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Hatching rate and larval growth variations in *Pseudoplatystoma punctifer*: maternal and paternal effects

Jesús Núñez[1,2], Diana Castro[3], Christian Fernández[2], Rémi Dugué[1], Fred Chu-Koo[4], Fabrice Duponchelle[1,2], Carmen García[3] & Jean-François Renno[1,3]

[1]IRD UR 175 CAVIAR, BP 5095, Cedex, France

[2]UNFV-FOPCA, Lima, Perú

[3]Laboratorio de Biología Molecular y Biotecnología, Instituto de Investigaciones de la Amazonía Peruana – IIAP, Iquitos, Perú

[4]Instituto de Investigaciones de la Amazonía Peruana – IIAP, Programa para el Uso y Conservación del Agua y sus Recursos (AQUAREC), Iquitos, Perú

Correspondence: J Nunez, 351 RUE J.F. BRETON, BP 5095, Montpellier Cedex 05, France. E-mail: jesus.nunez@ird.fr

Abstract

In *Pseudoplatystoma punctifer* (e.g. *Pseudoplatystoma fasciatum*) larvae, parental effects on hatching, growth of initial stages and dry feed adaptation were evaluated as they could influence fry heterogeneity, which is responsible for the enhancement of cannibalism, and which remains one of the main factors of mortality during larval stages. A full factorial experiment was carried out with 3 females × 3 males producing nine families of full siblings, raised separately in triplicates into 30 L tanks at 28 ± 0.5 °C in a water recirculating system. Paternal and maternal effects were observed on hatching success, yolk utilization efficiency and growth until 26 days post fertilization. Hatching success was generally over 80% except for one male × female combination (25%). Total length (TL) at hatching and during the first 4 weeks of exogenous feeding on live *Artemia* nauplii and dry feeds was determined in each family using digital photographs of larvae and NIH IMAGE J analysis freeware. Mean TL was calculated for each family at each sampling time and analysed using multifactorial analysis of variance tests. These results indicate not only dam but also sire effects at very early developmental stages as well as in subsequent stages of *P. punctifer*.

Keywords: *Pseudoplatystoma punctifer*, *Pseudoplatystoma fasciatum*, Doncella, reproduction, Peru, fish culture, parental effects

Introduction

The Doncella, known previously as *Pseudoplatystoma fasciatum*, has been renamed *Pseudoplatystoma punctifer* (Buitrago-Suárez & Burr 2007); however, recent genetic characterization of this species indicated that there was no genetic differentiation between the two described species in the Bolivian and Peruvian Amazon (Torrico, Hubert, Desmarais, Duponchelle, Nuñez Rodriguez, Montoya-Burgos, Garcia Davila, Carvajal-Vallejos, Grajales, Bonhomme & Renno 2009). Nevertheless, *P. punctifer* will be used in the text. *Pseudoplatystoma punctifer* is a large catfish widely distributed in the Amazon basin and one of the most commercially appreciated species (Goulding, Smith & Mahar 1996). As a piscivorous species (Barbarino Duque & Winemiller 2003), its meat is very popular in Amazonian markets because of its taste and the lack of intra-muscular spines. It reaches sizes of more than 100 cm, but its young are also praised on the ornamental market. All these traits have strongly increased the fishing pressure on this species.

The Doncella has been regarded as a potential candidate for the diversification of South American aquaculture for a while (Kossowski 1996), but the development of its aquaculture has been hampered by high mortality rates during the larval and early juvenile stages, essentially because of intense cannibalistic behaviour (Kossowski & Madrid 1985; Kossowski & Madrid 1991; Padilla Pérez, Alcántara Bocanegra & Ismiño Orbe 2001). This has fostered hybridization

attempts with other catfish species, mostly from the Pimelodidae family, with the objective of producing fry with reduced cannibalistic behaviour (Kossowski & Madrid 1991; Kossowski 1996). Cannibalism is described as the main cause of death in many fish species when it appears during early stages of fry production (Qin & Fast 1996; Kestemont, Xu, Hamza, Maboudou & Toko 2007; Arslan, Dabrowski & Portella 2009). Besides the cannibalistic behaviour in this species, the transition from live prey to inert food consumption in young stages was found equally difficult and this aspect continues to hamper the production of large catfish (Pimelodidae) in Latin America (Kossowski 1996; Nuñez 2009). In the last decade, several studies have provided significant contributions for fingerling production (Romagosa, Paiva, Godinho & Andrade-Talmelli 2003; Gervásio Leonardo, Romagosa, Borella & Batlouni 2004; Nuñez, Dugué, Corcuy Arana, Duponchelle, Renno, Raynaud & Legendre 2008; Diaz-Olarte, Cruz-Casallas, Marciales-Caro, Medina-Robles & Cruz-Casallas 2009). There are similar difficulties in *Pseudoplatystoma coruscans*, but significant progresses have been made especially with regard to weaning schedules (Martino, Cyrino, Portz & Trugo 2002; Segura, Hayashi, De Souza & Soares 2004). Nevertheless, as for *P. punctifer*, recent intra-generic hybrid attempts have been made to obtain faster growth or less cannibalistic behaviour (Faustino, Nakaghi, Marques, Makino & Senhorini 2007). Selection programmes are still largely undeveloped except for some species traditionally reared for centuries (carps) or more recently due to strong growth in aquaculture production (trout, tilapia). It has been shown that the gain obtained by selection after three generations could allow to nearly double the growth (Chevassus, Quillet, Krieg, Hollebecq, Mambrini, Faure, Labbe, Hiseux & Vandeputte 2004). The study of the variability of certain traits, such as growth, in larval or juvenile stage, has been initiated in several species, sole, *Solea solea* and herring, *Clupea harengus* (Panagiotaki & Geffen 1992); sea bass, *Dicentrarchus labrax* (Saillant, Chatain, Fostier, Przybyla & Fauvel 2001); haddock, *Melanogrammus aeglefinus* (Probst, Kraus, Rideout & Trippel 2006); winter flounder, *Pseudopleuronectes americanus* (Butts & Litvak 2007); atlantic halibut, *Hippoglossus hippoglossus* (Ottesen & Babiak 2007) and a coral reef fish, *Acanthochromis polyacanthus* (Donelson, Munday & McCormick 2009). In most cases, these studies have shown that the parentage of the offspring produced from factorial male–female crosses had a clear impact on larval growth performance or size heterogeneity, which is a very important effect in the culture of fish having cannibalistic behaviour that induce the necessity of periodically grading juveniles.

The aim of this study was to evaluate in *P. punctifer* larvae, the paternal or maternal effects on hatching and growth on initial stages and during an early dry feed adaptation period as they could influence fry heterogeneity, which is responsible for the enhancement of cannibalism in *P. punctifer* and remains one of the main factors of mortality in early larval stages.

Material and methods

The breeding stock consisted of 52 wild fish (28 females and 24 males) maintained for more than 3 years in the Instituto de Investigaciones de la Amazonía Peruana (IIAPs) Quistococha Research Station facilities. The fish were kept in a 2000 m^2 earthen pond and fed with live forage fish supplemented with beef liver distributed twice a day.

Males and females were checked periodically to determine their reproductive status. Males were tested for their capability of semen emission after a slight abdominal pressure, and for females, an intra-ovarian biopsy was performed. Females were selected based on oocyte modal diameter, coefficient of variation (CV%) [CV% = (SD/mean) × 100] of oocyte diameter distributions and percentage of atretic oocytes. For the three females used, the mean oocyte modal diameter, CV% and percentage of atresia ranged from 0.72 to 0.76 mm, 7.2–9.9% and 3.2–6.5% respectively.

Gamete characterization

For each female, a sample of stripped eggs was collected, then divided in three replicates and digital pictures were taken immediately in 9‰ saline solution in a Petri dish with a size reference. The photographs were then analysed with IMAGE J free software package (http://rsb.info.nih.gov/nih-image/).

Sperm was collected by gentle stripping with 5 mL syringes filled with 4 mL of 9‰ saline solution, to avoid sperm activation (1:5 sperm dilution). The collected sperm was checked for activation under microscope. The motility was evaluated by a 10 × dilution of the collected sperm with distilled water deposited directly on the microscope plate. Collected sperm must be completely immotile before activation with

distilled water, otherwise the sample was discarded. Spermatozoa counts were performed on a Thoma cell counting chamber using 100–500 × diluted sperm in 9‰ saline.

Spawning induction and gamete collection

Artificial reproduction of *P. punctifer* was performed according to (Nuñez *et al.* 2008). Briefly, females injected with Ovaprim® (Syndel Laboratories, Qualicum Beach, BC, Canada), received 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. Stripping occurred between 8 and 10 h after the second injection, depending on the average water temperature (28–27 °C).

Sperm was collected as described previously and stored at 4 °C until use.

Ovules are collected by gentle stripping of the females in dry 5 L plastic cups and used immediately.

Fertilization and incubation

For factorial crosses, 25 g of eggs (approximately 55 000 eggs) of each female were fertilized with 5 × diluted semen (∼ 10 000 spermatozoa egg^{-1}). Spermatozoa concentration was adjusted with 9‰ saline solution for the three males. Eggs and semen were gently mixed for 30 s and activation was performed with 25 mL of water. Fertilization was achieved after 1 min under gentle agitation and fertilized eggs were rinsed 3 × with 100 mL of water and distributed into nine individual 40 L incubators with a constant water flow of 1 L min^{-1} at 27.5 ± 0.5 °C.

Hatching

Larvae were not collected from the incubators before the age of 24 hours post fertilization (hpf), to ensure that hatching had ended in all crosses. Larvae were collected and concentrated into a 7 L aerated container and the total amount of hatched larvae was determined by a volumetric method. Fifty millilitres sample of concentrated larvae was counted in triplicate and the proportions of normal and deformed hatched larvae were calculated. For the experimental design, the desired number of larvae was sampled by a similar volumetric method. Then, larvae were quickly transferred to 1 m^3 tanks with aeration and flow-through water circulation (1.5 L min^{-1}).

Experimental design

At 1 days post fertilization (dpf), 3000 larvae (volumetric estimate) of each family were assigned randomly to 30 L tanks in an indoor recirculation water system (three replicate groups per cross). Water temperature (28.0 ± 0.5 °C) was kept constant during the experiment and all tanks were maintained in complete darkness (< 0.001 Lx during the day and night).

From the age of 3 dpf onwards, larvae were offered freshly hatched *Artemia* nauplii five times a day every 4 h from 06:00 to 22:00 hours. The feeding level at 3 dpf was five nauplii per larvae per meal and was increased by 25% every day thereafter. Feeding was *ad libitum* in slight excess, on the basis of the artemia feeding chart modified from previous work (Nuñez *et al.* 2008), and by controlling 30 min after artemia distribution that few *Artemia* nauplii were still present in the water tank, and that larvae stomachs were coloured with the typical orange artemia colour. From 9 dpf, artemia were progressively replaced by dry feeds (microparticulate shrimp feed and then trout pellets). For microparticulate and trout pellets, distributed *ad libitum*, nonconsumed feed was removed with a siphon 45 min after distribution. Initial size, yolk and corporal area were determined on 10 larvae at 1 and 3 dpf on triplicate samples. Larvae were randomly taken from each tank, anaesthetized with clove oil and photographed with a calibrated size marker. Pictures were analysed using the NIH IMAGE free software as described previously. Corporal (C_a) and yolk sac (V_a) areas were contoured with the hand-drawn closed polygon tool in NIH IMAGE J. As the image was calibrated with the size marker, the surface was automatically calculated by the freeware.

Yolk utilization efficiency (YUE) was calculated as follows:

$$\mathrm{YUE} = (C_{a3dpf} - C_{a1dpf})/(V_{a1dpf} - V_{a3dpf})$$

where C_a represents the corporal area in mm^2, V_a represents the yolk area in mm^2.

Thereafter, samples of at least 15 larvae were collected from each tank at 5, 9, 18 and 26 dpf. Their total length (TL) was measured on digital photographs as described previously.

Table 1 Density and feeding protocol of *Pseudoplatystoma fasciatum* larvae from 3 to 26 days post fertilization (dpf)

Days of breeding	3–5 dpf	6–9 dpf	10–13 dpf				14–18 dpf					19–26 dpf
Initial larvae density per tank	3000						1500					90
Feed type (%)												
Artemia	100		80	60	40	20	–					
Microparticulate shrimp feed	–		20	40	60	80	100	80	60	40	20	–
Trout pellets	–							20	40	60	80	100

Table 2 Body mass, fork length, mean egg size diameter and spermatozoa concentrations of *Pseudoplatystoma punctifer* breeders used for factorial crosses

	Weight (kg)	Fork length (cm)	Mean egg diameter (mm)	Spermatozoa concentration (spermatozoa mL^{-1})	SD	*N*
Female 1	5.4	78	0.825[a]	–	0.020	404
Female 2	5.1	77	0.817[a]	–	0.013	584
Female 3	4.1	76	0.822[a]	–	0.022	627
Male 1	1.8	66	–	4.87E+09[b]	1.10E+09	3
Male 2	2.5	60	–	4.93E+09[b]	1.76E+08	3
Male 3	1.4	57	–	5.43E+09[b]	1.50E+09	3

Identical superscript letters indicate no significant variations among oocyte diameter or spermatozoa concentration means at $P = 0.05$. *N*, the total number of measured eggs, and the number of semen concentration determinations.

At 5 and 19 dpf, densities and artemia or food rations were adjusted following the established protocol (Table 1). Three different successive decreasing densities were used, 3000, 1500 and 90 larvae $tank^{-1}$, corresponding to 100, 50 and 3 larvae L^{-1}. At the end of each of the three rearing periods, survival was determined by a total count of remaining larvae.

Results were analysed using one-way and multifactor analysis of variance (ANOVA) for experimental design procedure (Statgraphics Plus, StatPoint Technologies, Washington, DC, USA).

Results

Male, female and gamete characteristics

Male and female characteristics are summarized in Table 2. Body mass ranged from 4.1 to 5.4 kg for females and from 1.4 to 2.5 kg for males. Fork length varied from 76 to 78 cm in females and from 57 to 66 cm in males. Mean egg diameter, determined in triplicate samples for each female, revealed no significant differences among females ($P = 0.85$). Males have been chosen for similar sperm concentration (Table 2), determined in triplicates, and this parameter revealed no significant differences among the three males ($P = 0.79$) used in the 3×3 factorial crosses with females.

Hatching

Total hatching rates varied between 65% and 95%, except for one family whose hatching rate dropped to 25% (Fig. 1). Females 1 and 2 (crosses C1–C6) had similar hatching success (around 90%), whereas female 3 (families C7–C9) had a much more variable hatching rate. The ANOVA analysis (Table 3) indicated significant male ($P = 0.0001$) and female ($P < 0.0001$) effects, and a significant male–female interaction ($P = 0.0001$).

Over the three females tested (Fig. 1), two of them gave similar low rates of abnormal larvae (females 2 and 3) but female 1 gave significantly higher deformed larvae ($P < 0.05$) than the two other females. The proportion of abnormal larvae varied from 0.4% for family 9 to 17% for family 1. Analysis of variance (Table 4) indicated a strong female effect ($P < 0.01$) on deformed larvae at 24 hpf, but no male or male–

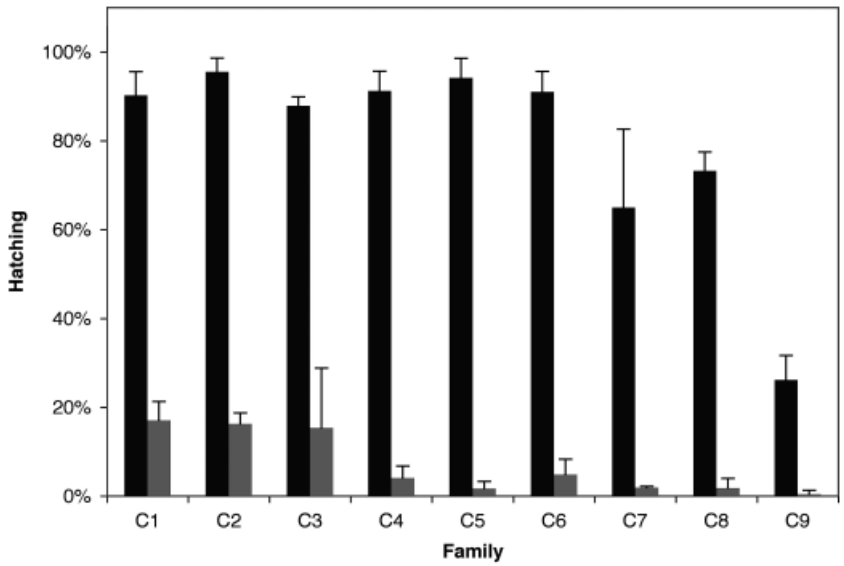

Figure 1 Percentage of total hatching (dark grey) and percentage of abnormal larvae (light grey) among total hatched of the nine crosses of *Pseudoplatystoma punctifer* (3 males × 3 females). Values represent the mean of three samples taken 20 h after fertilization. Error bars represent standard deviation. C1–C3, female 1 and males 1–3; C4–C6, female 2 and males 1–3; C7–C9, female 3 and males 1–3.

Table 3 Multivariate analysis for hatching – Type III sums of squares – *F*-ratios are based on the residual mean square error

Source	Sum of squares	d.f.	Mean square	*F*-ratio	*P*-value
Main effects					
A: female	0.815	2	0.407	80.68	0.0000
B: male	0.180	2	0.090	17.83	0.0001
Interactions					
AB	0.215	4	0.053	10.67	0.0001
Residual	0.090	18	0.005		
Total (corrected)	1.302	26			

Table 4 Multivariate analysis for abnormal larvae – Type III sums of squares – *F*-ratios are based on the residual mean square error

Source	Sum of squares	d.f.	Mean square	*F*-ratio	*P*-value
Main effects					
A: female	0.1157	2	0.0003	2.50	0.0000
B: male	0.0006	2	0.0057	0.11	0.8930
Interactions					
AB	0.0019	4	0.0004	0.19	0.9450
Residual	0.0462	18	0.0025		
Total (corrected)	0.1645	26			

female interaction effect was found ($P = 0.893$ and 0.945 respectively).

Results of initial (24 hpf), yolk area, final (96 hpf) yolk sac remaining area and YUE are summarized in Fig. 2. Female 1 (families 1–3) had larger initial and final yolk sac area, whereas females 2 and 3 had similar values. Yolk utilization efficiency tended to vary more homogeneously among females. The ANOVA indicated a significant female effect ($P < 0.05$) on the initial yolk area at hatching (24 hpf) but not on the final yolk area ($P = 0.900$) or on YUE ($P = 0.106$) at 3 dpf. A significant male effect was found on the final yolk area ($P < 0.05$) but not on the initial yolk area ($P = 0.67$) or on YUE ($P = 0.88$).

No male–female interaction was observed with any of the three variables (initial yolk area, final yolk area and YUE).

Growth

The TL was determined at 3 dpf, close to the end of yolk resorption process, and at 5 dpf, when all larvae had started exogenous feeding. At 3 dpf, the mean TL of the nine families varied from 3.18 to 4.34 mm (Fig. 3) and significant differences were found among them: the factorial ANOVA (Table 5) indicated both male ($P < 0.0001$) and female ($P < 0.0001$) effects as well as a significant male–female interaction ($P < 0.0001$).

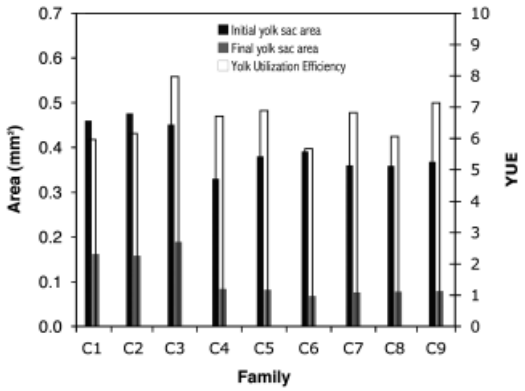

Figure 2 Variations of yolk area during yolk sac resorption in *Pseudoplatystoma punctifer*. Yolk utilization efficiency (YUE) is calculated between 1 and 3 days post fertilization. Values represent the mean of triplicates of 10 larvae per family. C1–C3, female 1 and males 1–3; C4–C6, female 2 and males 1–3; C7–C9, female 3 and males 1–3.

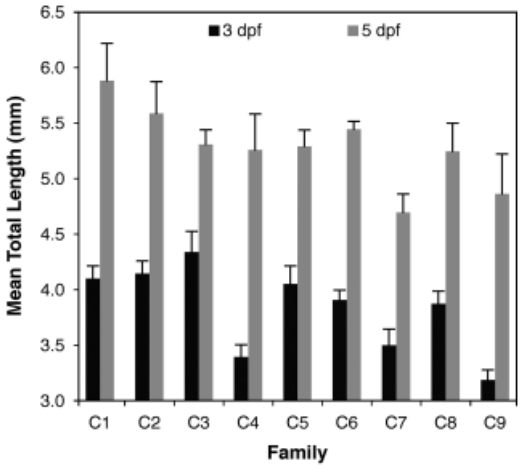

Figure 3 Mean total length of the nine families of *Pseudoplatystoma punctifer* larvae, at 3 and 5 days post fertilization (dpf). Values represent the mean and standard deviation of triplicate samples for each family (10 larvae per sample, $n = 270$). C1–C3, female 1 and males 1–3; C4–C6, female 2 and males 1–3; C7–C9, female 3 and males 1–3.

At 5 dpf, mean TL varied from 4.70 to 5.88 mm (Fig. 3) and as for 3 dpf, ANOVA (Table 6) indicated a significant male, female and male–female interaction effect ($P < 0.0001$).

At the end of the experiment (26 dpf), the mean TLs of the nine families differed substantially; they varied from 24.54 to 40.46 mm (Fig. 4), with a pronounced female ($P < 0.001$) effect, but no male effect or male–female interaction (Table 7) was observed. The growth differential at 26 dpf between the highest mean size (C8) and the lowest mean size (C5) represented 64%. When pooled per female, the growth difference between the progenies presented significant variations ($P < 0.01$). The growth difference was 47% ($P < 0.01$) between the progeny of females 3 (C7–C9) and 2 (C4–C6), 34% ($P < 0.01$) between the progeny of females 1 (C1–C3) and 2 (C4–C6), whereas the size (TL) difference between females 3 and 1 progenies represented only 9% and was not significant ($P = 0.21$).

The mean size of pooled males progenies represented only a maximum of about 9% total variation, and these differences were not significant ($P = 0.84$).

Results from individual family growth over the 3–26 dpf period are summarized in Fig. 5. From 3 to 9 dpf, growth is quite similar among the nine families, but important differences are observed on 18 dpf and these differences increase substantially on 26 dpf control.

The minimum–maximum overall family TL variability remained within the 4.82–12.56% of CV% at 3 dpf but increased from 7.26% to 13.20% at 5 dpf, from 8.87% to 18.29% at 18 dpf and from 16.92% to 38.05% at 26 dpf (Table 8). Another observation is that the family's hierarchy changed between 19 and 26 dpf. The most notable change concerned C5,

Table 5 Multivariate analysis for total length at 3 days post fertilization (dpf) – Type III sums of squares – *F*-ratios are based on the residual mean square error

Source	Sum of squares	d.f.	Mean square	*F*-ratio	*P*-value
Main effects					
A: female	1.107	2	0.553	276.9	0.0000
B: male	0.290	2	0.146	73.2	0.0000
Interactions					
AB	0.532	4	0.133	66.50	0.0000
Residual	0.036	18	0.002		
Total (corrected)	1.968	26			

Table 6 Multivariate analysis for total length at 5 days post fertilization (dpf) – Type III sums of squares – *F*-ratios are based on the residual mean square error

Source	Sum of squares	d.f.	Mean square	*F*-ratio	*P*-value
Main effects					
A: female	1.033	2	0.516	171.7	0.0000
B: male	0.290	2	0.145	48. 2	0.0000
Interactions					
AB	0.462	4	0.115	38.38	0.0000
Residual	0.054	18	0.003		
Total (corrected)	1.839	26			

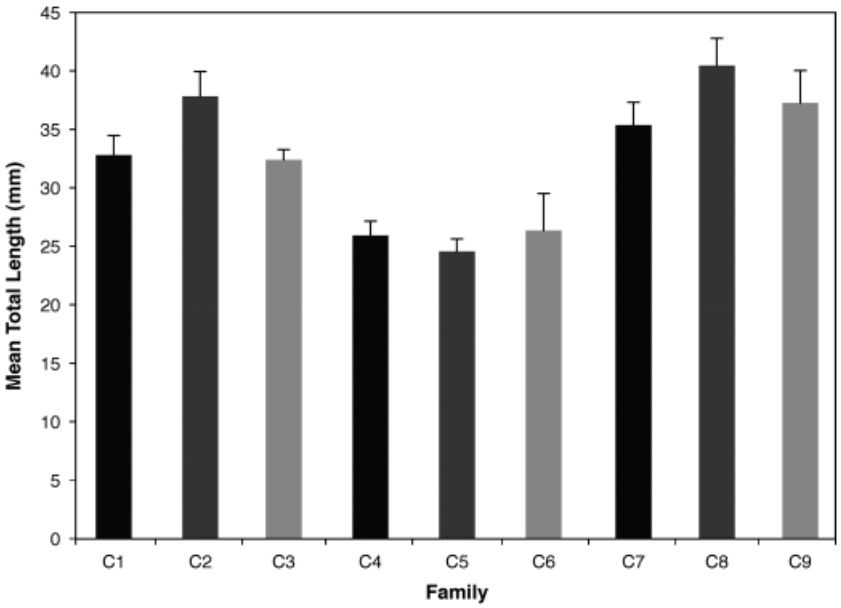

Figure 4 Mean total length of the nine families of *Pseudoplatystoma punctifer* larvae at 26 days post fertilization. Values represent the mean and standard deviation of triplicate samples for each family (minimum of 15 larvae per sample, $n = 717$). C1–C3, female 1 and males 1–3; C4–C6, female 2 and males 1–3; C7–C9, female 3 and males 1–3, similar shading of the bars correspond to the same male (solid black: male 1, dark grey: male 2, light grey: male 3).

Table 7 Multivariate analysis for total length at 26 days post fertilization (dpf) – Type III sums of squares – *F*-ratios are based on the residual mean square error

Source	Sum of squares	d.f.	Mean square	*F*-ratio	*P*-value
Main effects					
A: female	775.59	2	387.8	44.63	0.0000
B: male	23.68	2	11.8	1.36	0.2811
Interactions					
AB	72.50	4	18.12	2.09	0.1250
Residual	156.40	18	8.68		
Total (corrected)	1028.18	26			

which was the one that showed the best growth on 18 dpf but the worst on 26 dpf (Fig. 5). On the contrary, C7 had among the worst growth at 18 dpf but one of the highest at 26 dpf.

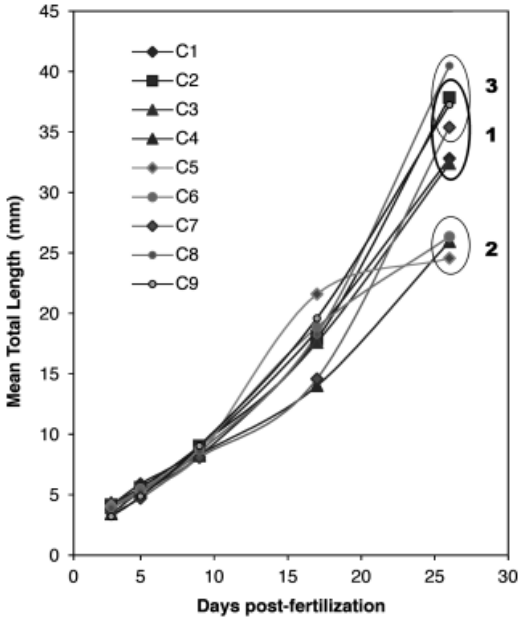

Figure 5 Mean total length of the nine families of *Pseudoplatystoma punctifer* larvae during the entire rearing period from 3 to 26 days post fertilization. Data points represent triplicate samplings of 10–30 larvae for each family ($n = 1517$). Each circle outlines the three progenies of each female (1–3).

Survival

The overall survival rates have been calculated at the end of each rearing density period (100, 50 and 3 larvae L^{-1}). Results of family survival are summar-

ized in Fig. 6 and analysed using multifactorial ANOVA and one-way ANOVA.

Family survival during the end of yolk resorption and the initiation of external feeding (3–5 dpf) varied from 52% to 91%, and there was no maternal or paternal effect ($P = 0.45$ and 0.946 respectively) or male–female interaction ($P = 0.632$). During this short period (3 days), larvae were kept at 100 larvae L^{-1} and were exclusively fed with live freshly hatched *Artemia* nauplii. One-way ANOVA indicated significant variations of mean survival among families ($P = 0.024$), but *post hoc* tests (Scheffé or Bonferroni) failed to identify significant difference between families. There was no significant correlation ($P = 0.528$) between survival and the mean TL (Fig. 7).

From 5 to 18 dpf, initial larvae density was set to 50 larvae L^{-1} and this period corresponded to the adaptation to microparticulate and trout pelleted feed. Survival varied from 22% to 43% and there was no paternal, maternal or male–female interaction effects ($P = 0.961$; 0.740 and 0.929 respectively).

Table 8 Coefficient of variation (CV%) of *Pseudoplatystoma punctifer* larvae total length of the nine crosses (C1–C9) during the rearing period from 3 to 26 days post fertilization (dpf)

	C1	C2	C3	C4	C5	C6	C7	C8	C9
3 dpf	5.11	7.60	4.82	7.22	5.57	7.68	9.74	5.33	12.56
5 dpf	10.04	9.15	7.26	10.66	8.14	7.40	8.49	10.13	13.20
18 dpf	11.43	13.06	14.45	14.18	9.18	8.87	15.92	18.29	16.62
26 dpf	19.90	24.36	16.92	25.33	28.56	38.05	21.87	22.97	30.76

There was no significant variations of mean survival among families ($P = 0.08$), or significant correlation ($P = 0.686$) between survival and the mean TL (Fig. 7).

From 19 to 26 dpf, initial larvae density was set to 3 larvae L^{-1} and corresponded to 100% trout pellets feeding. Survival varied from 17% to 40% and as there was no paternal or maternal or male–female interaction previously ($P = 0.816$; 0.758 and 0.11 respectively). One-way ANOVA indicated significant variations of mean survival among families ($P = 0.01$), but *post hoc* tests (Scheffé or Bonferroni) failed to identify significant difference between families. There was no significant correlation ($P = 0.102$) between survival and the mean TL (Fig. 7).

Discussion

It is accepted that the characteristics of the embryo and the larva depend on genetic and nongenetic (phenotype-based) factors like egg size or quality (Saillant *et al.* 2001; Rideout, Trippel & Litvak 2004; Ottesen & Babiak 2007; Donelson *et al.* 2009). The nongenetic effects (environmental effects) are often associated with maternal factors, because the female is responsible for 100% of the yolk characteristics of the egg. However, the contribution of males in the early stages of embryonic and larval development are sometimes highlighted (Rideout *et al.* 2004; Probst *et al.* 2006), although it may be suspected that the male effect is more related to genetic than to environmental factors (Saillant *et al.* 2001). Some authors, however, indicate that the maternal or paternal effect can be largely offset by factors of the

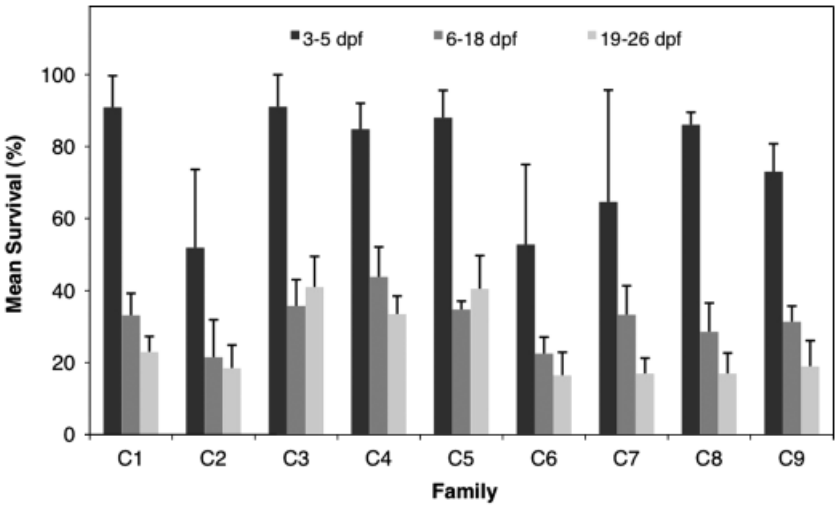

Figure 6 Variations of *Pseudoplatystoma punctifer* larvae survival, during the three breeding periods [3–5; 6–18 and 19–26 days post fertilization (dpf)]. Values represent the mean and standard deviation of triplicates for each family. Survival was calculated at the end of each breeding period. See 'Material and methods' for other details.

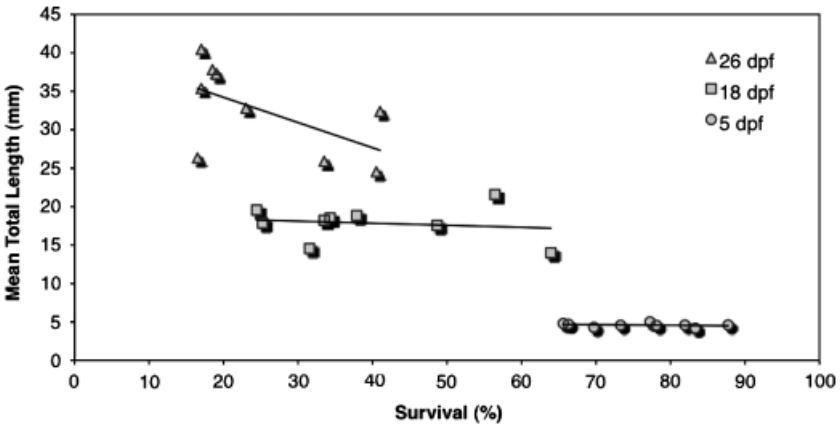

Figure 7 Linear regressions of total length (TL) vs. survival rate for the three successive breeding periods [3–5 days post fertilization (dpf), initial density: 100 larvae L^{-1}; 6–18 dpf, initial density: 50 larvae L^{-1}; 19–26 dpf, initial density 3 larvae L^{-1}).

rearing environment, such as the amount of food available (Donelson *et al.* 2009), and in some cases, differences that may occur in early breeding period can then disappear in weeks or months after birth (Ottesen, Babiak & Dahle 2009).

In this study, as in previous ones, genetic and environmental effects cannot be completely disentangled, as the male or female effect observed cannot be attributed solely to genetic or environmental factors acting on parental characteristics. Egg size is one of the main life-history trait involved with early embryo development and fry size variability at hatching (Donelson *et al.* 2009). As egg size did not differ significantly between the three females used, it is likely that the observed differences in hatching, growth and survival are rather linked to the genetic characteristics of parents; nevertheless, nongenetic effects related to yolk characteristics (i.e. protein or lipid content) cannot be totally excluded.

The variations in the observed variables on early larvae stages gave a good picture of larval development, growth and progressive early adaptation to dry feed characteristics for the nine families between 3 and 26 dpf.

Hatching

Even though total hatching success was very similar for the first six families (C1–C6), it was much lower for the last three (C7–C9) and dramatically low for family 9, suggesting some type of incompatibility between male 3 and female 3 and an overall lower performance at hatching for female 3. Nevertheless, this family had finally the best growth performance at 26 dpf.

The percentages of deformed larvae were low for females 2 and 3 but were substantially higher for female 1. However, this characteristic did not affect the overall growth performance of female 1 families. Female 2 had very high hatching rates, and very low proportions of abnormal larvae, yet the global performances of its progenies were the worst of the three females tested. These results strongly suggest that total hatching, or abnormal larvae percentages may not be good indicators of progeny growth performance in *P. punctifer*.

A weak but significant female effect was observed on the initial yolk area, but not on the final yolk area. As there was no significant differences of egg diameters among females, the difference of yolk area between female 1 and females 2 and 3, probably represents a different hydration process after fertilization and embryogenesis or differences in yolk quality among females, as already suggested for rainbow trout, *Oncorhynchus mykiss* (Kristjánsson & Vøllestad 1996). The observed male effect on the final yolk area may indicate that the male genome influences very early larvae metabolism as observed in sea bass, *D. labrax* (Saillant *et al.* 2001), winter flounder, *P. americanus* (Butts & Litvak 2007) and in Atlantic halibut, *H. hippoglossus* (Ottesen & Babiak 2007).

Growth

Total length varied slightly but significantly among families, at 3 and 5 dpf. Those variations are generally linked to female, but also to male and male–female interactions (Saillant *et al.* 2001). At 3 or 5 dpf, female 3 had the smallest progenies whereas female 1 had the largest ones. As mentioned previously,

however, the initial size does not appear to be correlated to growth performance later on (at 26 dpf).

After yolk resorption (4 dpf), exogenous feeding with artemia allowed homogenous size increase for all progenies with a relatively low size variability as confirmed by the CV% until 9 dpf. Between 9 and 14 dpf, artemia were progressively reduced to 0% while microparticulate food was increased in parallel from 0% to 100% during the same period. This period corresponds to the observed substantial increase in size variability among families as well as intra-family variability, probably because of different adaptation capabilities of the individuals at the intra- and inter-family levels. This variability increase was even higher between 19 and 26 dpf corresponding to the period of dry food pellets adaptation. During this period, the inter-family variability not only increased but the hierarchy in family sizes was also modified and family size differences were amplified during this period. Family 5 had the best growth at 18 dpf but became the worst on 26 dpf, suggesting poor adaptation capabilities to dry pelleted feed. It will be interesting to further study this trend to determine if the high growth potential can be recovered after the dry feed adaptation period; otherwise, the initial fast-growing advantage until 18 or 26 dpf cannot be used as an accurate indicator for selection purposes.

Survival

The survival observed during the three consecutive rearing periods were lower than those observed in previous experiments (Nuñez *et al.* 2008). This indicates that the adaptation time to microparticulate and to pelleted food was probably too short and that partial starvation induced lower growth rates and possibly higher mortality rates. Such high mortalities are probably enhanced by a pronounced cannibalistic behaviour, as described previously in *P. punctifer* (Kossowski 1996) and in other piscivorous fish species, like *Perca fluviatilis* or *Brycon* spp. (Baras & Jobling 2002). It is indeed likely that the size differential between individuals adapting at different rates to the new food probably reinforce, during the adaptation period, the natural cannibalistic tendency in this species. From our observations, even if at 19 dpf larvae had the possibility to absorb 1 mm pellets, in many cases, after partial ingestion, pellets were expulsed probably because of pellet hardness, because after sinking and hydration, humidified pellets were already partially consumed by some larvae at the beginning of the adaptation period. This behaviour possibly decreased feeding rates at the beginning of feed replacement and probably induced the high mortalities observed in all crosses. The mortality variations among the nine families during the last two periods of rearing may have influenced to some extent the growth performances because larvae density were not exactly the same in all families. Intra-cohort cannibalism may have also played an important role on growth performance because it may have contributed to a better food supply of the cannibals and disappearance of the smallest individuals. However, the linear correlation analysis between size (TL) and survival during the three rearing periods (3–5, 6–18 and 19–26 dpf) revealed no significant correlations between final density and TL, even if in the last period (19–26 dpf) there was a slight tendency suggesting that lower densities were associated with higher TL.

Conclusions

Finally, the results show that the parental origin significantly influences growth and probably dry feed adaptation capabilities during the first 4 weeks of life, generating an additional heterogeneity factor in multiple-family rearing groups. This heterogeneity has been shown to enhance aggressive and cannibalistic behaviour and this aspect has to be considered for multiple family communal rearing during early larval stages to avoid massive mortalities as mentioned previously for this species.

Family mean TL showed important differences after 1 month of rearing and assuming that at least part of the size differential originates from parental genomes, if growth performance is maintained or even amplified during further breeding months, selection of potential families with the highest growth rates may allow the improvement of species growth performance in culture conditions. However, further experiments should focus on studying the nature of this improved performance by some families as these higher TLs may be at least partly due to other factors like quicker adaptation to dry food consumption, higher cannibalism rates, variable aggressiveness or fish interactions. In addition, further studies are needed to fully document the persistence of such growth advantages over an extended period of time, ideally until marketable size, as some recent studies reported that initial growth advantage disappeared 50 days after hatching in a marine fish species, *A. polyacanthus* (Donelson *et al.* 2009). This approach has to be continued

until pre-adult stage with large numbers of 'fast'-growing families, authorizing conservation of breeding stock genetic variability in order to avoid possible 'bottleneck' effects induced by inappropriate selection protocols or insufficient number of breeders in the process of domestication of *P. punctifer*.

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