

Article

Enhancing Aquaculture Productivity via Polyculture with *Colossoma macropomum*: A Focus on Two Native Amazon Species

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Abstract

Rapid population growth has increased the demand for sustainable systems to produce protein-rich foods. Aquaculture with native species offers a strategic alternative to enhance food security in the Amazon region. This study evaluated the productive performance of *Liposarcus pardalis* and *Hypostomus hemicochliodon* in polyculture with *Colossoma macropomum* at different stocking densities (1, 3, and 5 fish/m²) for 120 days. Both loricariid species exhibited high survival (>96%) and satisfactory growth, with optimal performance at intermediate densities (3 fish/m²). Polynomial regression estimated optimal stocking densities of 2.45 and 2.42 fish/m² for *L. pardalis* and *H. hemicochliodon*, respectively. Polycultures with *H. hemicochliodon* yielded the highest biomass (22.11 ± 0.09 kg) and best feed conversion efficiency (FCE = 1.4), outperforming those with *L. pardalis* (19.36 ± 0.19 kg; FCE = 1.9). Although monoculture data for *C. macropomum* were not included, comparisons with published studies indicate that polyculture did not compromise its growth. The integration of native benthic, iliophagous species improves resource use and nutrient recycling, providing a sustainable strategy to enhance productivity and environmental efficiency in tropical aquaculture systems.

Keywords: *Hypostomus hemicochliodon*; iliophagous; *Liposarcus pardalis*; multitrophic; sustainability

Key Contribution: 1. *Liposarcus pardalis* and *Hypostomus hemicochliodon* have better production performance at 3 fish/m². 2. Polyculture with *H. hemicochliodon* improved biomass and feed efficiency. 3. Native loricariids support recycling and sustainability in Amazon aquaculture.

1. Introduction

Aquaculture is highly dependent on the interaction between the environment and the biological characteristics of the cultured organisms [1,2]. The development of diverse



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aquaculture systems has advanced significantly, incorporating factors such as stocking density, feeding strategies, and the integration of multiple species within shared environments [3]. This activity directly contributes to achieving the Sustainable Development Goals in aquaculture, as it not only ensures food security through the production of high-quality protein but also generates thousands of jobs in coastal communities worldwide, thereby strengthening local economies and promoting social development [4,5]. As one of the leading sources of essential nutrients, including high-quality proteins, vitamins, and minerals, aquaculture has experienced a notable increase in production levels over the past decade [6].

Monoculture is a more common method of culturing aquatic organisms, where they are raised individually in an aquaculture system [5]. On the other hand, there is the polyculture method, which focuses on sustainability and efficiency. This method integrates more than one species in the same environment, which differ in their feeding behaviors and ecological requirements [1,2,7,8]. In this method, a subordinate or secondary species is added that possesses different feeding habits and distribution in the water column to avoid direct competition with the main species for food and space [9,10]. This culture technique increases the maximum pond yield, promoting the efficient use of natural resources [2,3,7].

The selection and proportion of species in a polyculture are crucial to ensuring the viability of the system, as aspects such as feeding habits, distribution in the water column, and interspecific behavior must be considered [11]. A classic example is carp polyculture, where the combination of *Labeo rohita* (herbivorous, 20–30%), *Catla catla* (omnivorous, 30–40%), and *Cirrhinus mrigala* (detritivorous, 10–15%) has demonstrated successful trophic complementarity, establishing feeding hierarchies that optimize the use of available resources [12]. Similarly, tilapia of the genus *Oreochromis* has become one of the most widely used species in polyculture systems, typically comprising 50–60% of the total the species [13–16]. Shrimps of the genus *Macrobrachium* are often included as secondary species [10,17–19], as well as marine species such as *Penaeus* [20,21]. These combinations enhance trophic stratification and nutrient utilization, thereby improving the efficiency and sustainability of the production system. However, when either inappropriate planting densities or species combinations are used, excessive growth of phytoplankton, macrophytes, and benthic organisms can occur in the pond, creating an imbalance in the different trophic levels and affecting the performance of the system [1,16].

Colossoma macropomum, commonly known as Tambaqui, Cachama, or Gamitana [22], is a native South American species belonging to the order Characiformes and the family Serrasalminidae [23]. It is an omnivorous fish that feeds mainly on fruits, seeds, and leaves in the upper and middle strata of the water column [24]. Its ecological flexibility, rapid growth, and adaptability to captivity make it one of the most suitable native species for polyculture systems, where it can effectively utilize plant-based resources and contribute to nutrient recycling [25–27].

On the other hand, the Loricariidae family is a group of catfish characterized by ossified scales and a sucker-shaped mouth, which are generally of significant economic importance for aquarium use [28–30]. This family includes more than 600 species [31]. Among the species of Loricariidae, there are *Liposarcus pardalis* and *Hypostomus hemicochliodon*, which have iliophagous feeding habits and are generally located in the lower stratum of the water column [24]. Both *L. pardalis* and *H. hemicochliodon* display biological traits that suggest potential for aquaculture, including high tolerance to low oxygen levels, benthic feeding habits, and resistance to handling stress [24,30]. Their iliophagous diet and detritivorous behavior may enable integration into polyculture systems as bottom-cleaning species, aiding nutrient recycling and sediment control [24]. Despite these attributes, research on the cultivation of loricariid species remains scarce, and their functional role within poly-

culture systems has not been experimentally evaluated. Previous Amazonian polyculture studies have mainly focused on combining *Colossoma macropomum* with species such as *Prochilodus* sp., *Piaractus brachipomus*, or *Macrobrachium amazonicum*—species occupying mid- or surface strata [30–35].

Because the monoculture of *C. macropomum* has been well characterized in previous studies, this research did not include a monoculture treatment. Instead, it focused on developing a sustainable polyculture model that integrates complementary benthic species to improve nutrient recycling and reduce environmental impacts. For this reason, we hypothesized that incorporating these loricariids would enhance resource utilization, promote nutrient recycling, and improve overall growth performance within the polyculture system. Therefore, this study aims to optimize the cultivation of *C. macropomum* through the implementation of a polyculture system by integrating *L. pardalis* and *H. hemicochliodon* as complementary benthic species. By examining various population densities on the productive performance of these loricariids, as well as to understand their impact on the yield of *C. macropomum* and on the overall efficiency of polyculture systems.

2. Materials and Methods

2.1. Ethical Consent

The study was conducted in strict accordance with the EU 2010/63/EU [36] guidelines for animal experiments, following the 3Rs principle: Replacement, Reduction, and Refinement. It should be noted that no individual was sacrificed during the research, standard Commercial feed was used, and no drugs were used during the study.

2.2. Obtaining Biological Material and Experimental Design

The study was conducted at the facilities of the “Caña Brava” Asociación de Productores Agropecuarios y Servicios Múltiples, located in Lluncicate, Utcubamba, Amazonas, Peru (W 78°16'58" and S 5°44'45"). A total of 1241 fish were analyzed, including 372 loricariid fingerlings—186 *Liposarcus pardalis* (0.34 ± 0.25 g and 2.82 ± 0.20 cm) and 186 *Hypostomus hemicochliodon* (0.58 ± 0.25 g and 3.91 ± 0.20 cm), as well as 869 *Colossoma macropomum* fingerlings (7.81 ± 0.25 g and 7.71 ± 0.20 cm), which were sourced from the Ahuashiyacu fishing station in San Martín, Peru.

For 120 days under summer conditions, a randomized 2×3 factorial experimental design was conducted in a 413 m² earthen pond, where 18 experimental units of 22.95 m² each were delimited. Factor A corresponded to the species of loricariid in polyculture with *C. macropomum*: Lp = *L. pardalis* and Hh = *H. hemicochliodon*. Factor B corresponded to stocking densities: D1 = 1 fish/m², D3 = 3 fish/m², and D5 = 5 fish/m², with three replicates per combination. Two polycultures were established (Cm + Lp: *C. macropomum* + *L. pardalis*; Cm + Hh: *C. macropomum* + *H. hemicochliodon*). Following Costa et al. [37], who reported high growth rates in polyculture of *C. macropomum* with *iliophagous* species under a 70:30 ratio, and considering that the trophic and hierarchical relationship of both species is maintained [38], the same ratio was applied in this study, stocking 70% of individuals as the main species (*C. macropomum*) and 30% as the secondary species (*Loricariidae*) in each experimental unit. According to this ratio, the following were planted per experimental unit: D1 = 16 *C. macropomum* + 7 loricariids, D3 = 48 *C. macropomum* + 21 loricariids, and D5 = 81 *C. macropomum* + 34 loricariids (Figure 1).

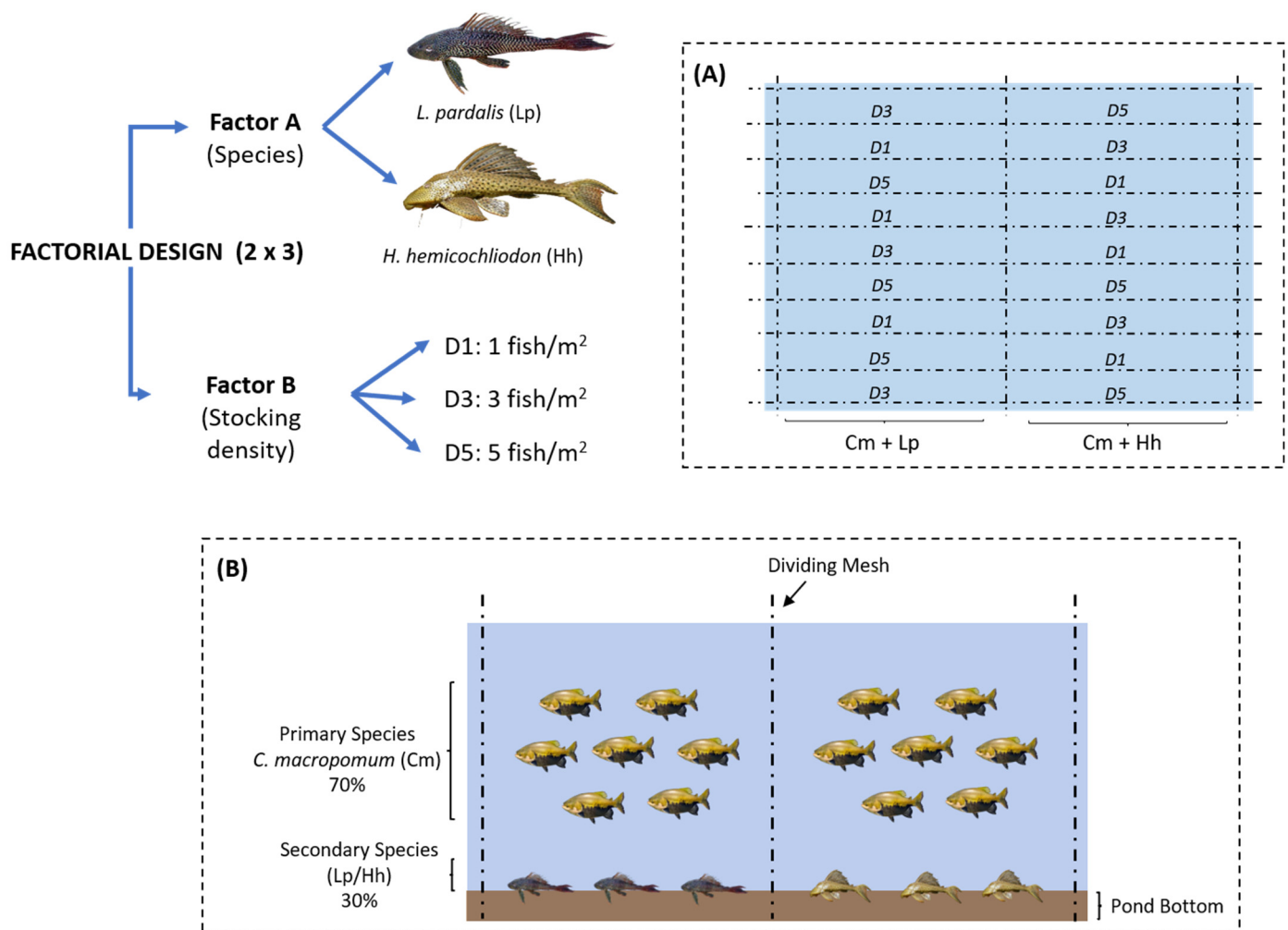


Figure 1. Schematic diagram of the factorial experimental design (factor A × B). **(A)** Spatial distribution of the experimental units. Cm + Lp: polyculture between *C. macropomum* + *L. pardalis*. Cm + Hh: polyculture between *C. macropomum* + *H. hemicochliodon*. **(B)** Proportion (70:30) and vertical distribution in the water column of both species.

The pond divisions were constructed using locally collected “pajuro” wood beams (200 cm × 20 cm × 20 cm) embedded at a depth of 50 cm. A net with a 12 mm mesh size was placed on the walls, and the intersections were secured with tarred twine. To prevent fish from moving between experimental units, the net was buried in the ground at a depth of 20 cm. Following the Fondo Nacional de Desarrollo Pesquero [39], CaO (calcium oxide; lime, Terrasur, Lima, Peru) was used to disinfect the area. It was scattered over the entire pond area. The next day, the pond was filled, and after two days, it reached a height of 20 cm. The foliar fertilizer (GREENZIT 20-20-20, AgroBesser, Lima, Peru) was applied. After three days, the pond was gradually filled to 120 cm (20 cm/day).

2.3. Stocking Densities, Feeding, and Sampling of Biological Material

L. pardalis, *H. hemicochliodon*, and *C. macropomum* fingerlings were acclimatized in polypropylene bags for more than fifteen minutes until water temperatures were homogenized at 27 °C. A balanced floating feed containing 28% crude protein (Aquatech, Peru) for tropical fish at the fingerling stage was provided (Table 1). The feeding rate and frequency were calculated based on feed data provided by Fondo Nacional de Desarrollo Pesquero [39] for the culture of *C. macropomum* (specific to each treatment and repetition) (Table S1).

Table 1. Formulation of the commercial diet.

Nutrients	Content (%)
Proteins	28
Fats	5
Fiber	8
Ashes	10
Humidity	12

Note: Proportioned by Aquatech. S.A.C. (Peru) (2025).

Eight completely random samples of the weight and length were taken every fifteen days, representing 10% of each population (D1: 2 Cm, 1, Lp/Hh; D3: 5 Cm, 2 Lp/Hh; D5: 8 Cm, 3 Lp/Hh), and the feeding rate was readjusted accordingly. A 12 mm mesh net was used for sampling, and weights were recorded using an electronic bowl-type scale (Ek2151h, max. 5 kg, Camry Scale, Hong Kong, China) with a precision of 0.05 g. Lengths were measured using an ichthyometer (KH-PISCIS-RIO-50B, Alpha Aquaculture S.A.C., Lima, Peru), which provided a precision of 1 mm for length. The data were then tabulated in a standardized sampling format.

2.4. Water Physical and Chemical Parameters

The pond was stabilized two days before stocking, and water quality parameters (temperature: 27.8 ± 0.4 °C, dissolved oxygen: 6.8 ± 0.7 mg/L, pH: 7.04 ± 0.06) were monitored periodically. The physical and chemical parameters of the polyculture pond water were evaluated daily at the center and corners of the pond, following a standard protocol established by Boyd et al. [40]. Multiparameter equipment (OAKLON WP 600 series meters) was used to record: dissolved oxygen (mg/L), pH, temperature (°C), and conductivity ($\mu\text{S}/\text{cm}^2$); the latter parameter was multiplied by 25 to align it with the scale of the other parameters. Data were recorded twice daily at 08:00 and 16:00, as this schedule allows evaluation of the extremes of water quality variations, providing critical information to adjust aeration, feeding, and culture management strategies [40]. Due to the summer season in the region, the water renewal rate was every 2 days.

2.5. Evaluation of Productive Performance

Variables such as weight, length, and initial biomass were recorded at the start of the experiment. Upon completion of the study, all fish were collected, and weight and length were measured to calculate the Absolute Weight Growth Rate (AWGR), Absolute Length Growth Rate (ALGR), and Relative Growth Rate (RGR) [41]. To determine Feed Conversion Efficiency (FCE) and the final biomass (kg), the conversion of each polyculture was evaluated, following Pardo-Carrasco et al. [42]. In addition, survival rates (SR) were determined [43–47].

The equations used in this study are presented as follows:

$$AWGR \text{ (g/day)} = (W_f - W_i) / t \quad (1)$$

$$ALGR \text{ (cm/day)} = (L_f - L_i) / t \quad (2)$$

$$RGR \text{ (%/day)} = (e^g - 1) \times 100 \quad (3)$$

$$FCE = (W_f - W_i) / Df \times 100 \quad (4)$$

$$SR \text{ (%) } = (Ind_f / Ind_i) \times 100 \quad (5)$$

where W_f is the final weight, W_i is the initial weight, L_f is the final length, L_i is the initial length, D_f is the feed supplied in grams, Ind_f is the initial individuals, Ind_i is the final individuals, g is $LnW_f - LnW_i$ and t is the duration of the study.

All parameters, including total biomass, growth rates, and FCE, were calculated per treatment group, as all experimental units had identical pond areas (22.95 m²), allowing for direct comparison among treatments.

2.6. Statistical Analysis

The data were analyzed using a two-way analysis of variance (ANOVA) with interaction, considering loricariid species (FA) and stocking density (FB) as factors, along with their interaction (FA $\times$ FB). This analysis allowed us to assess both the combined effect of loricariids and density (independent variables) on production parameters and the specific influence of loricariids on the performance of *C. macropomum*, including biomass and feed conversion ratio (FCR) in polyculture (dependent variables). Before conducting the two-way ANOVA, the normality and homogeneity of residual variances were verified using the Shapiro–Wilk ($\alpha = 0.05$) and Levene ($\alpha = 0.05$) tests. When significant differences were found ($p < 0.05$).

Furthermore, given that this is the first report on the productive performance of *L. pardalis* and *H. hemicochliodon* as secondary species in polyculture systems with *C. macropomum*, a second-order polynomial regression was applied in an exploratory manner to visualize possible nonlinear trends in the response of productive parameters (final weight) to optimal stocking density (SD_O). As established Xu et al. [48] and Pai et al. [49], in the cultivation of tilapia and shrimp, respectively. All statistical analyses were performed using R software (v.4.4.0).

3. Results

3.1. Water Physicochemical Parameters

During the evaluation, the trends of the physical and chemical parameters in the ponds were recorded (Figure 2). The mean values observed across both polycultures were 26.7 ± 0.4 °C for temperature, 6.89 ± 0.38 mg/L for dissolved oxygen, 3.8 ± 0.1 mS/cm² for conductivity ($\times 25$), and 6.22 ± 0.46 for pH. The temperature remained relatively stable throughout the culture period, while dissolved oxygen fluctuated between 6.0 and 7.2 mg/L. Conductivity exhibited slight variations across the sampling days, and pH ranged from 5.3 to 6.9, with a gradual increase toward the end of the trial.

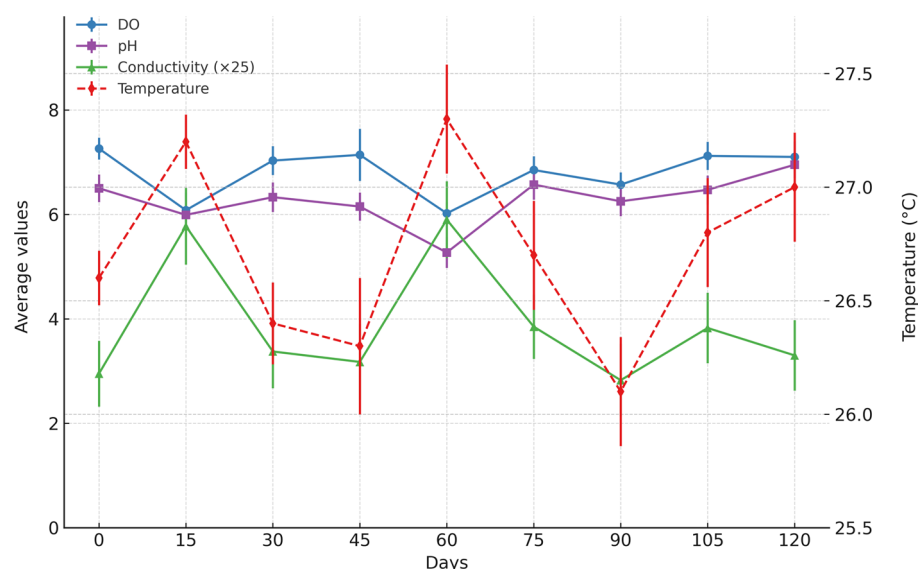


Figure 2. Trends in water quality parameters throughout the experiment.

3.2. Productive Performance

The two-way ANOVA (Table 2) confirmed that final weight and length were influenced by both species (FA) and stocking density (FB). However, the FA $\times$ FB interaction was only significant for *L. pardalis*, indicating that this species responded differently to changes in density, while no interaction effects were observed in *H. hemicochliodon*. RGR was determined almost exclusively by species, being significantly higher in *L. pardalis*, whereas density had no statistically significant effect. In contrast, survival responded only to density D1 and D5 (FB), although it remained high in all treatments overall.

Table 2. Coefficients and statistical significance of the factors evaluated in relation to the productive performance of loricariids.

Response	Factors	Coefficient	p-Value	Model Fitting
W _f (g)	FA-Lp	−0.304	0.021	R ² = 93.93%
	FB-D1	0.599	0.005	
	FB-D3	1.589	0.000	
	FA $\times$ FB-Lp $\times$ D1	−0.363	0.045	
	FA $\times$ FB-Lp $\times$ D5	−0.971	0.000	
L _f (cm)	FA-Lp	−0.189	0.040	R ² = 96.90%
	FB-D3	2.083	0.000	
	FA $\times$ FB-Lp $\times$ D1	−0.361	0.009	
	FA $\times$ FB-Lp $\times$ D5	−0.564	0.000	
RGR (g/days)	FA-Lp	−0.538	0.008	R ² = 61.19%
Survival (%)	FB-D1	1.933	0.000	R ² = 97.75%
	FB-D5	−1.266	0.000	

Notes: W_f: Weight final, L_f: Length final, RGR: Relative Growth Rate. FA: A factor. FB: B factor. Lp: *Liposarcus pardalis*. D1, D3 and D5: Stocking densities.

In terms of productive performance (Table 3), *Hypostomus hemicochliodon* recorded higher values than *Liposarcus pardalis*, reaching final weights of 14.0–17.5 g compared to 13.6–14.9 g and lengths of 14.0–17.8 cm compared to 15.6–18.4 cm, with maximums in the intermediate density (D3). Growth rates reflected the same trend, with *H. hemicochliodon* showing higher AWGR (0.08–0.14 g/day), ALGR (0.08–0.13 cm/day), and RGR (2.34–3.00%/day) than *L. pardalis* (0.11–0.12 g/day; 0.11–0.12 cm/day; 2.95–3.16%/day). Survival rates were high in both cases (>96%), with slight variations in density, but no significant differences were observed between species.

Table 3. Productive yield and survival of loricariids.

Species (FA)	Density (FB)	W _i (g)	W _f (g)	L _i	L _f	AWGR (g/day)	ALGR (cm/day)	RGR (%/day)	Survival (%)
<i>Liposarcus pardalis</i> (Lp)	D1	0.3 $\pm$ 0.0	14.8 $\pm$ 0.3	2.7 $\pm$ 0.1	16.7 $\pm$ 0.2	0.11 $\pm$ 0.02	0.11 $\pm$ 0.02	2.95 $\pm$ 0.25	100 $\pm$ 0.0
	D3	0.36 $\pm$ 0.01	14.9 $\pm$ 0.3	2.7 $\pm$ 0.5	18.4 $\pm$ 0.3	0.12 $\pm$ 0.02	0.12 $\pm$ 0.06	3.22 $\pm$ 0.15	96.8 $\pm$ 0.1
	D5	0.34 $\pm$ 0.02	13.6 $\pm$ 0.2	2.8 $\pm$ 0.7	15.5 $\pm$ 0.1	0.11 $\pm$ 0.07	0.10 $\pm$ 0.07	3.16 $\pm$ 0.13	97.4 $\pm$ 0.3
<i>Hypostomus hemicochliodon</i> (Hh)	D1	0.4 $\pm$ 0.0	15.9 $\pm$ 0.2	4.10 $\pm$ 0.03	17.8 $\pm$ 0.4	0.12 $\pm$ 0.01	0.11 $\pm$ 0.03	3.00 $\pm$ 0.01	100 $\pm$ 0.0
	D3	0.6 $\pm$ 0.2	17.5 $\pm$ 0.3	3.8 $\pm$ 0.9	19.9 $\pm$ 0.4	0.14 $\pm$ 0.01	0.13 $\pm$ 0.07	2.93 $\pm$ 0.23	97.4 $\pm$ 0.4
	D5	0.9 $\pm$ 0.1	14.0 $\pm$ 1.1	3.8 $\pm$ 1.1	14.0 $\pm$ 0.5	0.08 $\pm$ 0.09	0.08 $\pm$ 0.01	2.34 $\pm$ 0.19	99.8 $\pm$ 0.1

Notes: FA: A factor. FB: B factor. W_i: Weight initial, W_f: Weight final, L_i: Length initial, L_f: Length final, AWGR: Absolute Weight Growth Rate. ALGR: Absolute Length Growth Rate. RGR: Relative Growth Rate. D1: 1 fish/m². D3: 3 fish/m². D5: 5 fish/m². Values are the means $\pm$ S.D. of three replicates.

The two-way analysis of variance revealed varied effects of the factors Loricariidae species (FA), stocking density (FB), and their interaction on the productive variables of *Colossoma macropomum*. For final weight (W_f), a highly significant effect was observed for the association with *Liposarcus pardalis* (FA-Lp, $p < 0.001$) (Table 4), as well as for its

interaction with a density of 3 fish/m² (FA × FB-Lp × D5, $p < 0.001$). Similarly, in final length (L_f), a significant effect was recorded for the species (FA-Lp, $p < 0.001$) and density levels (FB-D1, D5; $p < 0.001$), with notable interactions in Lp × D5 ($p < 0.001$), indicating greater growth when associated with *L. pardalis*. For growth rates, both the absolute weight growth rate (AWGR) and the absolute length growth rate (ALGR) showed significant interactions between Lp and density levels ($p < 0.05$), suggesting that the performance of *C. macropomum* depended not only on the accompanying species but also on the stocking density used. In the relative growth rate (RGR), effects of FB (D5, $p < 0.001$) and the interaction FA × FB-Lp × D5 ($p = 0.001$) were noted, where combinations with *L. pardalis* at intermediate densities promoted the highest daily percentage increases in weight.

Table 4. Coefficients and statistical significance of the factors evaluated in relation to the productive performance of *Colossoma macropomum* associated loricariids.

Response	Factors	Coefficient	p-Value	Model Fitting
W _f (g)	FA-Lp	−6.567	0.000	R ² = 89.55%
	FA × FB-Lp × D5	8.300	0.000	
L _f (cm)	FA-Lp	2.738	0.000	R ² = 99.04%
	FB-D1	0.344	0.008	
	FB-D3	1.494	0.000	
	FA × FB-Lp × D5	−1.172	0.000	
AWGR (g/days)	FB-D3	0.016	0.000	R ² = 83.86%
	FA × FB-Lp × D1	−0.005	0.038	
AGLR (cm/days)	FB-D3	0.019	0.000	R ² = 82.30%
	FA × FB-Lp × D1	0.002	0.043	
RGR (%/days)	FB-D3	−0.065	0.004	R ² = 61.77%
	FA × FB-Lp × D1	−0.070	0.002	
	FA × FB-Lp × D5	0.050	0.018	
Survival (%)	FA-Lp	0.188	0.020	R ² = 97.75%
	FB-D1	1.183	0.000	
Biomass (kg)	FA-Lp	−0.455	0.000	R ² = 99.96%
	FB-D1	−8.322	0.000	
	FB-D5	0.144	0.008	
	FA × FB-Lp × D1	0.388	0.000	
	FA × FB-Lp × D5	0.522	0.000	
FCE	FA × FB-Lp × D1	−0.127	0.001	R ² = 88.11%
	FA × FB-Lp × D5	−0.111	0.002	

Notes: W_f: Weight final, L_f: Length final. AWGR: Absolute Weight Growth Rate. ALGR: Absolute Length Growth Rate. RGR: Relative Growth Rate. FCE: Feeding conversion efficiency. FA: A factor. FB: B factor. Lp: *Liposarcus pardalis*. D1, D3 and D5: Stocking densities.

Survival, in contrast, showed no interaction between factors, but only main effects of density (FB-D1, $p < 0.001$), indicating that this parameter remained stable and high in all treatments, regardless of the associated loricarid. On the other hand, total biomass reflected one of the most robust results: significant effects were found for FA-Lp ($p < 0.001$) and for the interactions FA × FB-Lp × D1 and FA × FB-Lp × D5 ($p < 0.001$) interactions, with a model fit greater than 99% (R² = 99.96%), confirming that the productivity of the system was strongly determined by the combination of species and density. Finally, feed conversion efficiency (FCE) was also modulated by the FA × FB-Lp interaction (D1 and D5, $p < 0.01$), showing that associations with *L. pardalis* at intermediate density favored more efficient feed use.

The association of *Colossoma macropomum* with Lp generally performed worse than its association with Hh, particularly in variables related to individual growth. Final weight (W_f) was significantly higher in the *C. macropomum* + Hh association (8.34 ± 0.1 g at

D5) than in *C. macropomum* + Lp (6.40 ± 0.3 g on D5), which was also reflected in the absolute weight growth rate (AWGR) and relative growth rate (RGR). Specifically, the highest AWGR (2.16 ± 0.01 g/day) and RGR ($3.00 \pm 0.06\%$ /day) values were observed in the *C. macropomum* + Hh on D5 combination, which were significantly higher than those obtained in the corresponding combinations with E1 (Table 5). In terms of stocking density, an increasing trend was observed in total biomass and feed conversion efficiency (FCE) as density increased, reaching the highest values at D5 for both associations. For example, biomass reached 22.11 ± 0.09 kg in *C. macropomum* + Hh on D5, compared to 13.96 ± 0.19 kg in *C. macropomum* + Lp on D5, representing a significant difference between associations. Similarly, the lowest FCE value was recorded in *C. macropomum* + Hh on D5.

Table 5. Productive performance of the *Colossoma macropomum*.

Species (FA)	Density (FB)	Wi (g)	Wf (g)	Li	Lf	AWGR (g/day)	ALGR (cm/day)	RGR (%/day)	Survival (%)	Biomass (kg) *	FCE *
<i>C. macropomum</i> + Lp	D1	7.88 ± 0.27	253.9 ± 2.1	7.7 ± 0.5	22.9 ± 0.1	2.05 ± 0.01	0.12 ± 0.03	2.89 ± 0.03	99.6 ± 0.3	4.16 ± 0.03	1.6 ± 0.0
	D3	7.8 ± 1.0	259.5 ± 3.4	7.5 ± 1.2	23.0 ± 0.2	2.09 ± 0.01	0.12 ± 0.08	2.92 ± 0.09	98.9 ± 0.2	12.76 ± 0.17	1.6 ± 0.0
	D5	6.4 ± 0.5	233.4 ± 2.3	6.8 ± 0.3	22.2 ± 0.2	1.89 ± 0.02	0.12 ± 0.04	2.99 ± 0.07	97.2 ± 0.3	19.36 ± 0.19	1.9 ± 0.0
<i>C. macropomum</i> + Hh	D1	7.3 ± 0.4	262.0 ± 6.5	7.6 ± 0.5	22.1 ± 0.4	2.12 ± 0.05	0.13 ± 0.02	2.98 ± 0.03	99.5 ± 0.4	4.30 ± 0.10	1.8 ± 0.1
	D3	9.2 ± 0.5	255.8 ± 4.2	8.4 ± 0.3	21.5 ± 0.4	2.05 ± 0.03	0.10 ± 0.03	2.77 ± 0.05	98.1 ± 0.2	12.16 ± 0.20	1.8 ± 0.0
	D5	8.3 ± 0.1	268.1 ± 1.5	8.3 ± 0.1	23.5 ± 0.1	2.16 ± 0.01	0.12 ± 0.08	2.90 ± 0.00	96.9 ± 0.2	22.11 ± 0.09	1.4 ± 0.0

Notes: FA: A factor. FB: B factor. Wi: Weight initial, Wf: Weight final, Li: Length initial, Lf: Length final. AWGR: Absolute Weight Growth Rate. ALGR: Absolute Length Growth Rate. RGR: Relative Growth Rate. FCE: Feeding conversion efficiency. D1: 1 fish/m². D3: 3 fish/m². D5: 5 fish/m². Values are the means ± S.D. of three replicates. * Biomass and FCE were considered with the data for both species.

3.3. Optimum Stocking Density

Although it is true that at densities of 3 fish/m², loricariids such as *L. pardalis* and *H. hemicochliodon* exhibited higher productive performance, the second-degree polynomial regression model revealed a significant quadratic trend of $R^2 = 90.7\%$ ($p < 0.001$) (Figure 3A) for *L. pardalis*, while for *H. hemicochliodon*, $R^2 = 95.98\%$ ($p < 0.000$) (Figure 3B). From the equations of the exploratory models, the optimal stocking densities in the polyculture were estimated to be 2.45 and 2.42 fish/m², respectively. These theoretical densities correspond to the maximum yield points in terms of weight gain.

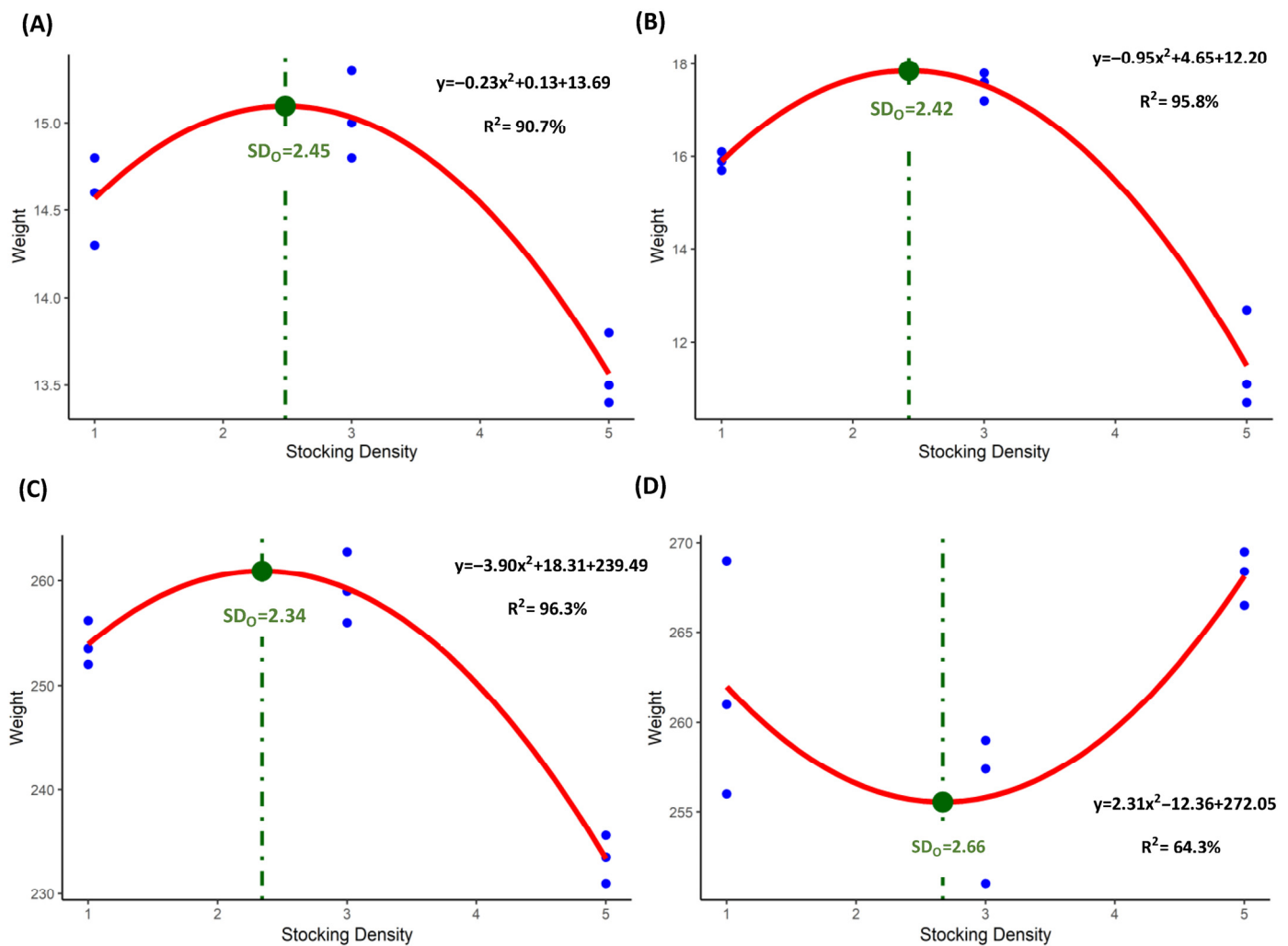


Figure 3. Schematic graph of the second-degree polynomial progression to determine optimum stocking density of loricariids in polyculture associated with *C. macropomum*. (A) *L. pardalis*, (B) *H. hemiochliodon*, (C) *C. macropomum* in PA, (D) *C. macropomum* in PB. SD₀: Optimum density. R²: Model fitting (%).

4. Discussion

This study provides the first assessment of the productive performance of *Liposarcus pardalis* and *Hypostomus hemiochliodon* as secondary species in polyculture systems with *Colossoma macropomum*. In general, both species of loricariids showed optimal growth at intermediate stocking densities (3 fish/m²), and their integration contributed to efficient feed conversion and biomass production, particularly in the polyculture with *H. hemiochliodon*.

Water quality parameters remained within optimal ranges for the species throughout the experiment. The temperature varied from 26.3 ± 0.1 to 27.3 ± 0.4 °C, consistent with previous studies on *C. macropomum* culture [30,50,51]. Conductivity averaged 3.8 ± 0.1 mS/cm² ($\times 25$) and showed a positive correlation with temperature, likely due to the increased solubilization of salts at higher temperatures [52–55]. Dissolved oxygen (6.02 ± 0.35 – 7.12 ± 0.45 mg/L) and pH (6.28 ± 0.46) also remained within the recommended ranges for *C. macropomum* and Loricariidae species in polyculture systems [56–59]. This stability, supported by daily water exchanges exceeding 30% of pond volume, indicates that environmental conditions were suitable and did not significantly affect fish performance or confound the experimental outcomes.

High stocking densities negatively affect individual well-being, leading to stress, growth inhibition, weakened immune responses, and increased susceptibility to pathogens [60–63]. Both *L. pardalis* and *H. hemicochliodon* feed not only on leftover feed from *C. macropomum* but also on phytoplankton and zooplankton present in the pond [64,65]. A clear difference was observed in the initial weights of the loricariid species, with *H. hemicochliodon* consistently exhibiting larger initial sizes than *L. pardalis*. This disparity could potentially confound direct comparisons of final weight or length. To minimize this effect, growth analyses were based on relative metrics (RGR, AWGR, ALGR), which normalize for initial size differences. Furthermore, statistical comparisons were made within density levels, ensuring that interspecific differences reflected true performance rather than initial biomass bias. For instance, *L. pardalis* in D3 had a lower final weight but a higher RGR due to its smaller initial size, reflecting greater biomass conversion efficiency at the individual level [37,60,61].

Although detailed biological information about *L. pardalis* and *H. hemicochliodon* is limited, especially regarding their performance under culture conditions, some general traits can be inferred from the Loricariidae family. Loricariids are primarily benthic and detritivorous, feeding on organic matter, periphyton, and biofilm present on pond substrates [30–35]. These feeding habits allow them to utilize uneaten feed and waste efficiently, contributing to nutrient recycling and sediment cleaning [24]. Therefore, the observed differences in growth performance may not solely reflect density effects, but also distinct ecological strategies within the Loricariidae family that influence energy allocation and interaction with *C. macropomum* in polyculture systems.

The introduction of complementary species into multitrophic aquaculture systems is a well-established technique, particularly when species with different feeding habits, such as omnivores and herbivores, are combined. This integration promotes a balance between waste accumulation and its utilization at the pond bottom, as benthic feeders can convert this waste into biomass, contributing to ecosystem stability [62,63]. The interaction between omnivorous species such as *C. macropomum* and iliophagous species such as shrimp has been widely studied. For instance, Franchini et al. [62] evaluated an integrated system with tambaqui and *M. amazonicum*, achieving a 35% increase in biomass production, while Dantas et al. [63] obtained up to 600 kg of biomass using only tambaqui waste and pond biota. Costa et al. [39] compared tambaqui monoculture and a 70:30 integrated system (*Prochilodus* sp.), reporting 23 kg of biomass in monoculture and 18.54 kg in polyculture after 120 days.

In the present study, although a monoculture treatment was not included, two polyculture systems (Cm + Lp and Cm + Hh) were evaluated under similar stocking densities. Polyculture Cm + Lp generated 19.32 ± 0.19 kg of biomass in D3, whereas Cm + Hh reached 22.11 ± 0.09 kg, values comparable to those reported in tambaqui monocultures. Slightly lower yields in polycultures are expected due to interspecific interactions and energy partitioning; however, maintaining comparable productivity while improving waste utilization and benthic activity highlights the sustainability and efficiency of integrating *L. pardalis* and *H. hemicochliodon* into Amazonian aquaculture [2,6,8]. Similarly, Teichert-Coddington [64] reported polycultures of *O. niloticus* and *C. macropomum* reaching 325 g and 270 g, respectively, consistent with the tambaqui weights observed in our study (268.1 ± 1.5 g).

C. macropomum is a wasteful feeder since, in a digestibility study of balanced feed reported by Meer et al. [65], only 71.5% was ingested, while 28.5% was wasted. This inefficiency provides an opportunity to develop polyculture systems more sustainably and productively. Our study demonstrated enhanced productive performance in a polyculture where Tambaqui served as the main species associated with *L. pardalis* at densities below

3 fish/m². This arrangement reduces stress, as *C. macropomum*'s omnivorous feeding habits minimize competition for food with *L. pardalis*, which has detritivorous feeding habits. These findings are supported by Tafur-Gonzales et al. [66], who evaluated the productive performance of *C. macropomum* and *Chaetobranchius semifasciatus* (bujurqui-tucunaré) in polyculture systems, observing a significant decrease in weight with increasing stocking density. Other studies, including Costa et al. [67] and Frisso et al. [68], indicate that culturing *C. macropomum* at high densities reduces weight and length gains while negatively affecting water quality and the hematobiochemical parameters of the fish. Likewise, Oliva et al. [69] emphasized the importance of stocking density in associated systems involving *C. macropomum* and *Piaractus brachipomus*. Consequently, indiscriminate increases in stocking density in aquaculture systems can compromise animal welfare, pond health, and overall production.

However, the performance of *C. macropomum* varied depending on the associated loricariid species. With *H. hemicochliodon*, *C. macropomum* achieved significantly higher final weights (268.1 ± 1.5 g) and biomass (22.11 ± 0.09 kg) compared to *L. pardalis*. This difference may be attributed to the more active feeding behavior of *H. hemicochliodon*, which could enhance *C. macropomum* feeding through increased pond movement and interaction, particularly at higher densities (D5) [61,70]. In contrast, *L. pardalis*, being more passive and benthic, may provide limited interspecific stimulation, resulting in optimal performance at intermediate densities (D3). These findings align with observations in polycultures involving benthic and less competitive species (*L. pardalis*), where the best growth is achieved at medium densities (D3), likely due to more efficient space utilization and reduced interference with the feeding behavior of *C. macropomum*. Overall, this underscores the importance of considering both stocking density and the ecological and behavioral traits of associated species when designing polyculture systems [71,72].

Feed conversion efficiency (FCE) is the primary economic indicator for management and profitability in aquaculture [73]. The advantage of the polyculture system is its potential to enhance FCE. However, inappropriate species combinations can significantly reduce efficiency, as demonstrated by Della et al. [74], who reported that cultivating *Prochilodus lineatus* with *Piaractus mesopotamicus*—both occupying the same water column stratum—led to resource competition. In contrast, the distinct vertical distribution of loricariids and *C. macropomum* in polycultures Cm + Lp and Cm + Hh resulted in FCE values of 1.6 (D1 and D3) and 1.4 (D5), representing the best outcomes for each stocking density. These results were achieved while maintaining water quality and pond sanitation within adequate ranges [39]. It should be noted that in the study reported by Matos Dantas et al. [75], *C. macropomum* cultivated in monoculture recorded an FCE of 1.33 when fed a basal diet (28% protein). This result is similar to that of our study (Cm + Hh, 1.4), demonstrating the potential of incorporating loricariids into the cultivation of *C. macropomum* to enhance crop productivity and sustainability.

A 100% survival rate was recorded at lower stocking density (D1) for both loricariid species, as reduced crowding minimized competition for space and food, thereby decreasing mortality from predation or pathogen proliferation [37]. Survival rates decreased as densities increased but remained above 96.97% in all treatments. Another factor contributing to the high survival was the vertical stratification of species in the water column: *C. macropomum* occupied the middle stratum, while loricariids remained in the lower stratum, reducing visibility by natural predators. *C. macropomum* had a lower survival rate compared to Loricariidae. This mortality can be attributed to the extruded nature of the feed, which caused it to remain buoyant. Consequently, the primary species, *C. macropomum*, had to ascend to the surface to feed, increasing its exposure to predators [63]. In contrast, the detritivorous loricariids remained at the bottom of the pond, thereby minimizing their risk

of predation [24,76]. Similar results were reported by Costa et al. [37], who observed comparable behavior in polycultures of tambaqui co-cultured with matrinxã (*Brycon amazonicus*) and curimatá (*Prochilodus* sp.).

As mentioned above, the highest productive performance of loricariids such as *L. pardalis* and *H. hemiochliodon* was observed at a density of 3 fish/m². However, our exploratory polynomial regression analysis estimated the optimal stocking densities as 2.45 and 2.42 fish/m², respectively, when these species were stocked as secondary species in a polyculture with *C. macropomum*. For *C. macropomum* in polyculture Cm + Lp, the optimal stocking density was calculated as 2.34 fish/m² (Figure 3C). In contrast, when associated with *H. hemiochliodon* (Cm + Hh), *C. macropomum* exhibited a quadratic response with a positive coefficient (Figure 3D). The model yielded a coefficient of determination of $R^2 = 64.3\%$, indicating that average weight gain was lower at intermediate densities (D3) and relatively higher at both low (D1) and high densities (D5). Moreover, the curve identified a minimum point at 2.66 fish/m², corresponding to the density at which *C. macropomum* exhibited the lowest average weight. The application of second-order polynomial regression as a statistical tool allows the estimation of theoretical optimal stocking densities [77,78], providing an initial reference for the design of polyculture systems involving loricariids. In the case of *C. macropomum* co-cultured with *H. hemiochliodon*, the model does not reveal a conventional optimum but rather a U-shaped trend, where growth is enhanced at both low and high densities. This pattern may be related to interspecific interactions, competition, or differential utilization of space and food resources [70,71].

The results suggest that the interaction between *C. macropomum* and loricariids is mainly mediated by indirect competition processes and complementary trophic effects. While their association with *C. macropomum* conditions the growth and performance of loricariids, the latter experiences a more subtle modulating effect, especially in combination with *L. pardalis*, where the benthic habit of loricariids could alter nutrient availability and feeding efficiency. Thus, the coexistence of both species reflects a dynamic balance: on the one hand, loricariids play a role in removing detritus and recycling organic matter; on the other hand, their presence may indirectly limit the growth of *C. macropomum* under certain density conditions. These findings underscore the importance of adjusting stocking densities in polyculture systems to minimize competition and enhance ecological complementarity between species.

5. Conclusions

The integration of native loricariid species (*Liposarcus pardalis* and *Hypostomus hemiochliodon*) as secondary species in polyculture with *Colossoma macropomum* demonstrated high survival and good growth. The optimal stocking densities were 2.45 fish/m² for *L. pardalis* and 2.42 fish/m² for *H. hemiochliodon*, achieving a feed conversion efficiency (FCE) of 1.6 (D3) and biomass of 19.36 kg for *L. pardalis* (D5), whereas *H. hemiochliodon* reached a higher performance with FCR of 1.4 and maximum biomass of 22.1 kg (D5). These values are similar to those reported in monocultures of *C. macropomum* according to the literature, indicating that the incorporation of benthic species such as these loricariids increases the productive efficiency of the pond by improving resource use, nutrient recycling, and sustainable aquaculture practices in the Amazon region.

However, since this study was conducted in small-scale experimental ponds, caution should be taken when extrapolating these results to commercial aquaculture systems. Environmental dynamics, management intensity, and spatial factors may differ substantially at larger scales. Therefore, further validation under replicated field and commercial conditions is required to confirm the scalability and long-term sustainability of these findings.

Supplementary Materials: The following supporting information can be downloaded at: <https://www.mdpi.com/article/10.3390/fishes10110563/s1>, Table S1: Feeding rate for *Colossoma macropomum*.

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