fishes

MDPI

Article

Zootechnical Parameters and Enzyme Activity in the Species *Brycon moorei* (Steindachner 1878)

Carlos David-Ruales [1,*], Débora Machado Fracalossi [2] and Felipe Collazos-Lasso [3]

[1] Animal Sciences Program, Faculty of Agricultural Sciences, Corporación Universitaria Lasallista, Caldas 055448, Colombia
[2] Department of Aquaculture, Federal University of Santa Catarina, Florianópolis 88034-001, SC, Brazil; debora.fracalossi@ufsc.br
[3] De los Llanos Aquaculture Institute (IALL), University de los Llanos, Villavicencio 500001, Colombia; lcollazos@unillanos.edu.co
* Correspondence: cdavid@unilasallista.edu.co

Abstract: *Brycon moorei* is an opportunistic omnivorous species. It is not known what its nutritional requirements are at any stage of its development; this study determined the influence of diet on the zootechnical parameters and enzyme activity of these fish. In a completely randomized quintuplicate design, an ad libitum dietary protocol was applied, which included *Prochilodus magdalenae* larvae, *Artemia salina* and balanced feed with increasing inclusion levels (35 to 55% crude protein (CP)). Larvae 22 h post-hatching (HPH), with an average initial weight and length of 3.07 ± 0.69 mg and 6.069 ± 0.22 mm, respectively, were seeded at a density of 17 larvae L^{-1} in tanks with an effective volume of 60 L. The main zootechnical parameters, water quality and enzyme activity were analyzed with respect to the experimental diets. Significantly, the best diet was that of 45% CP when compared with the other CP levels used. Specific serum enzyme activity was found from hatching, with fluctuating values, the specific pepsin-like activity started at 111 HPH. *B. moorei* larvae can receive a dry diet from 124 HPH and their requirement corresponds to 48.5% CP.

Keywords: dorada of the Magdalena basin; trypsin; chymotrypsin; amylase; pepsin-like; ontogeny

Key Contribution: The article outlines the feeding protocol and the best protein inclusion level to be used in diets for *B. moorei* and, being mainly the activity of the pepsin-like enzyme, it may mark the initiation of the dry diet in the early stages of development of this species.

check for updates

Citation: David-Ruales, C.; Machado Fracalossi, D.; Collazos-Lasso, F. Zootechnical Parameters and Enzyme Activity in the Species *Brycon moorei* (Steindachner 1878). *Fishes* **2023**, *8*, 592. https://doi.org/10.3390/fishes8120592

Academic Editors: Gisele Cristina Favero and Ronald Kennedy Luz

Received: 2 October 2023
Revised: 25 October 2023
Accepted: 3 November 2023
Published: 30 November 2023

1. Introduction

The constant production of quality larvae and juveniles in Neotropical species is a limiting factor for the development of aquaculture programs, as well as for studies related to the actions of preservation and recovery of these species in their environments [1,2]. In fact, the larviculture of Neotropical species is mainly carried out in semi-intensive systems [3] with rather poor results due to the availability of food and predation [4,5]. Similarly, larvae with altricial development [6] and exacerbated cannibalism have difficulty accepting dry diets, as is the case of *Brycon moorei* [7,8], a species endemic to Colombia and currently classified as vulnerable (A2d), due to factors such as the rapid loss of its natural environment [9]. Therefore, it is necessary to find new cultivation strategies and feeding techniques [10,11] and to rely on ontogenetic studies to understand the changes in structures related to feeding and digestive capacities that help to determine the type of nutrients that are best assimilated by the larvae [12,13]. In this sense, the evaluation of the presence and level of digestive enzyme activity can be used as an indicator for several productive parameters in fish larvae [14].

Little is known about the nutritional requirements in fish larvae [15], due to difficulties associated with size, variability, rapid changes, growth rates, among others [16]. Although

there are some reports on enzyme activity in marine [17] and freshwater [2] fish larvae, it has been shown that enzyme activity and its modulation may be related to dietary items [18]. In this regard, there are no known reports on the influence of diets on zootechnical performance and enzyme activity in larvae and juveniles of *B. moorei*. Therefore, the aim of this work was to understand the effect of the feeding protocol, the influence of diets on zootechnical parameters and the activity of some enzymes in this species.

2. Materials and Methods

2.1. Location

The experimental phase was carried out at the Doradal Fish Farming Station (Estación Piscícola Doradal) located at coordinates 5°52′59.5″ N and 74°47′03.6″ W; 165 km from Medellín, in the village of Doradal, in the Municipality of Sonsón, Antioquia, Colombia; at an altitude of 150 m.a.s.l.; average relative humidity of 75% and a temperature between 24 °C and 35 °C.

2.2. Environmental Parameters and Larval Collection

For larval collection and management, protocols were followed as proposed by David-Ruales and colleagues [9–11]. Larvae with an average initial weight and length of 2.97 ± 0.23 mg and 6.19 ± 0.029 mm, respectively, were kept in a recirculation system (RS) with 30 rectangular experimental units (EU) of 65 L effective volume. The RS maintained environmental parameters within comfort ranges for the species (Table 1); water quality monitoring was performed daily at 7:00 am, with a Hachh FF2 Kit and a YSI multiparameter probe (Professional plus). The inlet flow rate of each tank was controlled by flowmeters (Parker MR) and maintained at 3.5 L min^{-1}.

Table 1. Environmental parameters in the RS. DO: dissolved oxygen; Alk/Hard: alkalinity and hardness; T: temperature; NH3: non-ionized ammonia.

Parameter	Comfort Index
DO (mg L^{-1})	5.5 ± 0.5
pH	6.5 ± 0.8
T (°C)	26 ± 0.4
Alk/Hard (mg L^{-1} $CaCO_3$)	20.8 ± 1.7
NH_3 (mg L^{-1})	0.002 ± 0.00001

2.3. Feeding Protocol Design

The feeding protocol for *B. moorei* was carried out for a period of 15 days in the RS, using 1 day post-hatching (DPH) larvae; the fish were fed 5 times a day as follows: during the first 3 days with recently hatched *Prochilodus magdalenae* forage larvae (FL), from day 4 *Artemia salina* nauplii (A) were used and, from day 6, a dry diet (C) was offered with increasing inclusions of crude protein (CP) (35, 40, 45, 45, 50 and 55%); completing the transition to C from the thirteenth day onwards. All food items were offered ad libitum for a period of 5 min per EU; Table 2 shows the modified strategy of David and Castañeda [11]. Tanks were cleaned depending on the accumulation of residues or variations in water quality factors, mainly nitrogenous; each diet trial was performed in quintuplicate in order to sustain the number of larvae per number of samples for each treatment.

Table 3 displays the ingredients and the centesimal composition for each crude protein level; the diets were formulated based on the work of de Lazo (2000) [19] with a (CP) energy ratio of approximately 1:10. The product was developed in the Nutrition Laboratory of the Aquaculture Institute of the Universidad de los Llanos. Proximate and energy analyses were carried out in the Nutrition Laboratory of the Corporación Universitaria Lasallista, under the guidelines of the *"Association of Official Agricultural Chemists"* [20].

Table 2. Feeding protocol for *B. moorei*. DPH: (days post hatching); FL (forage larvae); A (*Artemia salina* nauplii) and Dd (dry diet).

DPH	Feeding Timing (Hours)				
	7	**9**	**11**	**13**	**15**
1	FL	FL	FL	FL	FL
2	FL	FL	FL	FL	FL
3	FL	FL	FL	FL	FL
4	A	A	A	A	A
5	A	A	A	A	A
6	A	A	A + Dd	A	A
7	A	A	A + Dd	A	A
8	A	A	A + Dd	A	A
9	A + Dd	A	A	Dd	A + Dd
10	A + Dd	Dd	Dd	A + Dd	Dd
11	Dd	Dd	A + Dd	Dd	Dd
12	A + Dd	Dd	Dd	Dd	Dd
13	Dd	Dd	Dd	Dd	Dd
14	Dd	Dd	Dd	Dd	Dd
15	Dd	Dd	Dd	Dd	Dd

Table 3. Dietary composition and proximate analysis of the experimental diets used in the experiment.

Ingredient	Protein Level%				
	55	**50**	**45**	**40**	**35**
Fish meal [a]	65.0	56.4	49.0	41.0	31.0
Earthworm meal [b]	11.7	11.7	11.7	11.7	12.7
Whole egg flour [c]	4.3	4.3	4.3	3.0	4.3
Pregelatinized starch [d]	0.3	8.9	20.8	34.3	37.0
Alpha-cellulose [e]	0.0	0.0	0.0	0.0	5.8
Fish oil [f]	6.5	6.5	3.0	0.5	0.1
Canola oil [g]	3.2	3.2	2.2	0.5	0.1
Soy lecithin [h]	1.1	1.1	1.1	1.1	1.1
Choline chloride [e]	3.2	3.2	3.2	3.2	3.2
Dicalcium phosphate [e]	0.5	0.5	0.5	0.5	0.5
Premiz Vit/Min [i]	4.1	4.1	4.1	4.1	4.1
Vit C [j]	0.2	0.2	0.2	0.2	0.2
Proximate analysis (dry basis)					
Dry matter	92.5	91.6	92.8	91.8	92.5
Crude protein	54.4	48.9	44.3	38.7	34.5
Crude fat	18.3	17.3	16.3	14.7	12.1
Ashes	7.6	7.5	7.1	7.0	6.9
Free nitrogen	11.1	10.1	9.1	7.9	5.5
Crude fiber	1.1	7.8	16.0	23.5	33.5
Energy kcal/kg	5022.0	4904.0	4546.0	4180.0	3805.0

[a] Fish meal (Agromat, Medellín, Colombia). Protein: 71.73%, fat: 11.09%, ashes: 16.74%. [b] Worms (Visalia, CA, USA-GMP certificated). Protein: 50 a 55%, fat: 6.56%, fiber: 3.3%, ashes: 7.59%, nitrogen-free extract: 17.6%, Ca: 0.5%, P: 0.9%, gross energy 4060 kcal Kg^{-1}. [c] ALSEC (La Estrella, Antioquia, Colombia). Protein 50%, fat 38%, ashes 5%. [d] Starch 1500. Protein 9.7% (Colorcon®, Bogotá, Colombia). [e] Sigma-Aldrich (St. Louis, MO, USA). [f] Scott's Emulsion® (GlaxoSmithKline, Medellín, Colombia). [g] Aceite Gourmet® Balance. (Team Foods, Bogotá, Colombia). [h] Team® Colombia (Bogotá, Colombia). [i] Rovimix vitamin (Lab. Roche S.A, Bogotá, Colombia): Vit. A 8 × 106 UI; Vit. D3 1.8 × 106 UI, Vit. E 66.66 g, Vit. B1 6.66 g, Vit. B213.33 g, Vit. B6 6.66 g, Pantothenic acid 33.33 g, Biotin 533.3 mg, Folic acid 2.66 g, Ascorbic acid 400.0 g, Nicotinic acid 100.0 g, Vit. B12 20.0 mg, Vit. K3 6.66 g, Mg 91.66, Zn 21.66, Fe 28.33 g, Cu 2.5 g, I 0.17 g, Selenium 66.66 g, Mn 2.5 g, Inositol F.G. 58.33 g, LuctanoxE 25 g; [j] Stay-C® DSM Nutritional Products, Bogotá, Colombia S.A.

2.4. Zootechnical Parameters

For the growth analyses, 10 larvae were randomly sampled (wet weight) before offering the first daily feed ration [21,22]. For each sample the following were recorded:

weight (mg), length (mm), weight gain (WG-mg); length gain (LG-mm), daily weight gain (DWG-mg), feed conversion (FC), specific growth rate (SGR-%), condition factor (K), survival (S%) and consumption (C mg/day/larva). The records and calculations of the zootechnical parameters were based on the work of several authors [23–25].

$$\text{WG, Weight gain} = \text{final weight (mg)} - \text{initial weight (mg)};$$

$$\text{DWG, Daily Weight Gain} = \text{Weight gain/T};$$

$$\text{LG, Length gain} = \text{final length (mm)} - \text{initial length (mm)};$$

$$\text{FCR, Feed Conversion Ratio} = \text{Total feed intake (mg)/Weight gain (mg)};$$

$$\text{SGR, Specific growth rate (\%/d)} = 100 \times (\text{lnBW2} - \text{lnBW1})/(\text{T2} - \text{T1});$$

$$\text{Survival Rate (\%)} = 100 \times \text{Nf/Ni};$$

$$\text{CF, Condition Factor (\%)} = (\text{Weight/Length\^3}) \times 100;$$

$$\text{I, Intake} = \text{feed intake (mg)/day/larva},$$

where BW1 and BW2 represent the initial and final body weights of each fish (in mg), T1 and T2 represent the times corresponding to those weights and Ni and Nf represent the initial and final numbers of fish, respectively.

2.5. Enzyme Extraction and Total Soluble Protein Dosing

For the analyses of each experiment, 1 g of larvae and/or juveniles (wet weight) was collected before offering the first daily feed ration. At time 0, and until the fifth day post-hatching, the complete larvae were arranged in perforated plastic conical cylinder tubes, assigned a code and immediately placed in liquid nitrogen, where they were preserved until the enzymatic assays. After the fifth day, samples were collected on a daily basis [26]. Larvae and tissues were homogenized in ultrapure cold water (1:10 weight/volume with Ultra-Turrax IKT25, AMEX® homogenizer); total soluble protein was obtained according to the protocol of Lowry and colleagues (1951) [27].

2.6. Enzyme Activity Assays

To measure trypsin activity, Na-Benzoyl-dl-arginine-4-p-nitroanilide (BAPNA) (Sigma-Aldrich St. Louis, MO, USA) was used as a substrate in a final concentration of 1 mM in 50 mM TRIS-HCL buffer, pH 8.2, following the protocol of Erlanger and colleagues [28]. To measure chymotrypsin, the substrate N-Succinyl-Ala-Ala-Pro-Phe-p-nitroanilide (SAPNA) (Sigma-Aldrich St. Louis, MO, USA) was used, at a final concentration of 1 nM, in 100 mM phosphate buffer, pH 7.8, according to the protocol of DelMar and colleagues [29]. For both enzymes, the reading was carried out at an absorbance of 410 nm. A unit of enzyme activity (U) was defined as the amount of enzyme catalyzing 1 µmol of substrate min^{-1} [29].

To determine amylase activity, the substrate used was 2% (m/v) starch in 100 mM acetate buffer, pH 7.5, following the protocol of Noelting and colleagues [30]. One unit of amylase activity (U) was defined as the amount of 1 µmol of glucose formed per min^{-1} determined from the standard glucose curve.

Pepsin-like activity, with 2% (*w*/*v*) bovine hemoglobin as a substrate, at a final concentration of citrate-phosphate substrate-buffer 100 mM, pH 3.0, was determined according to the protocol of Anson [31]. An enzyme unit (U) was defined as the amount of enzyme responsible for altering absorbance by 0.001 Optical Density Units (ODU/min). Readings were carried out in a microplate spectrophotometer (TECAN-Infinite pro, San Diego, CA, USA); all enzyme assays were performed in the Biochemistry laboratory of the Federal University of Santa Catarina (UFSC), Brazil.

2.7. Statistical Analysis

A completely randomized experimental design was applied, with 5 treatments and 6 replicates per treatment. To determine the optimal protein level, the polynomial regression model was used, reflected in the equation $Y = B_0 + B_1 t + B_2 t^2 + \mathcal{E}$, where: Y is the answer, B_0 is the model intercept, B_1 the linear regression coefficient associated with the treatment t, B_2 corresponds to the quadratic regression coefficient associated with the treatment t^2 and $\mathcal{E}$ to the experimental error [32]. The data of the treatments in terms of their zootechnical variables were tested for homoscedasticity by Levene's test, and normality by the Shapiro–Wilk test, then analyzed by one-way ANOVA, followed by Tukey's test. If normality was not found in the data, significant differences were determined using the nonparametric Kruskal–Wallis and Mann–Whirney U tests. Percentage data were transformed by Arc-Sin. The 5% significance level was used for all tests ($p < 0.05$). The software used was Statgraphics Centurion V7, licensed to the Corporación Universitaria Lasallista.

3. Results

The feeding protocol (P) led to satisfactory results for the management of the species, with a weight gain of 3258.7% and a length gain of 328% in 15 days. Table 4 shows the zootechnical variables that were analyzed in relation to the level of protein inclusion.

Table 4. Effect of protein levels on zootechnical parameters of the feeding protocol (n = 105 per CP level), different letters (a–c) indicate significant differences ($p < 0.05$).

Variables	Protein Levels (%)									
	35		40		45		50		55	
WG (mg)	62.8 ± 31.6	bc	61.5 ± 20.4	c	176.2 ± 61.3	a	69.3 ± 27.7	bc	114.0 ± 50.0	bc
DWG (mg)	4.1 ± 2.1	bc	4.09 ± 1.3	c	11.7 ± 4.1	a	4.6 ± 1.8	bc	7.6 ± 3.3	bc
LG (mm)	0.4 ± 0.2	b	0.5 ± 0.2	b	0.7 ± 0.2	a	0.5 ± 0.1	ab	0.3 ± 0.1	b
DLG (mm)	0.03 ± 0.01	b	0.03 ± 0.01	b	0.05 ± 0.01	a	0.03 ± 0.01	ab	0.02 ± 0.01	b
FC	0.9 ± 0.06	b	0.9 ± 0.04	b	0.6 ± 0.03	a	1.2 ± 0.06	c	0.5 ± 0.07	a
C (mg/day/larva)	5.6 ± 0.4	c	5.4 ± 0.2	c	10.2 ± 0.5	a	8.3 ± 0.4	bc	5.7 ± 1.6	c
SGR (%)	7.4 ± 3.2	b	6.6 ± 2.3	b	13.0 ± 1.7	a	7.4 ± 1.9	b	9.8 ± 3.9	ab
K	1.08 ± 0.2		1.09 ± 0.4		1.1 ± 0.06		1.1 ± 0.23		1.1 ± 0.2	
S%	43.7		42.8		45.6		39.9		43.5	

Comparing the different diets, for most parameters, the 45% CP diet was significantly the most effective; there were no significant differences in any of the diets for K and survival; thus the DWG was 1.54 times higher than the 55% diet and 2.87 times higher than the 40% one. The best feed conversion was found in the 55% diet, with a value of 0.5 ± 0.07; there was no significant difference with the 45% CP diet (0.6 ± 0.03), but there were significant differences with the 35%, 40% and 50% CP diets.

Protein requirements in the early development of *B. moorei* were estimated at a value of approximately 48.5% CP; the polynomial model presented a coefficient of determination of 75%. Figure 1 shows the results.

The lowest value for all diets was found at 71 HPH, when the item offered was forage larvae (FL) (0.025 ± 0.0038 mU mg of protein^{-1}). The specific activity for chymotrypsin was also present from hatching (12.1923 ± 1.66 mU mg of protein^{-1}). The maximum value found was 12.23 ± 0.41 mU mg of protein^{-1} at 111 HPH, when larvae were fed with *Artemia salina* (A). The lowest value was reached at 243 HPH (0.6264 ± 0.03 mU mg of protein^{-1}) and corresponds to the time of feeding with Dd for the 40% CP diet. The ratio between trypsin and chymotrypsin (T:C) decreased from 0.96 to 0.02 when the dietary item was changed from FL to A, that is, between 0 and 123 HPH, maintaining low values until the end of the experiment, with the lowest value for the 50% CP diet being 0.029—T:C and the highest value for the 45% CP diet, being 0.061—T:C, without any clear trend. Figure 2 shows the T:C ratio with respect to the HPH and the feeding protocol.

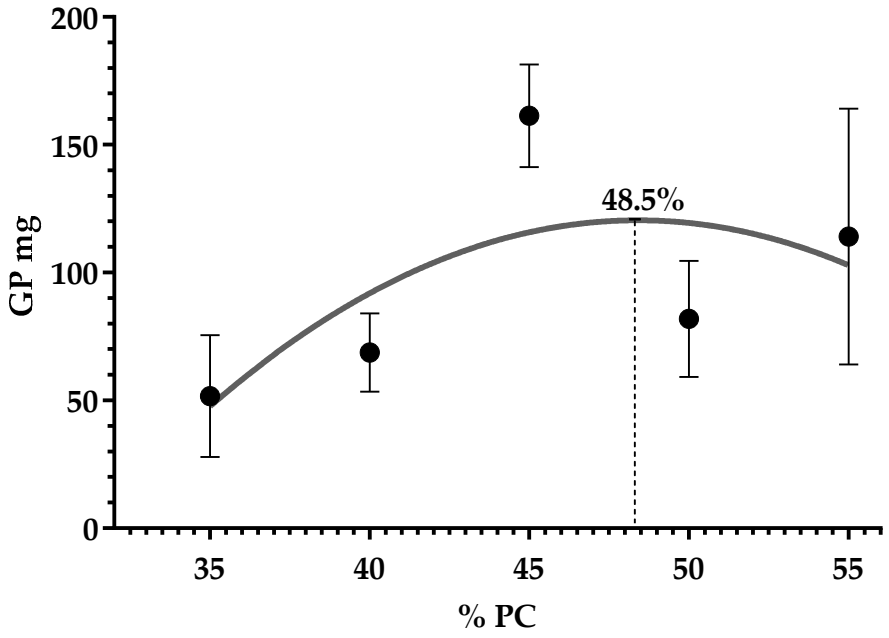

Figure 1. Mean comparison test using the polynomial regression model for the different CP levels (35 to 55% CP). Asterisk denotes a coefficient of determination of 75%.

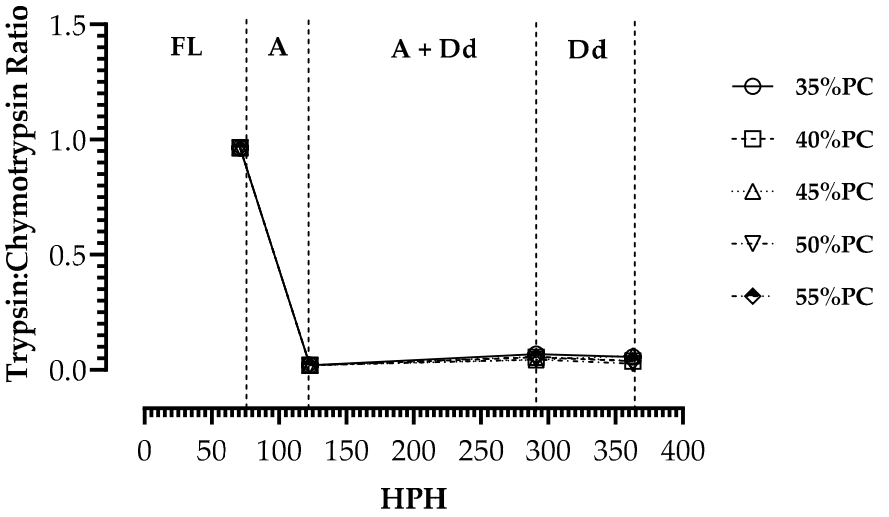

Figure 2. Trypsin:chymotrypsin ratio (T:C) in relation to the inclusion levels in the experimental diets (35 to 55% CP), the hours post-hatching (HPH) and the food item: forage larva (FL), *Artemia salina* nauplii (A); A + dry diet (Dd) and dry diet (Dd).

As for specific amylase activity, this was also present from the time of hatching (0.1895 ± 0.01 mU mg of protein^{-1}). The highest values are observed at the time of transition to the dry diet (A + Dd) (2.59 ± 0.09 mU mg of protein^{-1}), specifically, at 195 HPH for all diets and then at 219 HPH, only when Dd (2.09 ± 0.16 mU mg of protein^{-1}) is offered, for the 35% CP diet. The lowest values are observed for the 55% CP diet (0.0 mU mg of protein^{-1}) at 219 and 267 HPH, respectively. Figure 3 shows the variations in enzymatic activity of these three enzymes.

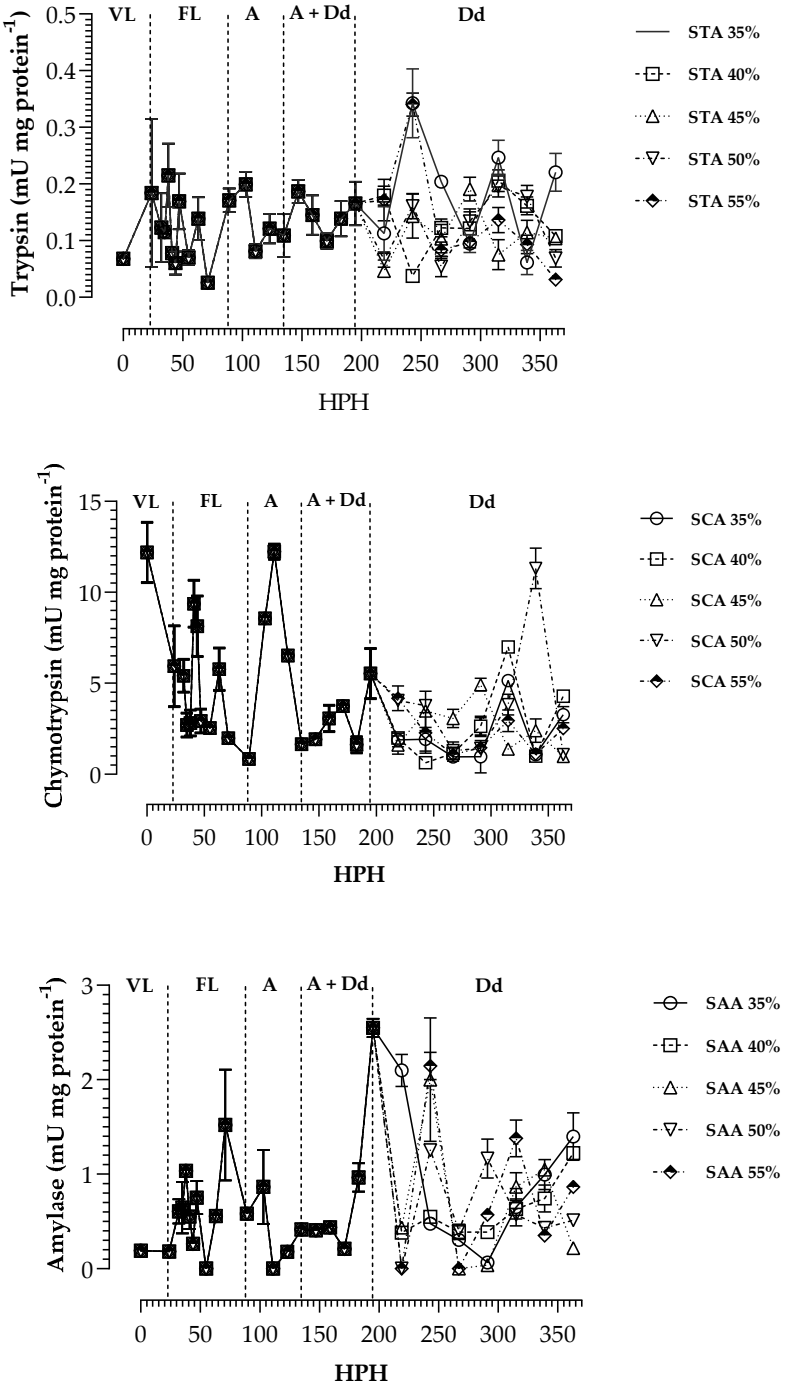

Figure 3. Specific trypsin, chymotrypsin and amylase activity (mU mg of protein^{-1}) in relation to the inclusion levels in the experimental diets (35 to 55% CP), the hours post-hatching (HPH) and the food item: vitelline larva (VL), forage larva (FL), *Artemia salina* nauplii (A); A + dry diet (Dd) and dry diet (Dd). STA: specific trypsin activity. SCA: specific chymotrypsin activity. SAA: specific amylase activity.

As for the specific pepsin-like activity, it can be seen that it was not initiated from the moment of hatching. Its activity starts to develop at 111 HPH, with A as the food item and with the lowest value recorded (0.00267 ± 0.0002 mU mg of protein^{-1}). This increases slightly up to 195 HPH (0.03798 ± 0.0007 mU mg of protein^{-1}) across the different diets. When the transition is made to a dry diet (*A* + DD) from 219 HPH, there is a strong tendency for an increase in specific activity, with the highest values at 243 HPH for the 50%

(0.9998 ± 0.17 mU mg of protein^{-1}) and 55% CP (0.9471 ± 0.03 mU mg of protein^{-1}) diets (Figure 4).

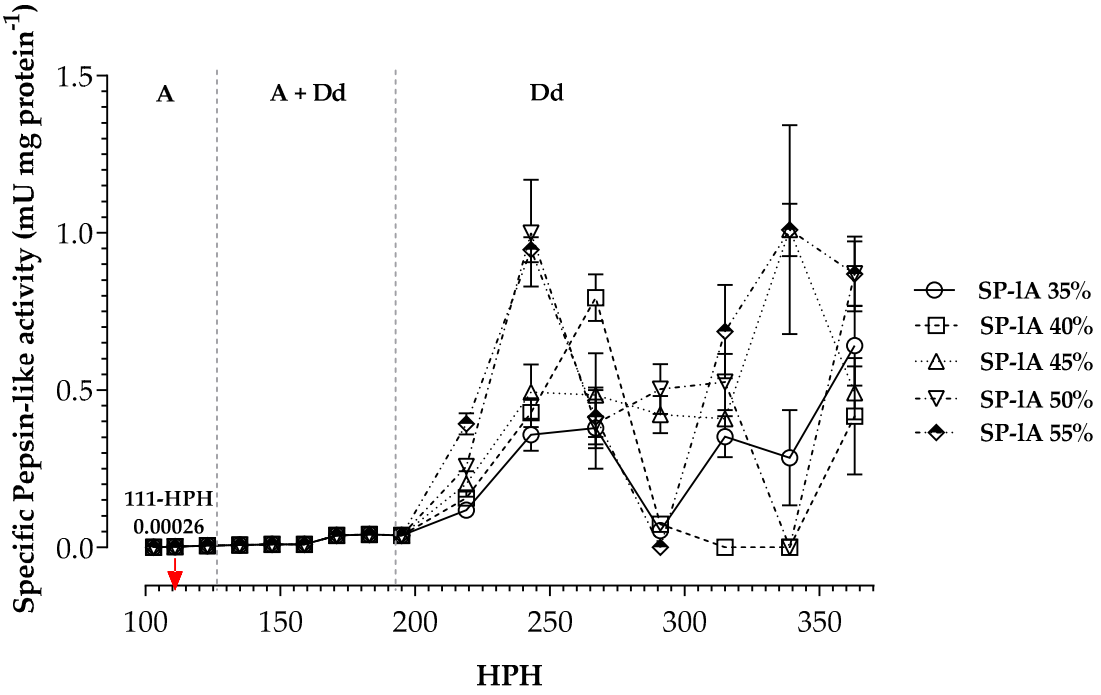

Figure 4. Specific pepsin-like activity (mU mg of protein^{-1}) in relation to CP inclusion levels in the experimental diets, the number of hours post-hatching and the food item: Artemia salina nauplii (A); A + dry diet (Dd) and dry diet (Dd); 111/HPE time at which pepsin-like activity becomes apparent.

4. Discussion

The larval state is the stage with the biggest transformations in the lives of fish [33]; these changes can be modulated in nature by environmental influences and ethological factors [34,35], but in closed systems, these factors can be controlled through feeding protocols that respond to the needs of the species; in this case, *P. magdalenae* larvae were used for several reasons. First of all, it is a species that cohabits with *B. moorei,* it belongs to a low trophic level (detritivorous) and in terms of association by fishery, it represents over 80% of the catches in the Magdalena river basin, forming the food base of species with superior habits [36]. The second reason is based on the success reported by several authors of the use of *P. magdalenae* larvae in first feeding protocols for fish of the genus *Brycon* [11,37,38]. The third reason can be found in the allometric growth ratios with respect to the maximum mouth opening for *B. moorei* [13], which imply for the species a differentiation in the size of the prey from larger to smaller proportions with respect to its development. The successful use of *Artemia salina* nauplii has been extensively tested as a first feeding strategy in freshwater [39] fish larvae, including *B. moorei* [7,11,40]. Finally, the design of dry diets must be in accordance with the capability of the larvae to assimilate them and with the quality of the ingredients in meeting the requirements of the species; it is understood that, in early stages of development, these needs are high [41]. In the present study, all diets were formulated with high quality ingredients in order to meet the needs of the species, relating their use to the time of development of the larvae's digestive system.

For *B. moorei*, in the present study, better survival and weight gain values were found (43% and 221.44 ± 64.56 mg, respectively), compared to those reported for the same species [11]; these differences can be explained by the variations in the feeding protocol. Although the survival value may be low compared to other Neotropical species, it is known that the family Bryconidae, and specifically *B. moorei*, shows the highest level of cannibalism recorded for freshwater species [7,8,38,42]. Therefore, it is important to establish a feeding

protocol that is in line with the nature of the species; in this regard, in *B. sinuensis* [37] and *Brycon siebenthalae* [43], these findings are also reported.

However, the production system can also affect these results. In general, larviculture of Neotropical species is mainly carried out in semi-intensive systems [3], but with low survival rates due to food availability and predation [4,5]. Nevertheless, recently, the focus has shifted to studies on recirculation for Neotropical freshwater species [26,44–47]. In this respect, the economic viability of intensive systems has been demonstrated for the species *Piaractus mesopotamicus* [4,48] and for *B. moorei* [11]. It has been shown that co-feeding strategies and gradual transition to a dry diet leads to better results than traditional production systems [49–54], and it is recognized that substituting live feed with commercial rations before day six generally leads to lower survival rates [52]. This study reports results consistent with those for species with indirect development.

Given that protein is the main nutrient for development in fish [12,55] and that determining amino acid requirements in larvae is complex [56], for better results, it is recommended that nutritional, physiological and management strategies be combined [15,55,57]. For example, in *Piaractus mesopotamicus* larvae, positive effects were found by managing a 12-day transition time to a dry diet [48], with a CP level of 38%, fed to larvae over 10 mg [58]. The results of the present study indicate a transition from day 6, with full Dd from day 13, reflecting positive effects with a 45% CP diet.

The main proteases responsible for protein hydrolysis in the intestine of fish are trypsin and chymotrypsin. Although their importance is recognized, there is still uncertainty regarding the specific contribution of each enzyme to the process of protein metabolism [59]; these enzymes, including amylase, may be present in the early stages of ontogenetic development, as has been described in several species [26,45,60]. In *B. moorei* these enzymes are present from hatching; the same behavior was observed in larvae of the species *Centropomus undecimalis* [61], *Centropomus nigrescens* [62], *Centropomus viridis* [63], *Pseudoplatystoma punctifer* [46] and *Pseudoplatystoma fasciatum* [64]. Each case, including the species being studied, showed peaks of activity which are specific to each species and may be the result of predetermined genetic factors [65], specific substrate requirements or variations in diet [66,67], feeding protocol [68], as well as physical factors such as temperature and species-specific differences, among others [18,69–75]. Fish are known to exhibit plasticity in the production of digestive enzymes in response to the type of diet [72,73], therefore, larvae could modulate enzyme production, reducing metabolic expenditure [16]. Furthermore, in larvae that do not have a stomach at the beginning of their ontogeny, the enzymes trypsin and chymotrypsin [14,66,73] are responsible for protein hydrolysis. Other authors state that these enzymes play an important role in the process of chorion rupture at hatching and in the cleavage of yolk proteins [74,75] or, in the case of amylase, it may be related to the presence of glycogen reserves in the yolk sac [76], which could explain the findings of this study. In the present study, no trends between variations in serum enzyme activity and protein inclusion levels were found in this regard; the contribution of each enzyme to protein hydrolysis in the fish intestine is still unclear and subject to debate [59,77]. However, with respect to the T:C ratio, a strong tendency to decrease was observed relative to the feeding protocol, specifically when switching from FL to A; it has been determined for other species that, when this ratio drops, it coincides with low growth rates [78,79].

This study showed a gradual increase in pepsin-like specific activity after five DPH and, with large variations in relation to protein inclusion from day eight onwards, indicating the presence of gastric glands and therefore a functional stomach; a morphological feature that could suggest that larvae of *B. moorei* are able to digest the formulated diet. Similar results have been found in other freshwater species with incomplete ontogenetic development, but with large differences in the time of gastric gland development [22,80–84]; in *B. moorei* the glandular stomach appears at 122 HPH [85] and, in this regard, several features associated with the maturity of this organ are considered. Some authors consider that certain species can develop a stomach early, as is the case in the species *Solea senegalensis*, without implying functionality [86]; in the species *Paralichthys olivaceus*, a storage function is suggested

first, prior to acid proteolytic activity [87]. Other authors point out that, although the gastric glands may indicate acid digestion, it may take some time before they are actually functional [88]. It is therefore suggested that a developed stomach implies functional gastric glands, in accordance with the increase of pepsin-like enzyme activity, which is when the transition to formulated diets can be initiated.

5. Conclusions

In *B. moorei*, a gradual transition to a dry diet is essential. Therefore, applying the feeding protocol as described above improves the results in terms of zootechnical parameters. The serum enzyme activity started at hatching and no relationship was found between the peaks of enzyme activity with respect to the inclusion of the protein level in the diet. However, a tendency to decrease was observed in the T:C ratio with respect to the feeding protocol. Also, the development and maturity of the digestive system must be taken into account, which, together with the onset of acid digestion, allows the larvae of this species to be fed from 123 HPH onwards, with a minimum of CP of 45% and a maximum of 50%, with larvae having a minimum total length of 11.38 mm and a minimum weight of 15.28 mg.

Author Contributions: Methodology, C.D.-R., D.M.F. and F.C.-L.; formal analysis, C.D.-R., D.M.F. and F.C.-L.; investigation, C.D.-R.; resources, C.D.-R. and D.M.F.; writing—original draft preparation, C.D.-R. and D.M.F.; writing—review and editing, C.D.-R., D.M.F. and F.C.-L.; funding acquisition, C.D.-R. and D.M.F. All authors have read and agreed to the published version of the manuscript.

Funding: This research was supported by the Research Center of the Universidad de los Llanos, as part of the doctoral training project entitled: Early development of the species *Brycon moorei*—Steindachner, 1878 (Dorada del Magdalena): embryonic, morphometric, histological and histochemical aspects, productive parameters and digestive enzyme activity in relation to diet, through rector resolution No.0005 de 2017.

Institutional Review Board Statement: The maintenance, handling and experiments conducted on fish during this study were carried out in strict accordance with the guidelines of the Experimental Animal Welfare Ethics Committee of the Universidad de los Llanos (Acta n° 04 legal document of 2 October 2017).

Data Availability Statement: The data presented in this study are available on demand from the first author at (cdavid@unilasallista.edu.co).

Acknowledgments: The authors express their gratitude to all the people who supported each stage of this study and to the institutions that participated by offering their installed capacity, to the Biochemistry and Fish Nutrition Laboratories of the Federal University of Santa Catarina, the Aquaculture Institute of the Universidad de los Llanos and the Corporación Universitaria Lasallista. We also thank the editors and reviewers of this paper for their work.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Atencio-García, V.; Zaniboni-Filho, E. El canibalismo en la larvicultura de peces. *Rev. MVZ Córdoba* **2006**, *11*, 9–19. [CrossRef]
2. David-Ruales, C.A.; Fracalossi, D.; Vásquez-Torres, W. Desarrollo temprano en larvas de peces, clave para el inicio de la alimentación exógena. *Rev. Lasallista Investig.* **2018**, *15*, 180–194. [CrossRef]
3. Mai, M.G.; Zaniboni-Filho, E. Efeito da idade de estocagem em tanques externos no desempenho da larvicultura do dourado *Salminus brasiliensis*. *Acta Sci.* **2005**, *27*, 287–296.
4. Jomori, R.K.; Carneiro, D.J.; Malheiros, E.B.; Portella, M.C. Growth and survival of pacu *Piaractus mesopotamicus* (Holmberg, 1887) juveniles reared in ponds or at different initial larviculture periods indoors. *Aquaculture* **2003**, *221*, 277–287. [CrossRef]
5. Senhorini, J.A.; Gaspar, L.A.; Fransozo, A. Crescimento, sobrevivência e preferência alimentar de larvas de matrinxã (*Brycon cephalus*) e de piracanjuba (*Brycon orbignianus*) em viveiros. *Bol. Téc. CEPTA Pirassununga* **2002**, *15*, 9–21.
6. Balon, E.K. Saltatory Processes and Altricial to Precocial Forms in the Ontogeny of Fishes. *Am. Zool.* **1981**, *21*, 573–596. [CrossRef]
7. Baras, E.; Ndao, M.; Maxi, M.Y.J.; Jeandrain, D.; Thomé, J.P.; Vandewalle, P.; Mélard, C. Sibling cannibalism in dorada under experimental conditions. I. Ontogeny, dynamics, bioenergetics of cannibalism and prey size selectivity. *J. Fish. Biol.* **2000**, *57*, 1001–1020. [CrossRef]
8. Baras, E.; Maxi, M.Y.J.; Ndao, M.; Mélard, C. Sibling cannibalism in dorada under experimental conditions. II. Effect of initial size heterogeneity, diet and light regime on early cannibalism. *J. Fish. Biol.* **2000**, *57*, 1021–1036. [CrossRef]

9. David-Ruales, C.A.; Castañeda-Álvarez, G.D. *Manual de Producción de Mueluda o Dorada el Magdalena (Brycon moorei)*, 1st ed.; Editorial Lasallista: Caldas, Colombia, 2018.
10. David-Ruales, C.A.; Castañeda-Álvarez, G.D. *Larvicultura de Peces Comerciales en Sistemas de Recirculación*, 1st ed.; En: Perspectivas y Avances en Investigación; Editorial Artes y Letras SAS: Itagüí, Colombia, 2010; pp. 199–215.
11. David-Ruales, C.A.; Castañeda-Álvarez, G.D. Recirculation system for larviculture stage of *Brycon moorei* Evaluation of a recirculation system for Dorada (*Brycon moorei*-Steindachner 1878) larvae in the Magdalena River. *Rev. CES Med. Vet. Zootec.* **2014**, *9*, 179–189.
12. Rønnestad, I.; Yúfera, M.; Ueberschär, B.; Ribeiro, L.; Sæle, Ø.; Boglione, C. Feeding behaviour and digestive physiology in larval fish: Current knowledge, and gaps and bottlenecks in research. *Rev. Aquac.* **2013**, *5*, S59–S98. [CrossRef]
13. David-Ruales, C.A.; Machado-Fracalossi, D.; Betancur-Gonzalez, E.M.; Rodríguez-Franco, N.; Castañeda-Álvarez, G.D.; Florez-Restrepo, C.; Vásquez-Torres, W. Relaciones alométricas en estadios tempranos de la especie *Brycon moorei* Steindachner (Characidae), en condiciones controladas. *Actual. Biol.* **2020**, *42*, 1–21. [CrossRef]
14. Zambonino-Infante, J.L.; Gisbert, E.; Sarasquete, C.; Navarro, I.; Gutierrez, J.; Cahu, C.L. Ontogeny and Physiology of the digestive system of marine fish larvae. In *Feeding and Digestive Functions in Fishes*; Cyrino, J.E.P., Bureau, D.P., Kapoor, B.G., Eds.; Science Publishers: Enfield, NH, USA, 2008; pp. 281–348.
15. Portella, M.C.; Leitão, N.J.; Takata, R.; Lopes, T.S. Alimentação e nutrição de larvas. In *NUTRIAQUA—Nutrição e Alimentação de Espécies de Interesse para a Aquicultura Brasileira*, 1st ed.; Fracalossi, D.M., Cyrino, J.E.P., Eds.; Sociedade Brasileira de Aquicultura e Biologia Aquática: Florianópolis, Brazil, 2012; pp. 185–216.
16. Gisbert, E.; Morais, S.; Moyano, F. Feeding and Digestion. In *Larval Fish Aquaculture*; Qin, J.G., Ed.; Nova Science Publishers, Inc.: New York, NY, USA, 2013; pp. 73–124.
17. Kolkovski, S.; Curnow, J.; King, J. Intensive rearing system for fish larvae research: I. Marine fish larval rearing system. *Aquac. Eng.* **2004**, *31*, 295–308. [CrossRef]
18. German, D.P.; Horn, M.H.; Gawlicka, A. Digestive Enzyme Activities in Herbivorous and Carnivorous Prickleback Fishes (Teleostei: Stichaeidae): Ontogenetic, Dietary, and Phylogenetic Effects. *Physiol. Biochem. Zool.* **2004**, *77*, 789–804. [CrossRef] [PubMed]
19. Lazo, J.P. Conocimiento actual y nuevas perspectivas en el desarrollo de dietas para larvas de peces marinos. In Proceedings of the Nutrición Acuícola V Memorias del V Simposium Internacional de Nutrición Acuícola, Mérida, Mexico, 19–22 November 2000; pp. 300–312.
20. AOAC Int. *Official Methods of Analysis of AOAC International*, 20th ed.; AOAC International: Gaithersburg, MD, USA, 2016.
21. Faustino, F. Desenvolvimento Embrionário e Larval de *Brycon gouldingi* (Teleostei, Characidae). Master's Thesis, Universidad Estadual Paulista, Bauru, Brazil, 2010.
22. Neumann, E. Desenvolvimento Inicial de Jatuarana, *Brycon amazonicus* (Teleostei, Characidae). Ph.D. Thesis, Centro de Aqüicultura da UNESP, Universidade Estadual Paulista, Bauru, Brazil, 2008.
23. Fracalossi-Machado, D.; Rodrigues-Oeda, A.P.; ESilva-De Castro, T.; Cyrino-Possebon, J.E. Técnicas experimentais em Nutrição de peixes. In *NUTRIAQUA—Nutrição e Alimentação de Espécies de Interesse para a Aquicultura Brasileira*; Fracalossi-Machado, D., Cyrino-Possebon, J.E., Eds.; Sociedade Brasileira de Aquicultura e Biologia Aquática: Florianópolis, Brazil, 2012; pp. 37–63.
24. Ricker, W.E. Growth rates and models. In *Bioenergetics and Growth*; Fish Physiology Book Series; Hoar, W., Randall, D., Brett, J., Eds.; Academy Press: New York, NY, USA, 1979; pp. 678–685.
25. Le Cren, E.D. The Length-Weight Relationship and Seasonal Cycle in Gonad Weight and Condition in the Perch (*Perca fluviatilis*). *J. Anim. Ecol.* **1951**, *20*, 201–219. [CrossRef]
26. Vega-Orellana, O.M.; Fracalossi, D.M.; Sugai, J.K. Dourado (*Salminus brasiliensis*) larviculture: Weaning and ontogenetic development of digestive proteinases. *Aquaculture* **2006**, *252*, 484–493. [CrossRef]
27. Lowry, O.H.; Rosebrough, N.J.; Farr, A.L.; Randall, R.J. Protein measurement with the folin phenol reagent. *J. Biol. Chem.* **1951**, *193*, 265–275. [CrossRef]
28. Erlanger, B.F.; Kokowsky, N.; Cohen, W. The preparation and properties of two new chromogenic substrates of trypsin. *Arch. Biochem. Biophys.* **1961**, *95*, 271–278. [CrossRef]
29. DelMar, E.G.; Largman, C.; Brodrick, J.W.; Geokas, M.C. A sensitive new substrate for chymotrypsin. *Anal. Biochem.* **1979**, *99*, 316–320. [CrossRef]
30. Noelting, G.; Bernfeld, P. Sur les enzymes amylolytiques III. La β-amylase: Dosage d'activité et contrôle de l'absence d'α-amylase. *Helv. Chim. Acta* **1948**, *31*, 286–290. [CrossRef]
31. Anson, M.L. The estimation of pepsin, trypsin, papain and cathepsin with hemoglobin. *J. Gen. Physiol.* **1938**, *22*, 79–89. [CrossRef]
32. Montes-Girao, P.J.; Fracalossi, D.M. Dietary Lysine Requirement as Basis to Estimate the Essential Dietary Amino Acid Profile for Jundiá, *Rhamdia quelen*. *J. World Aquac. Soc.* **2006**, *37*, 388–396. [CrossRef]
33. Fuiman, L.A.; Higgs, D.M. Ontogeny, growth, and the recruitment process. In *Early Life History and Recruitment in Fish Populations*; Chambers, R.C., Trippel, E.A., Eds.; Chapman and Hall: London, UK, 1997; pp. 225–249.
34. Osse, J.W.M.; van den Boogaart, J.G.M.; van Snik, G.M.J.; van der Sluys, L. Priorities during early growth of fish larvae. *Aquaculture* **1997**, *155*, 249–258. [CrossRef]
35. Osse, J.W.M.; van den Boogaart, J.G.M. Fish larvae, development, allometric growth and the aquatic environment. *ICES Mar. Sci. Symp.* **1995**, *201*, 21–34. [CrossRef]

36. Restrepo-Santamaria, D.; Herrera-Pérez, J.; Muñoz-Duque, S.; Ospina-Pabón, J.G.; Londoño, J.P.; Loaiza-Santana, C.A.; Álvarez-Bustamante, J.; Valencia-Rodríguez, D.; Jiménez-Segura, L. Inventarios de peces en la cuenca del río Magdalena (Colombia) como herramienta para la gestión de su conservación [Fish inventories in the Magdalena River basin (Colombia) as a tool for their conservation management]. *Caldasia* **2022**, *44*, 356–367. [CrossRef]
37. Atencio-Garcia, V.; Pertuz-Buelvas, V.M.; Pérez-Espitia, F.; Ortiz-Mestra, R.; Pardo-Carrasco, S.C. Manejo de la primera alimentación de dorada *Brycon sinuensis* ofreciendo larvas de bocachico *Prochilodus magdalenae*. *Rev. Colomb. Cienc. Pecu.* **2010**, *23*, 317–324.
38. Baras, E.; Jobling, M. Dynamics of intracohort cannibalism in cultured fish. *Aquac. Res.* **2002**, *33*, 461–479. [CrossRef]
39. Sales, J. First feeding of freshwater fish larvae with live feed versus compound diets: A meta-analysis. *Aquac. Int.* **2011**, *19*, 1217–1228. [CrossRef]
40. Baras, E. Minimización del canibalismo en especies de peces con larvas piscívoras: Estrategias y éxitos con el carácido *Brycon moorei*. In *Biología de las Poblaciones de Peces de la Amazonía y Acuicultura*; Renno, J.F., García, C., Duponchelle, F., Nuñez, J., Eds.; Red de Investigación sobre la Ictiofauna Amazónica: Iquitos, Perú, 2005; pp. 227–233.
41. Kolkovski, S. Digestive enzymes in fish larvae and juveniles—Implications and applications to formulated diets. *Aquaculture* **2001**, *200*, 181–201. [CrossRef]
42. Baras, E.; Lucas, M.C. Individual growth trajectories of sibling *Brycon moorei* raised in isolation since egg stage, and their relationship with aggressive behaviour. *J. Fish Biol.* **2010**, *77*, 985–997. [CrossRef]
43. Atencio Garcia, V.; Zaniboni-Filho, E.; Pardo Carrasco, S.; Arias-Castellanos, A. Influência da primeira alimentação na larvicultura e alevinagem do yamú Brycon siebenthalae (Characidae). *Acta Sci.* **2003**, *25*, 61–72. [CrossRef]
44. Gonçalves, L.U.; França, L.A.; Epifânio, C.M.; da Fonseca, F.A.L.; de Alcântara, A.M.; do Nascimento, R.G.; Silva, E.N.S.; da Conceição, L.E.C. Ostracoda impairs growth and survival of *Arapaima gigas* larvae. *Aquaculture* **2019**, *505*, 344–350. [CrossRef]
45. Silveira, J.; Silva, C.P.; Cargnin-Ferreira, E.; Alexandre, D.; Elias, M.A.; Fracalossi, D.M. Freshwater catfish jundiá (*Rhamdia quelen*) larvae are prepared to digest inert feed at the exogenous feeding onset: Physiological and histological assessments. *Fish Physiol. Biochem.* **2013**, *39*, 1581–1590. [CrossRef] [PubMed]
46. Castro-Ruiz, D.; Mozanzadeh, M.T.; Fernández-Méndez, C.; Andree, K.B.; García-Dávila, C.; Cahu, C.; Gisbert, E.; Darias, M.J. Ontogeny of the digestive enzyme activity of the Amazonian pimelodid catfish *Pseudoplatystoma punctifer* (Castelnau, 1855). *Aquaculture* **2019**, *504*, 210–218. [CrossRef]
47. Fernández-Méndez, C.; David, F.; Darias, M.J.; Castro-Ruiz, D.; Núñez-Rodríguez, J. Rearing of the Amazon catfish *Pseudoplatystoma punctifer* (Castelnau, 1855): Weaning with dry and moist diets. *J. Appl. Ichthyol.* **2015**, *31*, 83–87. [CrossRef]
48. Jomori, R.K.; Carneiro, D.J.; Martins, M.I.E.G.; Portella, M.C. Economic evaluation of *Piaractus mesopotamicus* juvenile production in different rearing systems. *Aquaculture* **2005**, *243*, 175–183. [CrossRef]
49. Beux, L.F.; Zaniboni-Filho, E. *Artemia* sp. Proportions and Effects on Survival and Growth of Pintado, *Pseudoplatystoma corruscans* Larvae. *J. Appl. Aquac.* **2008**, *20*, 184–199. [CrossRef]
50. David-Ruales, C.A.; Zapata-Berruecos, B.; Vásquez-Torres, W. Efectos de la primera alimentación en larvas de *Rhamdia sobre* la ganancia de peso y longitud. *Rev. Lasallista Investig.* **2009**, *6*, 7–13.
51. David-Ruales, C.; Lenis-Sucerquia, G.; Castañeda-Alvarez, G.; Lopera, A.; Restrepo, L. Initial diet composition affects weight gain and total length of Pacu (*Piaractus brachypomus*) larvae. *Rev. Colomb. Ciencias Pecu.* **2011**, *24*, 48–53.
52. Tesser, M.B.; Carneiro, D.J.; Portella, M.C. Co-Feeding of Pacu, *Piaractus mesopotamicus* Holmberg (1887), Larvae with Artemia Nauplii and a Microencapsulated Diet. *J. Appl. Aquac.* **2005**, *17*, 47–59. [CrossRef]
53. Cestarolli, M.; Portella, M.; Rojas, N. Efeito do nível de alimentação e do tipo de alimento na sobrevivência e no desempenho inicial de larvas de Curimbatá *Prochilodus scrofa* (Steindachner, 1881). *Bol. Inst. Pesca* **1997**, *24*, 119–129.
54. Pradhan, P.K.; Jena, J.; Mitra, G.; Sood, N.; Gisbert, E. Effects of different weaning strategies on survival, growth and digestive system development in butter catfish *Ompok bimaculatus* (Bloch) larvae. *Aquaculture* **2014**, *424–425*, 120–130. [CrossRef]
55. Hamre, K.; Yúfera, M.; Rønnestad, I.; Boglione, C.; Conceição, L.E.C.; Izquierdo, M. Fish larval nutrition and feed formulation: Knowledge gaps and bottlenecks for advances in larval rearing. *Rev. Aquac.* **2013**, *5*, S26–S58. [CrossRef]
56. Portella, M.C.; Jomori, R.K.; Leitão, N.J.; Menossi, O.C.C.; Freitas, T.M.; Kojima, J.T.; Lopes, T.S.; Clavijo-Ayala, J.A.; Carneiro, D.J. Larval development of indigenous South American freshwater fish species, with particular reference to pacu (*Piaractus mesopotamicus*): A review. *Aquaculture* **2014**, *432*, 402–417. [CrossRef]
57. Conceição, L.; Aragão, C.; Rønnestad, I. Proteins. In *Larval Fish Nutrition*, 1st ed.; Holt, J.G., Ed.; John Wiley & Sons, Inc.: Ames, IA, USA, 2011; pp. 83–116.
58. Jomori, R.K.; Ducatti, C.; Carneiro, D.J.; Portella, M.C. Stable carbon (δ 13 C) and nitrogen (δ 15 N) isotopes as natural indicators of live and dry food in *Piaractus mesopotamicus* (Holmberg, 1887) larval tissue. *Aquac. Res.* **2008**, *39*, 370–381. [CrossRef]
59. Solovyev, M.; Kashinskaya, E.; Gisbert, E. A meta-analysis for assessing the contributions of trypsin and chymotrypsin as the two major endoproteases in protein hydrolysis in fish intestine. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2023**, *278*, 111372. [CrossRef]
60. Frías-Quintana, C.A.; Álvarez-González, C.A.; Guerrero-Zárate, R.; Valverde-Chavarría, S.; Ulloa-Rojas, J.B. Changes in digestive enzymes activities during the initial ontogeny of wolf cichlid, *Parachromis dovii* (Perciformes: Cichlidae). *Neotrop. Ichthyol.* **2019**, *17*, e180161. [CrossRef]

61. Jimenez-Martinez, L.D.; Alvarez-González, C.A.; Tovar-Ramírez, D.; Gaxiola, G.; Sanchez-Zamora, A.; Moyano, F.J.; Alarcón, F.J.; Márquez-Couturier, G.; Gisbert, E.; Contreras-Sánchez, W.M.; et al. Digestive enzyme activities during early ontogeny in Common snook (*Centropomus undecimalis*). *Fish. Physiol. Biochem.* **2012**, *38*, 441–454. [CrossRef]
62. Yanes-Roca, C.; Toledo-Cuevas, M.E.; Sánchez, L.J.; Born-Torrijos, A.; Rhody, N.; Main, K.L. Digestive Enzyme Activity during Larval Development of Black Snook, *Centropomus nigrescens*. *J. World Aquac. Soc.* **2018**, *49*, 612–624. [CrossRef]
63. Hernández-López, I.A.; Ibarra-Castro, L.; Álvarez-González, C.A.; Martínez-Brown, J.M.; Maytorena-Verdugo, C.I.; Peña-Marín, E.S. Characterization of digestive enzymes during early ontogeny of white Snook (*Centropomus viridis*). *Aquaculture* **2021**, *535*, 736399. [CrossRef]
64. Castañeda-Alvarez, G.D. Desarrollo de la actividad de la tripsina, quimotripsina, proteasa ácida, lipasa y amilasa en la etapa larval de *Pseudoplatystoma fasciatum* alimentadas con *Artemia salina*. Master's Thesis, Instituto de Acuicultura de los Llanos, Universidad de los Llanos, Villavicencio, Colombia, 2009; 86p.
65. Zambonino Infante, J.; Cahu, C. Ontogeny of the gastrointestinal tract of marine fish larvae. *Comp. Biochem. Physiol. Part C Toxicol. Pharmacol.* **2001**, *130*, 477–487. [CrossRef]
66. Pérez-Jiménez, A.; Cardenete, G.; Morales, A.E.; García-Alcázar, A.; Abellán, E.; Hidalgo, M.C. Digestive enzymatic profile of *Dentex dentex* and response to different dietary formulations. *Comp. Biochem. Physiol. Part A Mol. Integr. Physiol.* **2009**, *154*, 157–164. [CrossRef] [PubMed]
67. Chakrabarti, R.; Rathore, R.M. Ontogenic changes in the digestive enzyme patterns and characterization of proteases in Indian major carp *Cirrhinus mrigala*. *Aquac. Nutr.* **2010**, *16*, 569–581. [CrossRef]
68. Gilannejad, N.; Moyano, F.J.; Martínez-Rodríguez, G.; Yúfera, M. Feeding Protocol Modulates the Digestive Process in Senegalese Sole (*Solea senegalensis*) Juveniles. *Front. Mar. Sci.* **2021**, *8*, 698403. [CrossRef]
69. Tong, X.H.; Xu, S.H.; Liu, Q.H.; Li, J.; Xiao, Z.Z.; Ma, D.Y. Digestive enzyme activities of turbot (*Scophthalmus maximus* L.) during early developmental stages under culture condition. *Fish. Physiol. Biochem.* **2012**, *38*, 715–724. [CrossRef]
70. Fernández, I.; Moyano, F.; Díaz, M.; Martínez, T. Characterization of α-amylase activity in five species of Mediterranean sparid fishes (Sparidae, Teleostei). *J. Exp. Mar. Bio Ecol.* **2001**, *262*, 1–12. [CrossRef]
71. López-Vásquez, K.; Castro-Pérez, C.A.; Val, A.L. Digestive enzymes of eight amazonian teleosts with different feeding habits. *J. Fish. Biol.* **2009**, *74*, 1620–1628. [CrossRef]
72. Dabrowski, K.; Portella, M.C. Feeding Plasticity and Nutritional Physiology in Tropical Fishes. *Fish Physiol.* **2005**, *21*, 155–224. [CrossRef]
73. Cara, B.; Moyano, F.J.; Zambonino, J.L.; Fauvel, C. Trypsin and chymotrypsin as indicators of nutritional status of post-weaned sea bass larvae. *J. Fish. Biol.* **2007**, *70*, 1798–1808. [CrossRef]
74. Gisbert, E.; Giménez, G.; Fernández, I.; Kotzamanis, Y.; Estévez, A. Development of digestive enzymes in common dentex *Dentex dentex* during early ontogeny. *Aquaculture* **2009**, *287*, 381–387. [CrossRef]
75. Sveinsdóttir, H.; Thorarensen, H.; Gudmundsdóttir, Á. Involvement of trypsin and chymotrypsin activities in Atlantic cod (*Gadus morhua*) embryogenesis. *Aquaculture* **2006**, *260*, 307–314. [CrossRef]
76. Gisbert, E.; Doroshov, S.I. Allometric growth in green sturgeon larvae. *J. Appl. Ichthyol.* **2006**, *22*, 202–207. [CrossRef]
77. Solovyev, M.M.; Kashinskaya, E.N.; Izvekova, G.I.; Gisbert, E.; Glupov, V.V. Feeding habits and ontogenic changes in digestive enzyme patterns in five freshwater teleosts. *J. Fish. Biol.* **2014**, *85*, 1395–1412. [CrossRef] [PubMed]
78. Rungruangsak-Torrissen, K.; Moss, R.; Andresen, L.H.; Berg, A.W.R. Different expressions of trypsin and chymotrypsin in relation to growth in Atlantic salmon (*Salmo salar* L.). *Fish. Physiol. Biochem.* **2006**, *32*, 7–23. [CrossRef]
79. Martínez-Llorens, S.; Peruzzi, S.; Falk-Petersen, I.B.; Godoy-Olmos, S.; Ulleberg, L.; Tomás-Vidal, A.; Puvanendran, V.; Odei, D.K.; Hagen, Ø.; Fernandes, J.M.O.; et al. Digestive tract morphology and enzyme activities of juvenile diploid and triploid Atlantic salmon (*Salmo salar*) fed fishmeal-based diets with or without fish protein hydrolysates. *PLoS ONE* **2021**, *16*, e0245216. [CrossRef]
80. Mai, M.G. Estudos da Ontogenia e da Alimentação Inicial de Larvas de Peixes, com Ênfase em Dourado *Salminus brasiliensis* (Characiformes, Characidae). Ph.D. Thesis, Universidade Federal de São Carlos, São Carlos, Brazil, 2009.
81. Santos, A.E.; Pedreira, M.M.; Santos, T.G.; Moura, G.d.S.; dos Santos, J.C.E.; Silva, R.C. Desenvolvimento do sistema digestório em larvas do peixe neotropical *Prochilodus argenteus* (Characiformes, prochilodontidae). *Acta Sci. Anim. Sci.* **2016**, *38*, 9–16. [CrossRef]
82. Gisbert, E.; Moreira, C.; Castro-Ruiz, D.; Öztürk, S.; Fernández, C.; Gilles, S.; Nuñez, J.; Duponchelle, F.; Tello, S.; Renno, J.; et al. Histological development of the digestive system of the Amazonian pimelodid catfish *Pseudoplatystoma punctifer*. *Animal* **2014**, *8*, 1765–1776. [CrossRef] [PubMed]
83. Santos, J.E.; dos Godinho, H.P. Ontogenic events and swimming behavior of larvae of the characid fish *Salminus brasiliensis* (Cuvier) (Characiformes, Characidae) under laboratory conditions. *Rev. Bras. Zool.* **2002**, *19*, 163–171. [CrossRef]
84. Tesser, M.B. Desenvolvimento do Trato Digestório e Crescimento de Larvas de Pacu, *Piaractus mesopotamicus* (Holmberg, 1887) em Sistemas de Co-Alimentação com Náuplios de Artemia e Dieta Microencapsulada. Master's Thesis, Aquaculture Center, Sao Paulo State University, Jaboticabal, Brazil, 2002; 59p.
85. David-Ruales, C.A. Desarrollo Temprano de la Especie *Brycon moorei*—Steindachner, 1878 (Dorada del Magdalena): Aspectos Embrionarios, Morfométricos, Histológicos e Histoquímicos, Parámetros Productivos y Actividad de las Enzimas Digestivas en Función de la Dieta. Ph.D. Thesis, Instituto de Acuicultura de los Llanos, Universidad de los Llanos, Villavicencio, Colombia, 2019.
86. Ribeiro, L.; Zambonino-Infante, J.L.; Cahu, C.; Dinis, M.T. Development of digestive enzymes in larvae of *Solea senegalensis*, Kaup 1858. *Aquaculture* **1999**, *179*, 465–473. [CrossRef]

87. Rønnestad, I.; Dominguez, R.P.; Tanaka, M. Ontogeny of digestive tract functionality in Japanese flounder, *Paralichthys olivaceus* studied by in vivo microinjection: pH and assimilation of free amino acids. *Fish. Physiol. Biochem.* **2000**, *22*, 225–235. [CrossRef]
88. Darias, M.J.; Murray, H.M.; Gallant, J.W.; Douglas, S.E.; Yúfera, M.; Martínez-Rodríguez, G. Ontogeny of pepsinogen and gastric proton pump expression in red porgy (*Pagrus pagrus*): Determination of stomach functionality. *Aquaculture* **2007**, *270*, 369–378. [CrossRef]