous work on this species reported good growth with natural zooplanktonic prey during the first 10 days of larval rearing (Padilla Pérez *et al.* 2001) but there was no indication as to whether survival was low or high. This study provided evidence that rearing Surubi larvae in green water until the age of 15 dph did not compromise their survival. After this period, natural zooplankton was apparently no longer sufficient to sustain larvae survival. This result offers perspectives for evaluating weaning schedules straight after plankton feeding. However, it is strongly suggested that larval rearing in green water be restricted to low stocking densities; otherwise, the plankton resources may rapidly become limiting, thereby causing growth heterogeneity and cannibalism, as was observed during this study. The early weaning of 15 dph larvae after natural zooplankton feeding has not yet been tested in this species. Nevertheless, this possibility has been tested successfully in marine species like Red porgy (*Pagrus pagrus*), with a weaning protocol (co-feeding) involving live prey and dry diets (Aristizábal & Suárez 2006), or Sole (*S. senegalensis*) (Ribeiro, Engrola & Dinis 2005).

Effect of light intensity on growth and survival

The effect of light intensity has already been tested in marine and freshwater cultured species, and there is generally a positive correlation of growth and survi-

val with ambient light intensity in most fish species studied (Boeuf & Le Bail 1999). However, in catfish the situation appears to be different because, most studies reported better survival and sometimes growth under short photoperiods or even in permanent darkness (Sundararaj, Nath & Halberg 1982; Kerdchuen & Legendre 1991; Britz & Pienaar 1992; Baras, Tissier, Westerloppe, Mélard & Philippart 1998; Giri *et al.* 2002; Almazan-Rueda, van Helmond, Verreth & Schrama 2005). This characteristic seems to be associated with their natural feeding behaviour, as adults usually feed around sunset or during the night (Belbenoit, Moller, Serrier & Push 1979; Sundararaj *et al.* 1982; Rodriguez, Richardson & Lewis Jr 1990; Kerdchuen & Legendre 1991; Ezenwaji 1999; Hossain, Batty, Haylor & Beveridge 1999; Spotte, Petry & Zuanon 2001; Almazan-Rueda, Schrama & Verreth 2004; Pohlmann, Atema & Breithaupt 2004). Catfish larvae become photophobic early during the larval period and exhibit a negative phototaxis (Blackshaw & Snyder 1997; Appelbaum & McGeer 1998; Hossain *et al.* 1999), but under artificial conditions larvae of many species can feed either in the light or in complete darkness. As in some other species, in most catfish, larvae exhibit aggressive behaviour, leading to numerous injuries and frequent cannibalism (Britz & Pienaar 1992; Appelbaum & Kamler 2000; Giri *et al.* 2002). Long periods of light in the African catfish, *Clarias gariepinus*, can induce increased plasma cortisol levels, indicating a physiological response to stressful conditions (Almazan-Rueda *et al.* 2005). According to our observations, the aggressive behaviour in *P. fasciatum* might be responsible for a significant part of larval mortality during the first 2 or 3 weeks of life. We observed that rearing larvae in the dark ensures less competition for space, because larvae are mainly distributed in the water column instead of being concentrated in the darker parts of the tank. Therefore, there are limited contacts between them, which finally improves survival rates, and to some extent growth. As Surubí larvae keep feeding normally in complete darkness, survival rates are improved under these conditions. We strongly recommend the use of complete dark conditions for the larval rearing of Surubí.

Effect of photoperiod and density on growth and survival

The effect of photoperiod and density on survival and growth in fish larvae has been demonstrated in some cultured species (Kaiser, Weyl & Hecht 1995; Almazan-Rueda *et al.* 2005). Nevertheless, in our experiments, with a 10-fold range of variation, the effect of density on survival was not significant. Survival at low densities was slightly better only in completely dark conditions, but this might be more related to the photoperiod (day length) than to density itself. However, larval growth was significantly affected by density: the larger larvae were observed at the lowest density. This situation is quite common among cultured species because each larva has more space and spends less energy competing for food with other congeners. In this study, the range of initial larvae density variation (10–100 larvae L^{-1} corresponding to 600–6000 larvae m^{-2}) was relatively high compared with the initial densities reported for marine species (0.1–30 larvae L^{-1}) (Hitzfelder, Holt, Fox & McKee 2006) but similar to other reported rearing densities for fresh water fish, *Sander lucioperca* (25–100 L^{-1}) (Szkudlarek & Zakęś 2007) or *Clarias batrachus* (1000–5000 m^{-2}) (Sahoo, Giri & Sahu 2004), indicating that *P. fasciatum* may be reared under high density conditions but also that survival will likely be improved using lower densities.

Effect of photoperiod and feeding regime on survival

There was a striking effect of both feeding and light regimes. In several fish species (visual-based feeders), the effect of long photophase generally has a positive effect on growth and survival (Barlow, Pearce, Rodgers & Clayton 1995; Ronzani Cerqueira & Macedo Brügger 2001; Downing & Litvak 2002; Fielder, Bardsley, Allan & Pankhurst 2002; Puvanendran & Brown 2002; Trotter, Battaglene & Pankhurst 2003; Moustakas, Watanabe & Copeland 2004; Pedro Canavate, Zerolo & Fernandez-Diaz 2006). As in other catfish species (Appelbaum & McGeer 1998; Appelbaum & Kamler, 2000; Giri *et al.* 2002), survival of Surubí larvae was higher under complete darkness and with a continuous food distribution. In complete darkness, larvae activity is mainly devoted to feeding and their aggressive behaviour is highly reduced because they remain quiet for long periods of time after feeding. The continuous distribution of food (over 24 h) also prevents long periods of food deprivation, which tend to stimulate foraging behaviour and increase contacts between fish. In some species, these higher survival rates were also associated with faster growth because the costs of swimming activity and aggres-

sive behaviour are reduced. However, this potential growth gain could not be detected under the present experimental conditions.

In conclusion, we have developed a reliable methodology for fish selection, and induced breeding for this very important, yet poorly studied Amazonian species. The overall survival rate obtained under the experimental conditions in this study was around 40% at 35 dph, which is compatible with mass production of fry owing to the high fecundity of this species. These preliminary results should help promote the culture of this promising species, which will be the only way to alleviate the increasing fishing pressure it endures in most Amazonian countries and to offer a new candidate for aquaculture of native species. Future research efforts should, focus among other things, on developing weaning protocols with efficient dry foods, for increasing growth rates and attaining a sustainable number of fingerlings for grow-out production of this species.

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References

Almazan-Rueda P., Schrama J.W. & Verreth J.A.J. (2004) Behavioural responses under different feeding methods and light regimes of the African catfish (*Clarias gariepinus*) juveniles. *Aquaculture* **231**, 347–359.

Almazan-Rueda P., van Helmond A.T.M., Verreth J.A.J. & Schrama J.W. (2005) Photoperiod affects growth, behaviour and stress variables in *Clarias gariepinus*. *Journal of Fish Biology* **67**, 1029–1039.

Almeida O.T.D. (2006) *A indústria pesqueira na Amazônia*. Ibama/Provarzea, Manaus-AM, Brazil p. 110.

Almeida O.T., Mcgrath D.G. & Ruffino M.L. (2001) The commercial fisheries of the lower Amazon: an economic analysis. *Fisheries Management and Ecology* **8**, 253–269.

Alok D., Krishnan T., Talwar G.P. & Garg L.C. (1998) Multiple induced spawnings of the Indian catfish *Heteropneustes fossilis* (Bloch) within a prolonged spawning season. *Journal of the World Aquaculture Society* **29**, 252–258.

Appelbaum S. & Kamler E. (2000) Survival, growth, metabolism and behaviour of *Clarias gariepinus* (Burchell 1822) early stages under different light conditions. *Aquacultural Engineering* **22**, 269–287.

Appelbaum S. & Mcgeer J.C. (1998) Effect of diet and light regime on growth and survival of African catfish (*Clarias gariepinus*) larvae and early juveniles. *Aquaculture Nutrition* **4**, 157–164.

Aristizábal E.O. & Suárez J. (2006) Efficiency of co-feeding red porgy (*Pagrus pagrus* L.) larvae with live and compound diet. *Revista de Biología Marina y Oceanografía* **41**, 203–208.

Baras E. & Jobling M. (2002) Dynamics of intracohort cannibalism in cultured fish. *Aquaculture Research* **33**, 461–479.

Baras E., Tissier F., Westerloppe L., Mélard C. & Philippart J.-C. (1998) Feeding in darkness alleviates density-dependent growth of juvenile vundu catfish *Heterobranchus longifilis* (Clariidae). *Aquatic Living Resources* **11**, 335–340.

Barlow C.G., Pearce M.G., Rodgers L.J. & Clayton P. (1995) Effects of photoperiod on growth, survival and feeding periodicity of larval and juvenile barramundi *Lates calcarifer* (Bloch). *Aquaculture* **138**, 159–168.

Barthem R. & Goulding M. (1997) *The Catfish Connection. Ecology, Migration and Conservation of Amazon Predators*. Columbia University Press, New York, NY, USA. 137 pp.

Belbenoit P., Moller P., Serrier J. & Push S. (1979) Ethological observations on the electric organ discharge behaviour of the electric catfish, *Malapterurus electricus* (Pisces). *Behavioral Ecology and Sociobiology* **4**, 321–330.

Blackshaw S. & Snyder S.H. (1997) Parapinopsin, a novel catfish opsin localized to the parapineal organ, defines a new gene family. *Journal of Neuroscience* **17**, 8083–8092.

Boeuf G. & Le Bail P.-Y. (1999) Does light have an influence on fish growth? *Aquaculture* **177**, 129–152.

Britz P. & Pienaar A. (1992) Laboratory experiments on the effect of light and cover on the behaviour and growth of African catfish, *Clarias gariepinus* (Pisces: Clariidae). *Journal of Zoology* **227**, 43–62.

Buitrago–Suárez U.A. & Burr B.M. (2007) Taxonomy of the catfish genus *Pseudoplatystoma* Bleeker (Siluriformes: Pimelodidae) with recognition of eight species. *Zootaxa* **1512**, 1–38.

De Leeuw R., Goos H.J.T., Richter C.J.J. & Eding E.H. (1985) Pimozide-LHRHa-induced breeding of the African catfish, *Clarias gariepinus* (Burchell). *Aquaculture* **44**, 295–302.

Downing G. & Litvak M.K. (2002) Effects of light intensity, spectral composition and photoperiod on development and hatching of haddock (*Melanogrammus aeglefinus*) embryos. *Aquaculture* **213**, 265–278.

Ezenwaji H. (1999) The abundance and trophic biology of *Clarias albopunctatus* Nichols & LaMonte, 1953 (Osteichthyes: Clariidae) in a tropical floodriver basin. *Hydrobiologia* **392**, 159–168.

FAO (2005) *Inland Fisheries management in Latin America and its contribution to food security and poverty alleviation*. FAO/COPESCAL/X/05/3E. Panamá, Republica de Panamá.

Fielder D.S., Bardsley W.J., Allan G.L. & Pankhurst P.M. (2002) Effect of photoperiod on growth and survival of snapper *Pagrus auratus* larvae. *Aquaculture* **211**, 135–150.

Gervásio Leonardo A.F., Romagosa E., Borella M.I. & Batlouni S.R. (2004) Induced spawning of hatchery-raised Brazilian catfish, cachara *Pseudoplatystoma fasciatum* (Linnaeus, 1766). *Aquaculture* **240**, 451–461.

Gilles S., Dugué R. & Slembrouck J. (2001) *Manuel de production d'alevins du silure africain Heterobranchus longifilis, Le Technicien d'Agriculture Tropicale*. IRD, Maisonneuve et Larose, Paris, France 128pp.

Giri S.S., Sahoo S.K., Sahu B.B., Sahu A.K., Mohanty S.N., Mukhopadhyay P.K. & Ayyappan S. (2002) Larval survival and growth in *Wallago attu* (Bloch and Schneider): effects of light, photoperiod and feeding regimes. *Aquaculture* **213**, 151–161.

Goos H.J.T., Joy K.P., De Leeuw R., Van Oordt P.G.W.J., Van Delft A.M.L. & Gielen J.T. (1987) The effect of luteinizing hormone-releasing hormone analogue (LHRHa) in combination with different drugs with anti-dopamine and anti-serotonin properties on gonadotropin release and ovulation in the African catfish, *Clarias gariepinus*. *Aquaculture* **63**, 143–156.

Hitzfelder G.M., Holt G.J., Fox J.M. & Mckee D.A. (2006) The effect of rearing density on growth and survival of Cobia, *Rachycentron canadum*, larvae in a closed recirculating aquaculture system. *Journal of the World Aquaculture Society* **37**, 204–209.

Hossain M.A.R., Batty R.S., Haylor G.S. & Beveridge M.C.M. (1999) Diel rhythms of feeding activity in African catfish, *Clarias gariepinus* (Burchell 1822). *Aquaculture Research* **30**, 901–905.

Hseu J.-R., Huang W.-B. & Chu Y.-T. (2007) What causes cannibalization-associated suffocation in cultured brown-marbled grouper, *Epinephelus fuscoguttatus* (Forsskal, 1775)? *Aquaculture Research* **38**, 1056–1060.

Kaiser H., Weyl O. & Hecht T. (1995) The effect of stocking density on growth, survival and agonistic behaviour of African catfish. *Aquaculture International* **3**, 217–225.

Kerdchuen N. & Legendre M. (1991) Effects of frequency and period of feeding on growth rate and feed efficiency of an African catfish, *Heterobranchus longifilis* (Teleostei, Clariidae). *Aquatic Living Resources* **4**, 241–248.

Kestemont P., Jourdan S., Houbart M., Melard C., Paspatis M., Fontaine P., Cuvier A., Kentouri M. & Baras E. (2003) Size heterogeneity, cannibalism and competition in cultured predatory fish larvae: biotic and abiotic influences. *Aquaculture* **227**, 333–356.

Kossowski C. (1996) Perspectives de l'élevage des poissons-chats (Siluroidei) en Amérique du Sud. *Aquatic Living Resources* **9**, 189–195.

Kossowski C. & Madrid F. (1985) Ensayo de la reproducción inducida en el bagre rayado cabezón *Pseudoplatystoma fasciatum* (Linnaeus 1766) (Pisces. Siluriformes). *Acta Científica Venezolana* **36**, 284–285.

Kossowski C. & Madrid F. (1991) Observaciones de los estadios embrionario y Iarval del bagre rayado cabezón *Pseudoplatystoma fasciatum* (Linnaeus, 1766) (Pisces, Siluriformes). *BioLlania* **8**, 9–15.

Legendre M., Linhart O. & Billard R. (1996) Spawning and management of gametes, fertilized eggs and embryos in Siluroidei. *Aquatic Living Resources* **9**, 59–80.

Lin H.R., Van der Kraak G., Liang J.-Y., Peng C., Li G.-Y., Lu L.-Z., Zhou X.-J., Chang M.-L. & Peter R.E. (1986) The effect of LHRH analogue and drugs which block the effects of dopamine on gonadotropin secretion and ovulation in fish cultured in China. In: *Aquaculture of Cyprinids* (ed. by R.E.M., J. Billard), pp. 139–150. INRA, Paris, France.

Loubens G. & Panfili J. (2000) Biologie de *Pseudoplatystoma fasciatum* et *P. tigrinum* (Teleostei: Pimelodidae) dans le bassin du Mamoré (Amazonie Bolivienne). *Ichthyological Exploration of Freshwaters* **11**, 13–34.

Mandiki S.N.M., Babiak I., Krol J., Rasolo J.F.R. & Kestemont P. (2007) How initial predator-prey ratio affects intra-cohort cannibalism and growth in Eurasian perch *Perca fluviatilis* L larvae and juveniles under controlled conditions. *Aquaculture* **268**, 149–155.

Moustakas C.T., Watanabe W.O. & Copeland K.A. (2004) Combined effects of photoperiod and salinity on growth, survival, and osmoregulatory ability of larval southern flounder *Paralichthys lethostigma*. *Aquaculture* **229**, 159–179.

Padilla Pérez P.P., Alcántara Bocanegra F. & Ismiño Orbe R. (2001) Reproducción inducida de la doncella *Pseudoplatystoma fasciatum* y desarrollo embrionario - larval. *Folia Amazonica* **12**, 141–154.

Pedro Canavate J., Zerolo R. & Fernandez-Diaz C. (2006) Feeding and development of Senegal sole (*Solea senegalensis*) larvae reared in different photoperiods. *Aquaculture* **258**, 368–377.

Peter R.E., Lin H.R. & Van Der Kraak G. (1988) Induced ovulation and spawning of cultured freshwater fish in China: advances in application of Gn-RH analogues and dopamine antagonists. *Aquaculture* **74**, 1–10.

Petrere M., Barthem R.B., Córdoba E.A. & Gómez B.C. (2004) Review of the large catfish fisheries in the upper Amazon and the stock depletion of piraíba (*Brachyplatystoma filamentosum* Lichtenstein). *Reviews in Fish Biology and Fisheries* **14**, 403–414.

Pohlmann K., Atema J. & Breithaupt T. (2004) The importance of the lateral line in nocturnal predation of piscivorous catfish. *The Journal of Experimental Biology* **207**, 2971–2978.

Puvanendran V. & Brown J.A. (2002) Foraging, growth and survival of Atlantic cod larvae reared in different light intensities and photoperiods. *Aquaculture* **214**, 131–151.

Qin J. & Fast A.W. (1996) Size and feed dependent cannibalism with juvenile snakehead *Channa striatus*. *Aquaculture* **144**, 313–320.

Reinert T.R. & Winter K.A. (2002) Sustainability of harvested pacú (*Colossoma macropomum*) populations in the Northheastern Bolivian Amazon. *Conservation Biology* **16**, 1344–1351.

Ribeiro L., Engrola S. & Dinis M.T. (2005) Weaning of senegalese sole (*Solea senegalensis*) postlarvae to an inert diet with a co-feeding regime. *Ciuencias Marinas* **31**, 327–337.

Richter C.J.J., Eding E.H., Goos H.J.T., De Leeuw R., Scott A.P. & Van Oordt P.G.W.J. (1987) The effect of pimozide/LHRHa and 17[alpha]-hydroxyprogesterone on plasma steroid levels and ovulation in the African catfish, *Clarias gariepinus*. *Aquaculture* **63**, 157–168.

Rodriguez J.A. (1996) Avances en la reproducción inducida de Siluridos. *Dahlia* **1**, 85–88.

Rodriguez M.A., Richardson S.E. & Lewis W.M. Jr. (1990) Nocturnal behavior and aspects of the ecology of a driftwood catfish, *Entomocorus gameroi* (Auchenipteridae). *Biotropica* **22**, 435–438.

Ronzani Cerqueira V. & Macedo Brügger A. (2001) Effect of light intensity on initial survival of fat snook (*Centropomus parallelus*, Pisces: Centropomidae Larvae. *Brazilian Archives of Biology and Technology* **44**, 343–349.

Ruffino M.L. (2004) *A pesca e os recursos pesqueiros na Amazônia brasileira*. Ibama/ProVárzea, Manaus, Brazil 262pp.

Ruffino M.L. (2005) *Gestão do uso dos Recursos Pesqueiros na Amazônia*. IBAMA, Manaus, Brazil 135pp.

Sahoo S.K., Giri S.S. & Sahu A.K. (2004) Effect of stocking density on growth and survival of *Clarias batrachus* (Linn.) larvae and fry during hatchery rearing. *Journal of Applied Ichthyology* **20**, 302–305.

Sato Y., Cardoso E.L., Sallum W.B. & Godinho H.P. (1997) Induçao experimental da desove do surubim *Pseudoplatystoma coruscans*. In: *Surubim* (ed. by M.O.T. Miranda), pp. 69–79. IBAMA, Belo Horizonte, Brazil.

Slembrouck J., Komarudin O., Maskur H. & Legendre M. (2004) *Propagation of the Indonesian Catfish, Pangasius Djambal*. IRD Éditions–DKP, Depok, Indonesia, 131pp.

Spotte S., Petry P. & Zuanon J.A.S. (2001) Experiments on the feeding behavior of the hematophagous candiru, *Vandellia* cf. *Plazaii*. *Environmental Biology of Fishes* **60**, 459–464.

Sundararaj B., Nath P. & Halberg F. (1982) Circadian meal timing in relation to lighting schedule optimizes catfish body weight gain. *Journal of Nutrition* **112**, 1085–1097.

Szkudlarek M. & Zakęś Z. (2007) Effect of stocking density on survival and growth performance of pikeperch, *Sander lucioperca* (L.), larvae under controlled conditions. *Aquaculture International* **15**, 67–81.

Tavares M.P.O. (1997) O surubim. In: *Surubim* (ed. by M.O.T. Miranda), pp. 9–25. IBAMA, Belo Horizonte, Brazil.

Trotter A.J., Battaglene S.C. & Pankhurst P.M. (2003) Effects of photoperiod and light intensity on initial swim bladder inflation, growth and post-inflation viability in cultured striped trumpeter (Latris lineata) larvae. *Aquaculture* **224**, 141–158.

Welcomme R.L. (1988) *International introductions of Inland Aquatic Species*, FAO fisheries technical paper T294, FAO, Rome, Italy, 318pp.