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Rearing of the Amazon catfish *Pseudoplatystoma punctifer* (Castelnau, 1855): weaning with dry and moist diets

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Summary

The aim of this study was to compare the effects of dry and moist diets at weaning on growth, survival and incidence of cannibalism in the Amazon catfish *Pseudoplatystoma punctifer*. Three moist diets (MCD: moist commercial diet; MCPD: moist commercial peptide enriched diet; BLD: bovine liver enriched diet) and a dry diet (CDD: commercial dry diet) were used to feed fingerlings reared in 40-L tanks (30-L water volume; three replicates per treatment; stocking density: initial 1500 larvae per tank, from 18 days post-fertilization, dpf (17 days post-hatch, dph) onwards 210 juveniles per tank; photoperiod 0L:24D) in a recirculation water system (27.9 ± 0.5°C). Fish were fed *Artemia* nauplii at 20% of the larval biomass from 4 to 19 days post-fertilization (dpf) and then weaned onto the four experimental diets within 3 days in the moist diets and within 6 days in the dry diet. At 33 dpf, feeding of groups fed the moist diets was switched to the dry diet within 2 days. Results showed higher (58.6 ± 10%) and lower (4.7 ± 0.7%) survival rates in BLD and CDD groups, respectively, the latter showing the highest occurrence of type II cannibalism. Although weaning was achieved in all dietary groups, the moist diets MCD and MCPD showed better growth results both in terms of total length and wet weight. This study showed that, among the tested diets, the moist diets seem to be more adequate for early juvenile *P. punctifer*.

Introduction

Commercial fishing for big catfish species is one of the main economic activities in the Peruvian Amazon (Barthem and Goulding, 1997). Among these large catfish, *Pseudoplatystoma punctifer* is one of the most prized, with great acceptance among the Amazonian population due to the quality of its meat. High demand has caused heavy fishing pressure on the natural populations, resulting in an increase in the capture of individuals below minimum fishing size (Tello and García, 2009). This situation has led to a growing interest in the cultivation of this species as it presents a great potential for commercial aquaculture in the Peruvian Amazon (Kossowski, 1996; Nuñez, 2009). The reproduction of *P. punctifer* by hormonal induction in captivity has been mastered (Kossowski and Madrid, 1985; Padilla et al., 2001; Leonardo et al., 2004; Nuñez et al., 2008); however, the larval stage is shown to be a critical step in the culture of this species. The first larval rearing trials showed strong cannibalism among individuals (Kossowski and Madrid, 1991), with a high level of aggressiveness due to piscivorous feeding habits favoured by size heterogeneity of individuals and high rearing densities (Atencio-García and Zaniboni-Filho, 2006). Use of live food (*Artemia* nauplii) at first feeding has improved survival (Díaz-Olarte et al., 2009) as in other freshwater and seawater species, due to the high n-3 and n-6 fatty acid content (Prieto and Atencio, 2008). Previous studies have also shown that complete darkness and low densities can greatly reduce cannibalism (Nuñez et al., 2008; Díaz-Olarte et al., 2009; Baras et al., 2011).

Intensive farming of carnivorous species requires live feeds, which represent high production costs; therefore, their replacement by compound diets is one of the main objectives in fish larval nutrition. Weaning success depends on many factors including the developmental degree of the digestive system, nutritional quality and composition of the diet, palatability of the feed, weaning strategy, initial size of the fish and the diet and the cannibalistic activity (Kubitza and Lovshin, 1999; Gisbert et al., 2014; Darias et al., 2015). Studies on *Pseudoplatystoma* sp. showed the importance of using moist diets (mostly using beef liver or heart) during weaning to reduce the levels of cannibalism at this stage (Guerrero, 2003; Takata, 2007; Maia, 2011; Marciales-Caro et al., 2011; Fernández et al., 2012).

The aim of this study was to compare at weaning the effects of dry and moist diets on growth, survival and incidence of cannibalism in the Amazon catfish *P. punctifer*.

Material and methods

Fish origin and experimental design

Larvae of *P. punctifer* were obtained by hormonal induction of a male and a female at the Instituto de Investigaciones de la Amazonia Peruana (IIAP) Iquitos, Peru, as described in Nuñez et al. (2008). Hatching occurred at 20 ± 2 h post-fertilization (hpf) at 27.5 ± 0.5°C. At 4 days post-fertilization-dpf (3 days post hatching-dph) fish were transferred to twelve 40-L tanks (30-L water volume) connected to a recirculating water system and reared at an initial density of 50 larvae L^{-1}. Water conditions remained constant throughout the

Table 1
Pseudoplatystoma punctifer fish density, feeding schedule and weaning protocol, 4–41 days post-fertilization at 27.9 ± 0.5°C and total darkness under different feeding regimes: MCD, moist commercial diet; MCPD, moist commercial peptide enriched diet; BLD, beef liver diet; CDD, commercial dry compound diet (three replicates per treatment)

Feeding periods	I	II	III								IV		
Days post-fertilization (dpf)	4–17	18–19	20	21	22	23	24	25	26	27–32	33	34	35–41
Density (fish L^{-1})	50	7	7	7	7	7	7	7	7	3	3	3	3
Meals per day	7	5	5	5	5	5	5	5	5	5	3	3	3
Feeding with moist diets (%)													
Artemia nauplii	100	100	75	50	25	–	–	–	–	–	–	–	–
Moist diet (MCD, MCPD, BLD)	–	–	25	50	75	100	100	100	100	100	66.6	33.3	–
Dry diet (CDD)	–	–	–	–	–	–	–	–	–	–	33.3	66.6	100
Feeding with dry diet (%)													
Artemia nauplii	100	100	87.5	75	62.5	50	37.5	12.5	–	–	–	–	–
Dry diet (CDD)	–	–	12.5	25	37.5	50	62.5	87.5	100	100	100	100	100

experiment (41 days): 27.9 ± 0.5°C, pH 7.1 ± 0.2, 7.2 ± 0.6 mg L^{-1} dissolved oxygen, N-NO_2 < 0.05 mg L^{-1}, N-NH_4 0.2 ± 0.07 mg L^{-1}. Water temperature, pH and dissolved oxygen were measured daily and N-NO_2 and N-NH_4 weekly at 06.00 h in six randomly-chosen tanks. Larvae were kept in complete darkness in order to reduce cannibalism and fed seven times a day every 2 h (from 06.00 to 18.00 hours) with newly hatched *Artemia* nauplii at 20% of the larval biomass (on a wet weight basis). At 18 dpf, fish density was reduced to seven individuals per litre. Fingerlings were weaned at 20 dpf onto the four experimental diets within 3 days in the moist diets and within 6 days in the dry diet. At 33 dpf, feeding of groups fed the moist diets was switched to the dry diet within 2 days (Table 1). Particle size was increased (0.8, 1.5 and 2.6 mm) according to the growth of the fish. Fish were fed *ad libitum* from weaning onwards. Feeding frequencies throughout the experimental period are given in Table 1. During the experiment, 15 individuals per tank were randomly sampled at 13, 18, 32 and 41 dpf (three replicates per treatment) and anesthetized with clove oil (0.05 μl/ml; Moyco®, Lima, Peru) prior to the determination of the total length (TL). This was done on photographed fingerlings with a calibrated size mark using ImageJ software (Rasband, 1997–2012).

Experimental diets and proximate composition analysis

Three moist experimental diets were generated: moist commercial diet (MCD) and moist commercial peptide enriched diet (MCPD) were elaborated using a dry commercial diet (PURINA®, Cargill Incorporated, Lima, Peru) containing 50% protein to which water and neutral gelatine was added. Fish hydrolysate (Peptides Peru S.A., Lima, Peru) was also incorporated to the MCPD diet. The beef liver diet (BLD) consisted of a mix of beef liver and cornstarch (Duryea®, Unilever, Lima, Peru). Moist diets were sieved to 600 μm to produce small pellets that could be ingested by fingerlings. The commercial dry compound diet (CDD) used was Aquaxcel® (Cargill Animal Nutrition, Franklinton, LA, USA; particle size 600 μm).

Proximate composition of the diets was determined by the AOAC method (AOAC, 1990): moisture was calculated as the weight loss after subjecting the sample to 105°C in an oven, total lipid content was determined as the weight of oil extracted with the Soxhlet method through organic solvent (hexane), total protein content was determined by the Kjeldahl method, and the ash content was calculated as the weight of ashes after subjecting the sample to 600°C in a muffle furnace. Composition of the moist and dry diets is described in Tables 2 and 3, respectively.

Table 2
Proximate composition (in % dry weight of diet), experimental moist diets used to feed *Pseudoplatystoma punctifer* fingerlings reared at 27.9 ± 0.5°C and total darkness (three replicates per treatment). MCD, moist commercial diet; MCPD, moist commercial peptide enriched diet; BLD, beef liver diet; CDD, commercial dry compound diet. Biochemical composition of prepared diets determined by AOAC (1990) method

Dietary treatments	MCD	MCPD	BLD
Purina® (50% protein)[a]	48	48	0
Beef liver (fresh)	0	0	95
Fish hydrolysate[b]	0	1	0
Cornstarch[c]	0	0	5
Water	50	49	0
Gelatin (neutral)	2	2	0
Analyses of the diets (% DM)			
Proteins	26	32	17
Lipids	3	4	2
Ashes	5	6	1
NFE	10	10	25
Moisture	56	48	55

NFE, Nitrogen free elements.
[a]Purina® (Cargill Incorporated, Lima, Peru).
[b]Peptides Peru S.A. (Lima, Peru).
[c]Duryea® (Unilever, Lima, Peru)

Incidence of cannibalism

Cannibalism in larvae is commonly divided into two types: type I or incomplete, characterized by bites on the fins or body, and type II or complete, when prey is completely ingested (Hecht and Appelbaum, 1988). In the present study, only type II cannibalism was evaluated. This was done every 2 h from 07.30 to 19.30 hours by counting those fish in whose mouths the caudal fin of the cannibalized sibling poked out. Headlamps at the lowest light intensity mode were used during the counting.

Table 3
Proximate composition (% dry weight of diet) of commercial diets

Ingredients[a]	Aquaxcel	Purina
Protein	50	50
Lipids	16	10
Fibres	3	2
Calcium	2	–
Phosphorous	1.2	–
Ash	11	10
Moisture	11	12

[a]Source: Aquaxcel® (Cargill Animal Nutrition, Franklinton, LA, USA) and Purina® (Cargill Incorporated, Lima, Peru).

Statistical analysis

Results were expressed as mean ± standard deviation. An analysis of variance (ANOVA) was performed using the SPSS v.20 software and Tukey's test was used to determine significant differences between means for multiple comparisons. Survival values were transformed by the square root method before analysis. When normality of data was not achieved, the non-parametric Kruskal–Wallis test, followed by the Steel-Dwass-Critchlow-Fligner test for pair comparisons, was performed using XLSTAT. Differences were considered statistically significant at $P < 0.05$.

Results

Total length and wet weight of *P. punctifer* during the *Artemia* feeding phase was monitored at 13 and 18 dpf in order to calculate growth for each culture period. Fish reached a similar average TL in all experimental groups ($P > 0.05$) of 15.1 ± 1.3 mm (13 dpf) and 18.2 ± 1.9 mm (18 dpf), respectively. Similarly, there were no statistical differences in survival among tanks ($P > 0.05$) at 18 dpf, the average being 89.3 ± 3.5%. Survival rate at 32 dpf (Fig. 1a), corresponding to the end of the 20–32 dpf feeding phase, was higher in the BLD group than in the rest of the dietary treatments ($P < 0.05$), being almost two times higher than in the CDD group. Survival of the MCD group was higher than in the CDD group ($P < 0.05$), with the MCPD group presenting intermediate values. At the end of the 33–41 dpf feeding period, the best survival results (Fig. 1a) were obtained in the BLD group followed by the MCD and MCPD groups, which presented similar survival rates ($P > 0.05$), and finally by the CDD group ($P < 0.05$).

TL measurements (Fig. 1b) performed at the end of the 20–32 feeding phase revealed MCD and MCPD as the most efficient diets, TL being significantly higher than in the two other groups ($P < 0.05$). TL in the BLD group was significantly lower than in the CDD group ($P < 0.05$). At the end of the 33–41 dpf feeding period, the MCPD and MCD groups presented

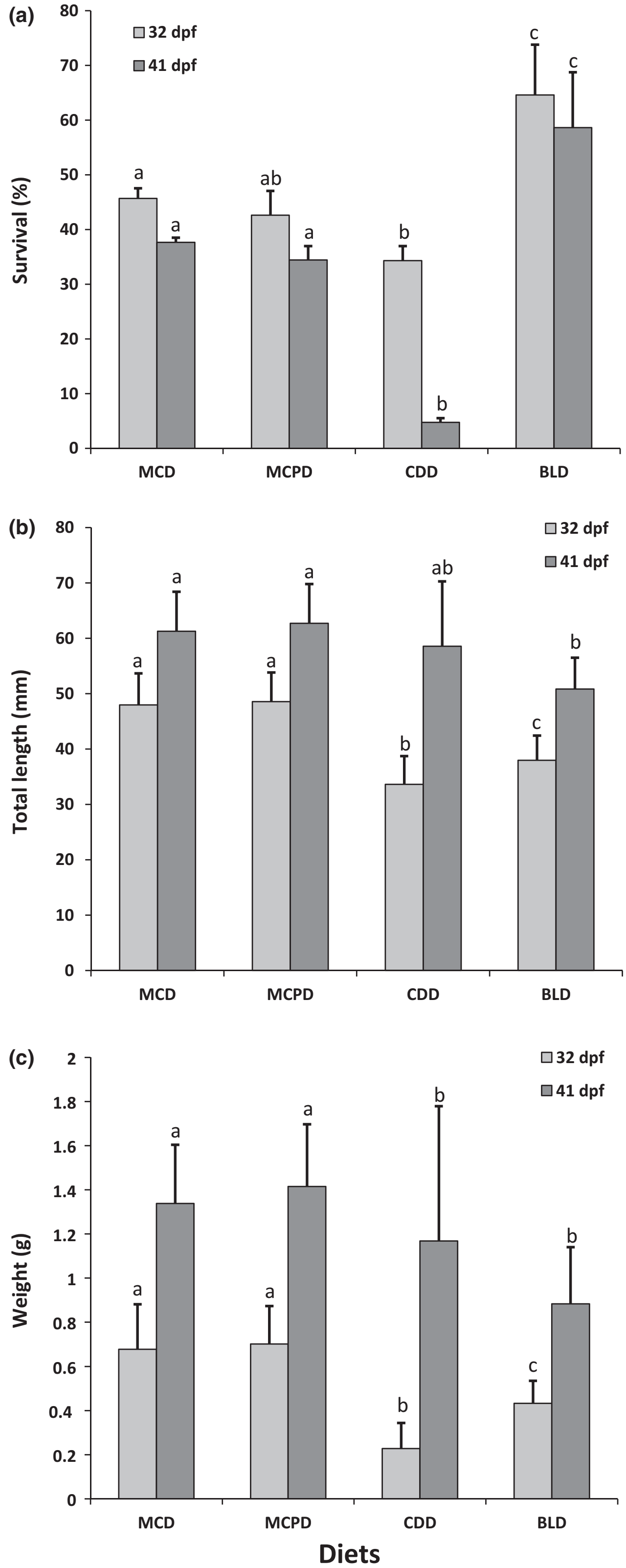

Fig. 1. Rearing of *Pseudoplatystoma punctifer* at 27.9 ± 0.5°C and total darkness under different feeding regimes: MCD, moist commercial diet; MCPD, moist commercial peptide enriched diet; BLD, beef liver diet; CDD, commercial dry compound diet (three replicates per treatment). (a) survival, (b) total length, (c) wet weight at end of feeding periods III and IV (32 and 41 days post fertilization – dpf, initial n = 210 and 90, respectively). Values represent mean and standard deviation (n = 90 for TL, n = 30 for WW per dietary treatment). Different superscript letters = significant differences between dietary treatments for the same age ($P < 0.05$).

Table 4
Type II or complete cannibalism observed 21–29 days post-fertilization during feeding period III. Data is sum of cannibals observed in three replicates per dietary treatment. Percentage of cannibals calculated with respect to number of individuals at beginning of feeding period III (n = 210, three replicates). Values expressed as mean ± SD

Days post-fertilization	21	22	23	24	25	26	27	28	29	Total	%
Diets											
MCD	–	1	–	–	–	–	–	–	–	1	0.2 ± 0.3
MCPD	3	1	–	2	–	1	–	1	–	8	1.3 ± 0.3
BLD	1	–	1	–	1	–	–	–	–	3	0.5 ± 0.8
CDD	2	2	2	1	–	–	–	1	2	10	1.7 ± 0.9

higher TL than the BLD group ($P < 0.05$), whereas the CCD group displayed intermediate values ($P > 0.05$).

Body weight between dietary treatments showed a similar pattern at 32 dpf to that of TL, where fish that consumed the MCPD and MCD diets had the highest wet weights, followed by the BLD and CDD groups ($P < 0.05$). At 41 dpf, fish fed the MCD and MCPD diets showed similar weights (1.35 ± 0.07 g average, $P > 0.05$) but presented higher weights than those fed the CDD and BLD diets ($P < 0.05$). CDD and BLD weight groups were similar (1.00 ± 0.14 g average, $P > 0.05$, Fig. 1c).

Type II cannibalism was observed only during feeding period III (20–32 dpf), being more frequent at the beginning of this period and declining from 25 dpf onwards, especially in the MCD and MCDP groups. The incidence of type II cannibalism was similar in all dietary treatments (Table 4).

Discussion

Weaning of carnivorous fish from live prey to compound food is generally difficult in many species due to their non-acceptance of dry diets. For this reason, it is very common to use palatable (i.e. moist) weaning diets and then gradually replace them with less palatable (i.e. hard) diets (Kubitza and Lovshin, 1999).

In this study, weaning was achieved with all tested diets, but better results, based on wet weight of individuals at the end of the experiment, were obtained with the MCD and MCPD moist diets. However, direct weaning onto the commercial dry diet tested did not completely succeed as proved by the extremely low survival (4.7%). Improvement of fish adaptation to dry diets through a previous feeding period with moist diets has been also demonstrated in *Hoplias lacerdae* (Luz et al., 2002). For the genus *Pseudoplatystoma*, the importance of gradual change from live food to compound feeds using moist diets has been reported in previous studies (Guerrero, 2003; Ayres, 2006; Maia, 2011; Marciales-Caro et al., 2011). However, factors other than moisture could also be influencing growth and survival of *P. punctifer,* such as a higher particle size than required (i.e. with respect to the oesophagus size), abrasive particles associated with the irregular form obtained after sieving, or inadequate nutritional quality and composition. The higher weaning time used for the CDD treatment (two times longer than for the other groups) could also negatively influence weaning, and therefore survival and growth, by at least three possible reasons: (i) longer co-feeding period allowing individuals to choose *Artemia* against the compound diet, then favouring a lower adaptability to compound diets; (ii) weaning age too late, reducing the capacity of an earlier adaptation of individuals to compound diets; and (iii) longer time of *Artemia* supply, which seems not to be nutritionally adequate for *P. punctifer* (Gisbert et al., 2014; Darias et al., 2015).

Best survival was observed in the BLD group. The use of liver and beef heart diets before weaning onto dry diets has shown to improve survival in several *Pseudoplatystoma* species (Guerrero, 2003; Ayres, 2006; Maia, 2011; Marciales-Caro et al., 2011). This could be related to an earlier and better acceptance of these feeds, perhaps due to enhanced chemical stimuli (smell, taste and palatability) (Kolkovski et al., 1997, 2000).

The group weaned directly to the CDD diet showed the lowest results in terms of length and weight at 32 dpf, but at the end of the second feeding period (41 dpf), compensatory growth was observed leading individuals to reach similar growth values than those in the BLD group. However, the low final survival (4.7%) observed in the CDD group suggests that growth recovery was likely due to the feeding on congeners rather than on the dry feed. Although no signs of cannibalism were observed from 29 dpf onwards, the important size heterogeneity observed in this group at the end of the experimental period (almost twice that compared to the other groups) would allow bigger individuals to ingest smaller ones without being noticed using the methodology to detect type II cannibals. An increase in cannibalistic behaviour can be associated with an inadequate diet, as has been observed for other piscivorous fish species including *P. punctifer* (Atencio-García and Zaniboni-Filho, 2006; Gisbert et al., 2014; Darias et al., 2015).

The proximate composition of MCD and MCPD diets differed mainly in the higher protein level in the latter due to the inclusion of fish hydrolysate. The lower growth observed in fish fed the BLD diet might be due to the lower protein level of the BLD diet. The similar growth and survival observed in the MCD and MCPD groups indicated that peptide enrichment did not favor those parameters in early *P. punctifer* juveniles, likely because their digestive systems might already be prepared to digest complex proteins at this developmental stage (Gisbert et al., 2014).

In conclusion, this study showed that among the tested diets, moist feeds seemed better suited for *P. punctifer* during the early stages of development. Factors such as particle size, form and texture, and nutritional composition of the dry diet should be considered in order to improve *P. punctifer* growth and survival and reduce cannibalism.

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