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Influence of dietary protein and lipid levels on growth performance and the incidence of cannibalism in *Pseudoplatystoma punctifer* (Castelnau, 1855) larvae and early juveniles

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Summary

The aim of the study was to evaluate the influence of different dietary protein and lipid levels and their ratios on larval growth, survival and the incidence of cannibalism in *Pseudoplatystoma punctifer*. Larvae were raised in a recirculation system from 3 to 26 days post-fertilization (dpf) (2–25 days post hatching, dph) at an initial density of 40 larvae L^{-1}, 27.8 ± 0.65°C and 0L : 24D photoperiod. Larvae were fed from 4 to 12 dpf with *Artemia* nauplii and weaned onto four different compound diets from 13 dpf within 3 days, then fed exclusively with these diets until 26 dpf. These diets contained 30 : 15, 30 : 10, 45 : 15 or 45 : 10 protein : lipid (P : L) (in % of dry matter) levels. A control group was fed *Artemia* nauplii until 17 dpf and weaned thereafter with the 45P : 10L compound diet. The experiment was carried out in triplicate. Results showed higher growth and survival rates and lower incidence of cannibalism in the group fed the 45P : 15L diet than in the other treatments. Differences in larval survival and growth performance were associated with the higher protein and lipid content rather than the protein : lipid ratio of this diet. When comparing diets with the same protein level, the increase in dietary lipid led to an improvement in growth, suggesting that energy from lipids spares protein for growth in *P. punctifer* fingerlings. An *Artemia* feeding period longer than 12 dpf did not improve larval growth or survival.

Introduction

Fish represent one of the main sources of protein for human consumption in the Peruvian Amazonia; the rapid demographic growth during the last decades has led to an increased exploitation of fisheries resources (Garcia et al., 2009). In this context, the development of a sustainable aquaculture has become essential to satisfy increasing demands. *Pseudoplatystoma punctifer* is a catfish species that suffers from high fishing pressures but has been considered to have high potential for aquaculture diversification in South America for almost 20 years (Kossowski, 1996). However, although research efforts have been made to control the complete life cycle in captivity (Padilla et al., 2001; Nuñez et al., 2008; Baras et al., 2011; Núñez et al., 2011), low survival at the end of the larval and juvenile stages continues to be the principal hindrance in its culture. Mortality is attributed to the high incidence of cannibalism and the low acceptability of compound diets at weaning (Baras et al., 2011; Núñez et al., 2011; Gisbert et al., 2014). However, there is no information on the nutritional needs of this species during the early life stages.

Establishing an adequate feeding protocol adapted to the digestive capacities and nutritional needs during early development while also addressing options to reduce cannibalism is of primary importance to improve survival and growth. We have already described the ontogeny of the digestive system of *P. punctifer* in order to synchronize the stage of development and maturation of their digestive organs with the feeding protocol and rearing practices (Gisbert et al., 2014). We found a clear correlation between the feeding protocol and the incidence of cannibalism, suggesting that improving feeding strategies and nutrition could reduce cannibalistic behaviour. In this context, the first signs of cannibalism were observed at 11 dpf (10 days post-hatching, dph) and coincided with the formation of the gastric glands of the stomach and the oral valves, fully equipped with taste buds for screening the quality of food, suggesting that *Artemia* might not be covering the nutritional needs of the larvae (Gisbert et al., 2014). Moreover, the incidence of cannibalism clearly increased at weaning, especially at the end of co-feeding, also showing the nutritional inadequacy of the diet used in that study. These results highlighted the need to study the nutritional requirements of larvae and juveniles as this may reduce cannibalism.

During the larval and early juvenile stages, proteins constitute the main macronutrient for growth (Rønnestad et al.,

1999) and lipids represent the main energy source during morphogenesis (Sargent et al., 1999). There is little information on nutritional requirements of *Pseudoplatystoma* species, and most refers to the juvenile stage (Martino et al., 2002a, b; Lundstedt et al., 2004; Campos et al., 2006; Arslan et al., 2009, 2013; Bicudo et al., 2012; Silva, 2013; Cornélio et al., 2014; Gonçalves, 2014). The goal of the present study was to evaluate the influence of different dietary lipid and protein levels and their ratios on growth, survival and the incidence of cannibalism of *P. punctifer*. Since carbohydrates were used to obtain the desired dietary protein : lipid proportions, the variation of carbohydrate content was also considered in the interpretation of the results.

Materials and methods

Spawning and larval and early juvenile rearing

Larvae were obtained by hormonally-induced spawning of a sexually mature pair of *P. punctifer* (♀: 3.6 kg; ♂: 1.85 kg body weight, BW) from a broodstock maintained in captivity at the Instituto de Investigaciones de la Amazonía Peruana (IIAP, Iquitos, Peru). A pair of mature females and males was transferred from the pond to a 500-L indoor tank where they were kept at 27.5 ± 0.5°C and 12L : 12D photoperiod during the induction process. Females and males were injected intramuscularly with Carp Pituitary Extract (Argent Chemical Laboratories, Inc., Redmond, WA) at 5 mg kg^{-1} and 1 mg kg^{-1} BW, respectively. Hormone injections were administered in one dose for the male and two doses for the female: the first at 10% of the total dose and the second 12 h later at 90% of the total dose. Stripping of the female, sperm collection and the fertilization procedure were performed following the protocol described by Nuñez et al. (2008). Spawned eggs (fertilization rate = 99.9%) were incubated at 27.7 ± 0.6°C in four 60-L tanks (50-L water volume; temperature measured daily at 07.00 h in each tank) connected to a clear water recirculating system, whereby hatching occurred 18 ± 2 h later (hatching rate = 96%). Larvae were transferred at 4 days post-fertilization – dpf (3 days post-hatching – dph; 5.6 ± 0.7 mm total length – TL, n = 30) into 40-L tanks (30-L water volume) connected to a water recirculation system provided with mechanical and biological filters. Water conditions throughout the experiment were: 27.8 ± 0.7°C, pH 7.0 ± 0.5, dissolved oxygen 7.4 ± 0.2 mg L^{-1}, $N–NO_2$ 0.38 ± 0.27 mg L^{-1}, $N–NH_4$ 0.26 ± 0.13 mg L^{-1}. Water temperature, pH and dissolved oxygen were measured daily and $N\text{-}NO_2$ and $N\text{-}NH_4$ weekly at 07.00 h in six tanks. Water supply was adjusted in each tank to assure a water flow rate of 0.2 L min^{-1}. In this study we considered the previously used feeding protocol for *P. punctifer* (Gisbert et al., 2014) for the control group, in which weaning took place at 18 dpf (17 dph at 28°C). However, we decided to advance the weaning age for the remainder of the treatments to 13 dpf in order to coincide with the gastric gland formation of the stomach (Gisbert et al., 2014) and improve the use of the experimental compound diets. Thus, larvae were reared in triplicate (initial density = 40 larvae L^{-1}, n = 1200 larvae per replicate) from 4 to 26 dpf under 0L : 24D photoperiod (<0.001Lx at the water surface) and fed five times per day with *Artemia* spp. nauplii in slight excess from 4 to 17 dpf (0.6–12.2 nauplii ml^{-1}) in the control group and from 4 to 12 dpf in the other treatments (0.6–9 nauplii ml^{-1}) considering larval density, weight increase and the daily food ration (Baras et al., 2011). At 13 dpf, larvae were weaned within 3 days onto four compound diets containing different protein and lipid levels (Fig. 1), the control group onto the 45 : 10 protein : lipid (P : L) diet from 17 dpf, and all treatments fed up to 26 dpf exclusively with these diets.

Proximate composition and lipid class analyses

Four experimental diets were prepared at the Ifremer (Plouzané, France) as described in Cahu et al. (2003) including different percentages of proteins and lipids in their formulation (Table 1). Total lipids of the compound diets were extracted in chloroform : methanol (2 : 1, v : v) according to the method of Folch et al. (1957) and quantified gravimetrically after evaporation of the solvent under a nitrogen flow

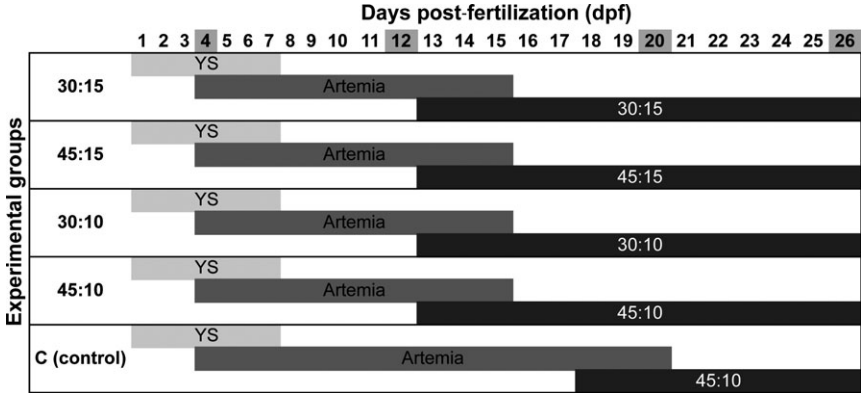

Fig. 1. Experimental nutrition design, larval and early juvenile stage, *Pseudoplatystoma punctifer*. Four experimental groups were weaned from 13 days post fertilization (dpf) within 3 days with compound diets containing different levels of proteins and lipids. Dietary treatment code corresponds to protein : lipid (P : L) level included in tested diets. The control group was weaned onto 45P : 10L compound diet at 18 dpf. YS, yolk-sac stage. Days marked in grey = sampling points.

followed by vacuum desiccation overnight. Analysis of lipid classes was performed according to Olsen and Henderson (1989). Protein and carbohydrate contents were determined following the Lowry et al. (1951) and Dubois et al. (1956) methods, respectively.

Larval and early juvenile performance

Groups of larvae and early juveniles were sampled from each tank at 4 (n = 30), 12 (n = 15), 20 (n = 15) and 26 (n = 15) dpf and anaesthetized using Eugenol (0.05 μl ml^{-1}; Moyco®, Moyco, Lima, Peru) for growth measurements. Individual wet weight (WW) was determined using an analytic microbalance (Sartorius BP 211 D, Data Weighing Systems, Inc., Elk Grove, IL, ±0.01 mg). Specific growth rate (SGR, in % day^{-1}) was calculated as SGR = (ln WW_f − ln WW_i)/(t_f − t_i) x 100; where WW_f, WW_i, t_f and t_i represented final and initial WW and time of the experiment, respectively. For total length (TL) measurements, specimens were placed on a Petri dish and photographed using a scale bar. TL was measured on the pictures using ImageJ software (Rasband, 1997–2012).

The number of cannibals was counted in each tank twice a day (08.00 h and 17.00 h) and the incidence of cannibalism expressed as the percentage of fish displaying cannibalistic behaviour at each feeding period. Two types of cannibalism were recorded: type I, when larvae were partially damaged (pectoral fins and/or stomach bitten), and type II, when individuals were completely ingested by their siblings (Fig. 2). Survival was evaluated by counting the individuals surviving at 12 and 26 dpf with respect to the number of individuals at the beginning of each feeding period and calculated considering the number of individuals sampled at each feeding period.

Table 1
Composition of experimental diets. Dietary treatment code corresponds to protein : lipid level included in tested diets. DM, dry matter

Dietary treatments	30 : 15	30 : 10	45 : 15	45 : 10
Ingredients[1] (in % DM)				
Fishmeal	36	36	53	53
Hydrolysed fishmeal (CPSP)	9	9	14	14
Lipids	14	8	12	7
Marine lecithin	3	8	3	7
Soybean lecithin	11	0	9	0
Gelatin	15	15	15	15
Wheat starch	20	26	0	5
Vitamin mix[2] (×4)	2	2	2	2
Mineral mix[3]	3	3	3	3
Betain	1	1	1	1
Analyses of the diets (% DM)				
Proteins	30.07	30.90	43.13	42.86
Total lipids	12.50	7.43	12.46	10.36
Neutral lipids	6.99	5.42	7.29	6.93
Phospholipids	6.35	2.20	4.80	2.60
Carbohydrates	24.87	31.31	2.34	7.58
Moisture	18.89	22.35	17.48	15.25

[1]All dietary ingredients obtained commercially. Fishmeal hydrolysate CPSP 90 : 10% lipids; Soluble Fish Protein Concentrate (SopropÊche, Boulogne sur Mer, France); soy lecithin (Ets Louis François, St Maur des Fossés, France); marine lecithin LC 60 (Phosphotech, St Herblain, France).
[2]Composition per kilogram of vitamin mixture: choline chloride 60%, 333 g; vitamin A acetate, (4000 IU g^{-1}) 2 g; vit. D_3 (1920 IU g^{-1}) 0.96 g; vit. E (40 IU g^{-1}) 20 g; vit. B_3 2 g, vit. B_5 4 g; vit. B_1 200 mg; vit. B_2 80%, 1 g; vit. B_6 600 mg; vit. B_9 80%, 250 mg; vit. concentrate B_{12} (10 g kg^{-1}), 0.2 g; biotin, 1.5 g; vit. K_3 51%, 3.92 g; meso-inositol 60 g; cellulose, 543.3 g.
[3]Composition per kilogram of mineral mixture: 90 g KCl, 40 mg KIO_3, 500 g $CaHPO_4$ $2H_2O$, 40 g NaCl, 3 g $CuSO_4$ $5H_2O$, 4 g $ZnSO_4$ $7H_2O$, 20 mg $CoSO_4$ $7H_2O$, 20 g $FeSO_4$ $7H_2O$, 3 g $MnSO_4$ H_2O, 215 g $CaCO_3$, 124 g $MgSO_4$ $7H_2O$, and 1 g NaF.

Statistical analysis

Results were expressed as mean ± SD. Statistical tests were conducted using SIGMASTAT 3.0 (Systat Software Inc., Richmond, VA). All data were checked for normality (Kolmogorov–Smirnov test) and homogeneity of variance (Bartlett's test) and evaluated by one-way ANOVA followed by

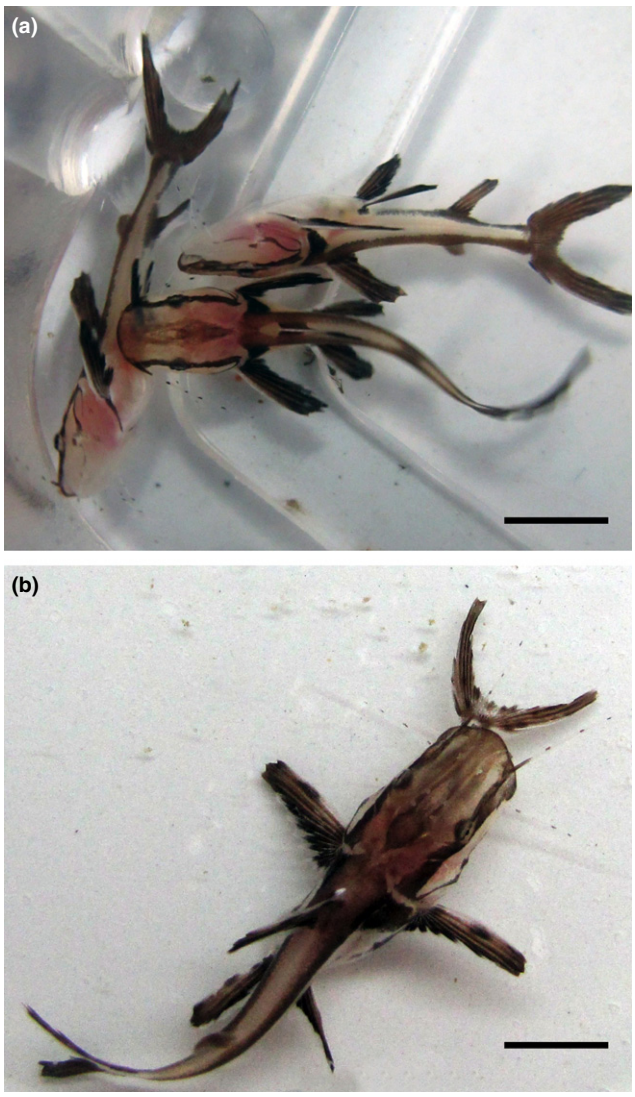

Fig. 2. Images showing type I (a) and type II (b) cannibalism in reared *Pseudoplatystoma punctifer* juveniles. Scale bar = 5 mm.

the Holm–Sidak method for all pairwise comparisons ($P < 0.05$). Data were previously arcsine-transformed for survival and cannibalism variables. The incidence of type I and type II cannibalism within the same dietary group was compared using a *t*-test ($P < 0.05$).

Results

Proximate composition and lipid class analyses

Analyses of proximate composition and lipid classes of the compound diets are shown in Tables 1 and 2, respectively. Diets were formulated for designing two feeds with high protein levels (about 45%) and two feeds with low protein levels (about 30%), crossed with high lipid levels (around 15%) or low lipid levels (around 10%). The four diets exhibited similar neutral lipid (NL) levels, when phospholipid (PL) was higher in diets with higher lipid levels. Phosphatidylcoline was the major component of PL and together with phosphatidylserine and phosphatidylinositol accounted for most of the differences. Triacilglicerids (TAG) were the most predominant NL, followed by cholesterol and free fatty acids. TAG levels were higher in the 45P : 15L and 45P : 10L diets (Table 2). Carbohydrates were added as wheat starch in order to maintain the four diets with a similar energy level, around 1350 KJ g^{-1} wet weight.

Growth performance and incidence of cannibalism

P. punctifer larvae from the five dietary treatments did not show significant differences in TL, WW and survival during the *Artemia* feeding phase, being on average 12.0 ± 0.13 mm TL (n = 45), 7.3 ± 0.55 mg WW (n = 45) and 87.5 ± 6.6% survival (initial n = 3600) ($P > 0.05$, Figs 3a, 4a and 5a). However, differences in growth were observed at 20 dpf, 5 days after weaning was completed ($P < 0.001$). Larvae fed the 45P : 15L diet presented significantly higher TL and WW values (two times higher) than the rest of the treatments ($P < 0.001$, Figs 3b and 4b).

At 26 dpf, early juveniles fed the 45P : 15L diet had the highest TL ($P < 0.05$), followed by those fed the 45P : 10L, 30P : 15L and C diets, which presented similar TL ($P > 0.05$), and those fed the 30P : 10L, which were the smallest ones ($P < 0.05$, Fig. 3c). Regarding WW, larvae from the 45P : 15L group were also heavier, followed by those fed the 45P : 10L diet, by the 30P : 15L and C groups that had similar WW values ($P > 0.05$), and finally by the 30P : 10L group ($P < 0.05$, Fig. 4c).

No significant differences in SGR between treatments were observed during the *Artemia* feeding period. However, SGR of larvae fed the 45P : 15L diet was significantly higher than in the other treatments, larvae from the 30P : 10L and C groups showing the lowest SGR values ($P < 0.001$, Table 3). These results indicate that SGR was preferentially affected by the protein level and then by the lipid level.

Survival at the end of the experiment (26 dpf) was significantly higher in larvae fed the 45P : 15L diet, followed by those fed the 45P : 10L, 30P : 15L, C and 30P : 10L diets ($P < 0.05$, Fig. 5b).

No cannibalism was observed during the *Artemia* feeding period. Incidence of cannibalism during the compound diet feeding period (13–26 dpf) is shown in Fig. 6. Larvae from the 30P : 10L group showed a significantly higher incidence of type I cannibalism than the 30P : 15L, 45P : 15L and C groups ($P < 0.05$), whereas the 45P : 10L group presented intermediate values. Regarding type II cannibalism, the 30P : 10L and 30P : 15L groups showed higher incidence of cannibalism than the rest of treatments ($P < 0.05$). There was higher incidence of type I cannibalism than type II ($P < 0.05$) in the 30P : 10L group, whereas values of type I

Table 2
Lipid classes (in % of dry matter) analysed in the prepared experimental diets. Data expressed as mean ± SD (n = 3). Different superscript letters denote differences statistically significant between dietary treatments (one-way ANOVA, $P < 0.05$). Dietary treatment code corresponds to the protein : lipid level included in the tested diets

	Dietary treatments			
	30 : 15	30 : 10	45 : 15	45 : 10
SM	0.28 ± 0.0^{c}	0.61 ± 0.25^{b}	0.00 ± 0.00^{d}	0.92 ± 0.00^{a}
Lyso PC	1.37 ± 0.02^{a}	0.90 ± 0.13^{b}	1.31 ± 0.10^{a}	1.41 ± 0.07^{a}
PC	21.18 ± 0.15^{a}	16.63 ± 0.34^{c}	18.04 ± 0.24^{b}	16.67 ± 0.52^{c}
PS/PI	6.94 ± 1.86^{a}	3.54 ± 0.13^{b}	5.45 ± 1.04^{a}	2.94 ± 0.15^{b}
Lyso PE	2.70 ± 0.62^{a}	0.00 ± 0.00^{c}	1.81 ± 0.13^{b}	0.00 ± 0.00^{c}
PE	9.73 ± 1.61^{a}	0.00 ± 0.00^{b}	8.05 ± 0.45^{a}	0.00 ± 0.00^{b}
Uk1	5.06 ± 0.89^{c}	7.55 ± 0.28^{a}	3.78 ± 0.49^{d}	6.49 ± 0.04^{b}
Uk2+3	2.69 ± 0.13^{a}	0.00 ± 0.00^{c}	2.15 ± 0.29^{b}	0.00 ± 0.00^{c}
Total PL	50.12 ± 3.56^{a}	29.23 ± 0.46^{c}	40.78 ± 1.46^{b}	27.97 ± 0.99^{c}
CHOL	14.41 ± 1.64^{b}	23.83 ± 1.63^{a}	15.32 ± 0.71^{b}	22.81 ± 1.09^{a}
FFA	8.63 ± 1.45^{b}	14.59 ± 1.66^{a}	11.82 ± 0.08^{a}	14.09 ± 2.14^{a}
TAG	16.86 ± 0.39^{b}	18.20 ± 1.31^{b}	21.29 ± 0.58^{a}	22.51 ± 1.73^{a}
SE+W	9.98 ± 2.98	14.15 ± 1.80	10.79 ± 1.26	12.61 ± 3.77
Total NL	49.88 ± 3.56^{c}	70.77 ± 0.46^{a}	59.22 ± 1.46^{b}	72.03 ± 0.99^{a}

CHOL, Cholesterol; FFA, Free Fatty Acids; Lyso PC, LysoPhosphatidylColine; NL, Neutral Lipids; PC, PhosphatidylColine; PE, PhosphatidylEthanolamine; PI, PhosphatidylInositol; PL, Phospholipids; PS, PhosphatidylSerine; SE, Sterolesters; SM, Sphingomieline; TAG, Triacilglicerids; Uk, Unknown; W, wax.

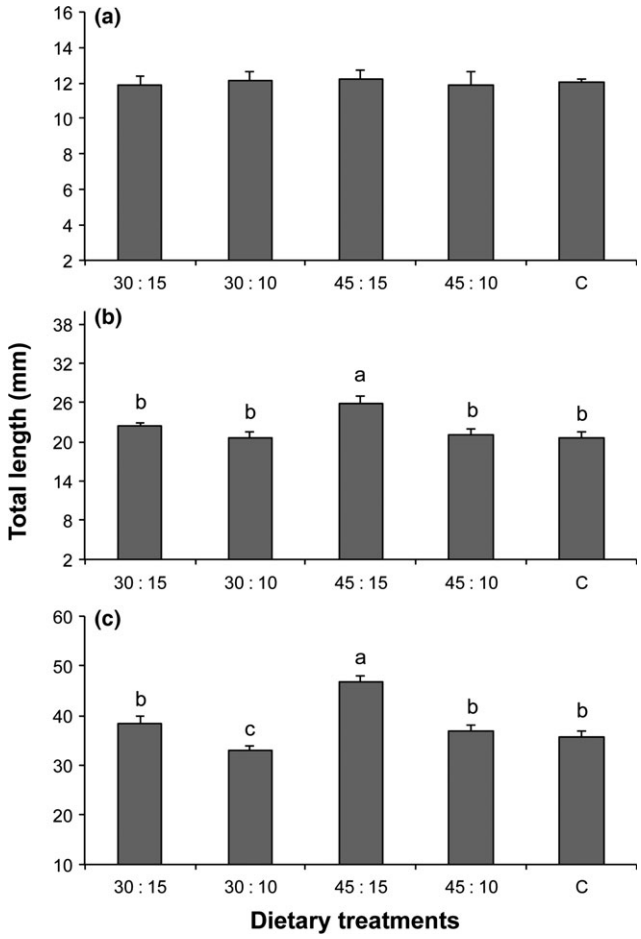

Fig. 3. Total length (TL), *Pseudoplatystoma punctifer* at (a) 12 days post fertilization – dpf (n = 45), (b) 20 dpf (n = 45) and (c) 26 dpf (n = 45) reared at 27.8 ± 0.7°C and in complete darkness. Data expressed as mean ± SD. Different superscript letters = statistically significant differences between dietary treatments (one-way ANOVA, $P < 0.05$). Dietary treatment code corresponds to the protein : lipid level included in the tested diets.

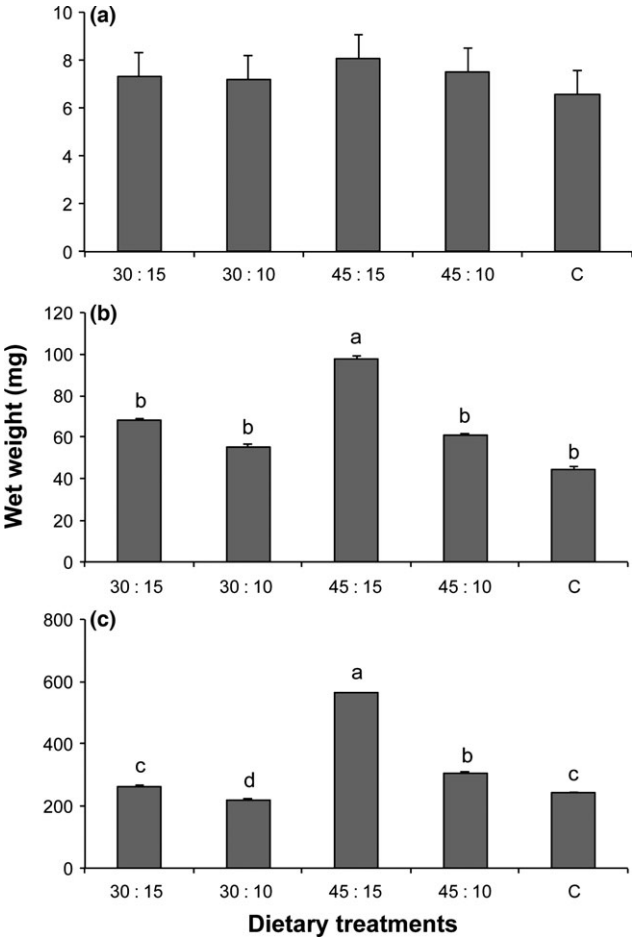

Fig. 4. Wet weight (WW), *Pseudoplatystoma punctifer* at (a) 12 days post fertilization – dpf (n = 45), (b) 20 dpf (n = 45) and (c) 26 dpf (n = 45) reared at 27.8 ± 0.7°C and in complete darkness. Data expressed as mean ± SD. Different superscript letters = statistically significant differences between dietary treatments (one-way ANOVA, $P < 0.05$). Dietary treatment code corresponds to the protein : lipid level included in the tested diets.

and type II cannibalism were similar among the rest of the diets ($P > 0.05$).

Discussion

Growth performance

Extending the *Artemia* feeding phase did not provide any advantage in terms of larval growth and survival in view of the results observed in the C and 45P : 10L groups, despite being fed the same compound diet. As larvae grew, the differences in growth between both groups increased (see WW at 20 and 27 dpf), group C showing no signs of compensatory growth. Whether this developmental delay generated during the early stages would be irreversible remains to be elucidated. This finding is of special relevance considering the high cost of *Artemia* and the associated costs of nauplii production. The results are also consistent with our hypothesis that *Artemia* nauplii do not cover the nutritional needs of *P. punctifer* larvae (Gisbert et al., 2014), as survival and growth performance of specimens from this group (C) were similar to those of the 30P : 15L group and only higher than the group fed the 30P : 10L diet. These results indicate that the feeding protocol used previously (Gisbert et al., 2014) allowed larvae to grow normally, but did not let them fully exploit their growth potential. In addition, the large accumulation of lipid deposits found in the intestine and liver suggested a nutritional imbalance with regard to protein and lipid content of the administered compound diet (Gisbert et al., 2014). The 45P : 15L diet soon showed an evident positive effect on growth (1 week after weaning) and at the end of the experiment allowed the amelioration of larval growth performance (six times in terms of WW and two times in terms of TL) and survival (two times) compared to preceding protocols under similar rearing conditions (Gisbert et al., 2014; M.J. Darias, unpublished data). Nevertheless, parental origin, which significantly affects growth during the early development of this species (Núñez et al., 2011), might also account for such differences.

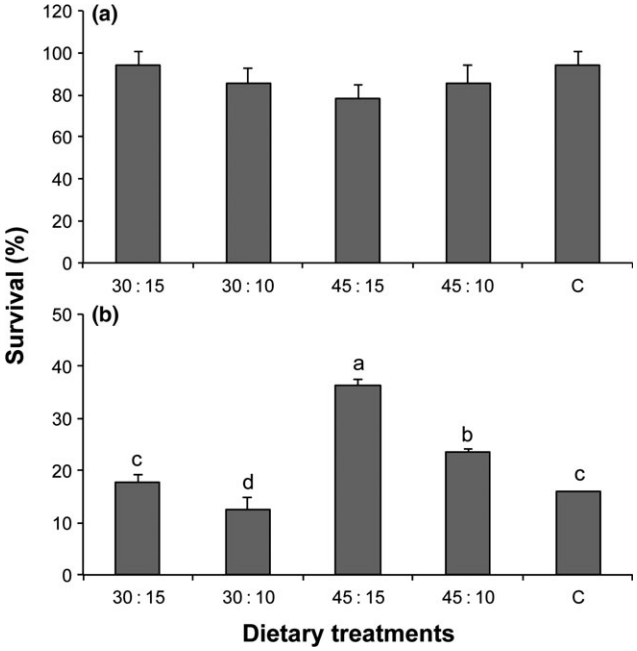

Fig. 5. Survival (%) of *Pseudoplatystoma punctifer* at (a) 12 days post fertilization – dpf and (b) 26 dpf (initial n = 3600 per treatment) reared at 27.8 ± 0.7°C and in complete darkness. Data were expressed as mean ± SD. Different superscript letters denote statistically significant differences between dietary treatments (one-way ANOVA, $P < 0.05$). Dietary treatment code corresponds to the protein : lipid level included in tested diets.

Table 3
SGR (in % day^{-1}) of *Pseudoplatystoma punctifer* larvae during *Artemia* and compound diet feeding periods in each dietary treatment (rearing temp. 27.8 ± 0.7°C, photoperiod 0L : 24D). Data expressed as mean ± SD (n = 45). Different superscript letters denote differences statistically significant between dietary treatments (one-way ANOVA, $P < 0.05$). Dietary treatment code corresponds to the protein : lipid level included in the tested diets

	Feeding periods	
Dietary treatments	*Artemia* (4–12 dpf)	Compound diets (13–26 dpf)
30 : 15	0.20 ± 0.01	0.53 ± 0.02^{bc}
30 : 10	0.20 ± 0.00	0.52 ± 0.01^{c}
45 : 15	0.21 ± 0.00	0.61 ± 0.01^{a}
45 : 10	0.20 ± 0.02	0.55 ± 0.01^{b}
C	0.19 ± 0.00	0.53 ± 0.01^{c}

Incidence of cannibalism

A variable incidence of cannibalism during the early juvenile stage was observed between treatments (3.6–6.4%), although this was considerably reduced compared to previous studies (16–30%, Arslan et al., 2009; Baras et al., 2011; Gisbert et al., 2014). In the present study, type I cannibalism was predominant, but not exclusive, at early stages; type II cannibalism was more important from 15 dpf onwards, coinciding with the formation of the gastric glands and the end of weaning. Two peaks of cannibalism were observed: the first day of weaning (13 dpf) and 2 days after the end of weaning

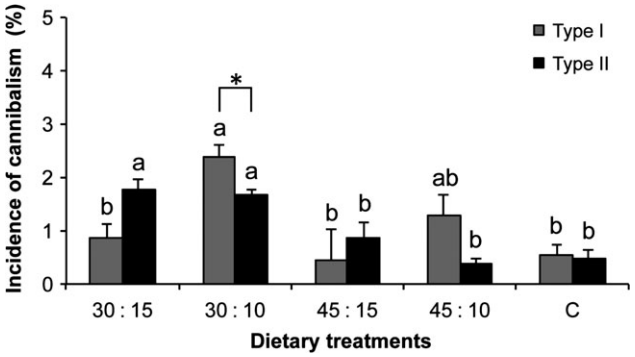

Fig. 6. Incidence of cannibalism (%) in *Pseudoplatystoma punctifer* during compound diet feeding period (13–26 days post fertilization – dpf; rearing temperature 27.8 ± 0.7°C; photoperiod 0L : 24D; initial n = 400). Data expressed as mean ± SD. Different superscript letters and asterisks = statistically significant differences between dietary treatments within each type of cannibalism and between the two types of cannibalism, respectively (one-way ANOVA, $P < 0.05$). Dietary treatment code corresponds to protein : lipid level included in tested diets.

(17 dpf). Cannibalism then gradually decreased until disappearing at 21 dpf (data not shown). Although weaning had previously been a critical stage promoting cannibalistic behaviour (Gisbert et al., 2014), the lower incidence of cannibalism noted in the present study and, in particular, its gradual disappearance after weaning, showed that a better-suited diet could greatly reduce this behaviour. Cannibalism was not consistently related to growth or survival, the smaller specimens (30P : 10L group) presenting the higher incidence of cannibalism and groups showing low incidence of cannibalism displaying low survival rates (30P : 15L and C groups). Although higher size distribution was generally found in groups displaying higher cannibalism in preceding experiments, no effect of cannibalism on size distribution of the cohort among the experimental groups was observed in the present study (data not shown). In any case, its influence in those parameters might have been very limited due to the low registered incidence of cannibalism. Indeed, a beneficial effect of all tested diets in the behaviour of *P. punctifer* was observed, fish being calmer and less aggressive than in previous experience. This suggests that, besides the overall nutritional value of the diets, the inclusion of specific nutrients inducing relaxing effects, such as PL, could participate to attenuate cannibalism. In fact, dietary PL have been shown to reduce the locomotor activity, stress and aggressiveness in humans and rats when provided in sufficient amounts (Chalon et al., 1998; DeMar et al., 2006; Hamazaki and Hamazaki, 2008), while its deficiency promotes an anxious behavioural profile in fish (Lund et al., 2014). Research on the nutritional effect on behaviour would be useful for understanding the incidence of cannibalism in this species. Besides being essential for growth and performance in many fish species, including juveniles of *P. fasciatum* (Arslan et al., 2009; Cahu et al., 2009), PL could also account for improving the palatability of the diets (Hadas et al., 2003; Tocher et al., 2008; Gong et al., 2014). This is particularly important for *P. punctifer* since taste and smell seem to have a key

role in feeding, as suggested by the presence of large size oral valves equipped with taste buds believed to serve for screening the food quality (Yashpal et al., 2006; Gamal et al., 2012), and large development of the olfactory organ (Gisbert et al., 2014). Indeed, larvae and early juveniles of *P. punctifer* demonstrated a sensitivity to food texture, being more attracted by humid than by dry compound diets (Fernández-Méndez et al., 2015), this being likely linked to the palatability and/or smell associated to the attractants released.

Macronutrient requirements in larvae and early juveniles

To our knowledge, this is the first report on the nutritional requirements during the larval and early juvenile stage of the genus *Pseudoplatystoma*. Reports in the literature show a notable difference in protein, lipid and carbohydrate requirements for older juveniles (initial wet weight ranging from 1 to 120 g) of several *Pseudoplatystoma* species. Thus, optimal levels range from 36 to 49% for proteins (Campos et al., 2006; Zanardi et al., 2008; Silva, 2013; Cornélio et al., 2014; Gonçalves, 2014), from 8 to 19% for lipids (Martino et al., 2005; Campos et al., 2006; Arslan et al., 2013; Silva, 2013) and from 13% to 25% for carbohydrates (Lundstedt et al., 2004; Okamura, 2009; Gonçalves, 2014) depending on the quality of ingredients and their relative proportions. In particular, Gonçalves (2014) working with *P. reticulatum* found that protein content could be reduced up to 36% protein when other energetic nutrients were balanced (15% carbohydrate and 8% lipids). Our study showed that a very good larval growth can be obtained with a diet containing 45% protein, brought as fishmeal (native and hydrolysed), and 15% lipids, brought as phospholipid (marine and soybean lecithin) and neutral lipid (oil included in fishmeal). Independently of the energy level that was the same in all diets, protein and/or lipid levels were insufficient in the other three diets for promoting similar growth. Besides the apparent insufficient protein and lipid content of the 30P : 10L diet, its high carbohydrate level (30%) could also be responsible for the impaired growth observed in this group. Indeed, Gonçalves (2014) did not find a protein-sparing effect by carbohydrates in juveniles of *P. reticulatum* fed two dietary carbohydrate (15 and 25%) and three protein (44, 40 and 36%) levels. Okamura (2009) found that juveniles of *Pseudoplatystoma* spp. hybrid (*P. corruscans* × *P. fasciatum*) presented persistent hyperglycemia when fed 20% cornstarch and concluded that the optimum level would be around 15%. The capacity of *P. punctifer* larvae and early juveniles to digest carbohydrates needs to be determined. Meanwhile, results from this study indicate that there is room for optimizing the balance of energetic compounds for *P. punctifer* larvae and early juveniles to spare protein for growth.

Indeed, one of the goals in fish nutrition science is to reduce the dietary protein content through incorporation of other energy sources, such as lipids and carbohydrates, allowing improvement of protein utilization for growth. The protein-sparing effect of lipids has been reported in many fish species, including in fingerling stages (Vergara et al., 1996; Li et al., 2012). However, a lipid level higher than 19% did not improve growth, nor had protein-sparing effects in *P. corruscans* juveniles, but resulted in increased visceral lipid content (Martino et al., 2005). Differences in larval and early juvenile survival and growth observed in the present study seemed to be associated with the higher protein and lipid content rather than the protein : lipid ratio. Comparing diets with the same protein level, the increase in dietary lipids led to an improved growth, suggesting that energy from lipids spares protein in *P. punctifer* fingerlings. These results indicate that the growth potential for this species might not have been fully exploited. Thus, further research to optimize the balance of proteins and energetic compounds (lipids and carbohydrates) is needed in *P. punctifer,* especially during the larval stage.

Conclusions

In conclusion, *P. punctifer* larvae were successfully weaned at 13 dpf but *Artemia* spp. did not satisfy the nutritional requirements of individuals, at least after the completion of the digestive system development. Among the tested diets, the 45P : 15L diet greatly improved growth and survival of *P. punctifer* larvae and early juveniles and reduced the incidence of cannibalism compared to previous feeding protocols used for this species. Differences in larval survival and growth were associated with the higher protein and lipid content of the diet rather than its protein : lipid ratio. Further studies on the digestive enzyme activity response to these dietary treatments will allow determination of a suitable macronutrient composition of the diet during the early development of this species.

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