

## Article

# Intensification of Amazon River Prawn Hatchery

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**Abstract:** The effects of the intensification on the performance of the Amazon River prawn hatchery carried out in a simple recirculation system were investigated. Newly hatched larvae were stocked in 120 L tanks at 80, 100, 120 and 140 larvae L<sup>-1</sup> in a closed recirculating system. The experiment used a randomized block design with five replicates. An exponential equation was adjusted to express the relationship between the stocking density and productivity (postlarvae L<sup>-1</sup>). The development, larval quality, survival and postlarval (PL) dry weight did not significantly differ among the treatments ( $p > 0.05$ ). When 80 larvae were stocked, the productivity ( $54 \pm 11$  PL L<sup>-1</sup>) was lower than those at higher densities ( $p < 0.05$ ). Stocking 120 and 140 larvae L<sup>-1</sup> resulted in higher productivities ( $75 \pm 18$  and  $80 \pm 17$  PL L<sup>-1</sup>, respectively) with a lower use of *Artemia* nauplii to produce each postlarvae ( $\sim 1200$  *Artemia* nauplii PL<sup>-1</sup>). The maximum mean *M. amazonicum* postlarval production estimated by the exponential model was 93 PL L<sup>-1</sup>. This means that despite the increase in stocking density, productivity tends to stabilize. The results showed that *M. amazonicum* tolerates high intensification in recirculating hatchery systems based on a crushed shell bed biofilter, and the intensification optimizes *Artemia* use.

**Keywords:** stocking density; productivity; recirculation system; larviculture; *Macrobrachium amazonicum*; *Artemia* nauplii

**Key Contribution:** *M. amazonicum* postlarvae can be produced in simple RAS systems stocked at 100 to 140 larvae L<sup>-1</sup>, and productivity may reach around 93 PL L<sup>-1</sup> in about 20 days. As the culture intensifies, the use of *Artemia* nauplii per production unity is reduced by  $\sim 20\%$ .



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## 1. Introduction

Aquaculture may play an essential role in reaching the Sustainable Development Goals defined in Agenda 2030 [1]. Thus, more sustainable aquaculture systems should be implemented as a form of nature-positive food production that serves the people and the planet. The use of native low trophic species (LTSs) and integrated multitrophic aquaculture (IMTA) systems has been considered more environmentally sustainable than the culture of exotic or high trophic level species and monocultures [2]. Native species showed a lower impact on surrounding biodiversity; LTSs generally fed on detritus and small natural biota, recovering organic matter to trophic webs; and IMTAs use the wastes of one species to feed others, according to the principles of the circular economy. The Amazon River prawn, *Macrobrachium amazonicum*, is a LTS widely distributed in rivers and lakes of South America [3–5]. This species has been primarily exploited by artisanal fisheries [6,7] and has great potential for use in aquaculture [8,9]. The Amazon River prawn has been demonstrated to adapt very well to IMTA systems [10–12]. There is a large local market for *M. amazonicum*, mainly in the Amazon and northeastern Brazil [3,6].

An essential constraint to producing native LTS organisms is the lack of seed availability [13]. Therefore, developing hatchery technology to provide the postlarvae of *M. amazonicum* for sustainable grow-out farms is relevant. Prawn larviculture is generally performed in recirculating aquaculture systems (RASs) [8]. These systems are more conservative of water and heat, generate fewer effluents than flow-through systems and are less exposed to climate changes. However, they are more expensive to set up and manage than most aquaculture systems.

An RAS is an intensive aquaculture that uses about 1 to 10% of the water used in conventional aquaculture systems and allows for the total control of water variables and effluents [14]. Generally, intensification has been associated with unsustainable systems; however, intensification may be a way to save financial and natural resources and decrease the harmful effluents per unit of product. Additionally, job positions may be created if planned accordingly. High productivity is essential to reduce unit production costs. The challenge is finding the intensification level that maximizes productivity and increases job positions while minimizing costs and the environmental impact.

Increasing intensification leads to an increasing stocking density. It depends on the carrying capacity of the system [15] and the intrinsic characteristics of the cultured species. High densities may increase the levels of ammonia and intraspecific competition, which may result in low animal welfare, poor growth, survival and production. Low stocking densities may result in lower productivity. Therefore, it is essential to know the effect of the levels of intensification on the water quality and prawn development to define the best stocking densities. Papers that have focused on the stocking densities of the freshwater prawn *M. rosenbergii* in the larviculture phase were found in the literature [16–18]. However, for *M. amazonicum*, intensification has only been studied in the nursery [19–21] and grow-out [6] phases. Considering the above rationale, the objective of this paper was to evaluate the effect of the intensification of an *M. amazonicum* hatchery performed using simple and cheap recirculating aquaculture systems.

## 2. Materials and Methods

### 2.1. Experimental Conditions

Intensification was evaluated based on the increase in the larval stocking density in the rearing tanks. This experiment was set up according to a randomized block design with four treatments (stocking densities) and five replicates. The tested stocking densities were 80, 100, 120 and 140 larvae L<sup>-1</sup>. The evaluated variables were the larval development, larval quality, *Artemia* nauplii utilization, survival, metamorphosis rate, productivity, dry weight and average maximum productivity.

The *Macrobrachium amazonicum* larvae were obtained from females maintained in earthen ponds in a semi-intensive system. This stock was generated by animals from a diadromous population captured in Pará State (01°13'25" S, 48°17'40" W) in 2001, and it has been maintained for research purposes since then. Females with transparent eggs were collected and placed into larval hatching tanks (70 female m<sup>-2</sup>). The water was maintained at a salinity of ~5, a temperature of ~29 °C and with constant aeration.

After hatching, all the larvae were counted and transferred to rearing tanks at densities of 80, 100, 120 and 140 larvae L<sup>-1</sup>. Larvae were reared in 120 L cylindrical tanks with conical bottoms and under a closed recirculation aquaculture system (RAS) [8]. Crushed shell bed biofilters with 30 L (25% of that of the rearing tanks), provided with a heater and intense aeration were used for each tank. The water was moved via an air-lift pump and the recirculation rate was about 24 times per day. Larvae were fed beginning on the 2nd day with newly hatched *Artemia* nauplii supplied "ad libitum" in the afternoon (17:00 h). A moist inert diet (egg-custard-based; see Mallasen and Valenti [22] for composition) was supplied from the 9th day after stocking twice a day (at 8:00 h and 11:00 h). The feeding rate was adjusted daily and corresponded to consumption. The temperature (°C), water recirculation rate (% day<sup>-1</sup>), total ammonia nitrogen (TAN, mg L<sup>-1</sup>) and nitrite concentration (N-NO<sub>2</sub> mg L<sup>-1</sup>) were monitored daily. The dissolved oxygen (DO, mg L<sup>-1</sup>) and DO saturation (%), salin-

ity and pH were monitored weekly. Nitrogen compounds were analyzed using colorimetric tests. The dissolved oxygen was determined using a YSI Model 55 oxygen meter (Yellow Springs Instruments Co., Inc., Yellow Springs, OH, USA), and the salinity and pH were measured using a YSI Model 63 digital pH meter (Yellow Springs Instruments Co., Inc., Yellow Springs, OH, USA). The mean and standard deviation of the variables for each treatment are presented in Table 1. The photoperiods were kept constant at 12:12 h (light:dark) with ~1000 lux.

**Table 1.** Water data (means  $\pm$  standard deviations) obtained during *Macrobrachium amazonicum* larviculture at different stocking density treatments. The means did not significantly differ ( $p > 0.05$ ). TAN = total ammonia nitrogen; DO = dissolved oxygen.

Variables	Stocking Density (Larvae L <sup>-1</sup> )			
	80	100	120	140
Temperature (°C)	29.8 $\pm$ 0.1	29.8 $\pm$ 0.1	29.9 $\pm$ 0.1	29.9 $\pm$ 0.1
Recirculation rate (% day <sup>-1</sup> )	22.4 $\pm$ 0.4	25.0 $\pm$ 6.0	21.5 $\pm$ 2.9	22.5 $\pm$ 3.5
TAN (mg L <sup>-1</sup> )	0.23 $\pm$ 0.02	0.23 $\pm$ 0.02	0.24 $\pm$ 0.07	0.25 $\pm$ 0.05
N-NO <sub>2</sub> (mg L <sup>-1</sup> )	0.05 $\pm$ 0.01	0.05 $\pm$ 0.01	0.05 $\pm$ 0.03	0.04 $\pm$ 0.01
DO (mg L <sup>-1</sup> )	7.84 $\pm$ 0.17	8.08 $\pm$ 0.15	7.83 $\pm$ 0.60	7.84 $\pm$ 0.13
DO Saturation (%)	103.3 $\pm$ 4.0	106.3 $\pm$ 1.1	103.1 $\pm$ 4.6	106.8 $\pm$ 4.4
Salinity	10.2 $\pm$ 0.4	10.4 $\pm$ 0.5	10.1 $\pm$ 0.2	9.9 $\pm$ 0.4
pH	7.71 $\pm$ 0.56	7.71 $\pm$ 0.58	7.76 $\pm$ 0.56	7.75 $\pm$ 0.53

## 2.2. Larval Stage Index and Larval Condition Index

Samples containing 10 larvae were taken from each tank, and analyses of the larval stage index (LSI) and larval condition index (LCI) were performed using stereomicroscopy every two days. Larval stages were identified according to Guest [23]. The LSI was determined using the weighted average method described by Manzi et al. [24]:

$$LSI = \left( \sum Si \times ni \right) N^{-1}, \quad (1)$$

in which:  $Si$  is the larvae PL<sup>-1</sup> stage ( $I = 1-10$ ),  $ni$  = number of animals in stage  $Si$ , and  $N$  = total number of animals observed.

The LCI was determined following the criteria developed by Tayamen and Brown [25] for *M. rosenbergii* adapted to *M. amazonicum*. The criteria for determining the condition index for evaluating larval quality were the gut fullness, gut lipid content, pigmentation, body coloration, setation, muscle-to-gut ratio, abdominal muscle appearance, melanization, fouling organisms, the photopositive response between stage I and V, and, between stage VI and IX, swimming behavior was added. Each criterion was given a score, where 0 = poor, 1 = fair, and 2 = excellent.

## 2.3. Use of *Artemia* nauplii

The feed ratios are expressed as the *Artemia* nauplii density in the rearing water (nauplii mL<sup>-1</sup> day<sup>-1</sup>). The estimated use of nauplii mL<sup>-1</sup> was obtained daily. Feeding was monitored by estimating the concentration of nauplii mL<sup>-1</sup> using a 5 mL pipette (mean of five replicates). After 24 h, another estimation was conducted to quantify the amount of remaining nauplii inside the tank. The daily *Artemia* consumption, in nauplii mL<sup>-1</sup> concentration, was obtained by subtracting both these values for each treatment. The mean value of the five samples was then multiplied by the volume of water in the tank to estimate the total number of nauplii in the tank.

As we had a single cohort inside the tanks, no mass mortality was observed during the experiment, and survival was high, it is reasonable to suppose that the mortality rate was almost constant [26]. Thus, we can compute the instantaneous mortality rate ( $m$ ), using

the number of prawns stocked ( $N_0$ ) and harvested ( $N_T$ ), and the culture duration ( $T$ , in days) as Equation (2):

$$m = \ln(N_T/N_0)/T, \quad (2)$$

Then, based on Wineliller and Dailey [27], we determined the populational decline curve for each tank as Equation (3):

$$N_t = N_0e^{-mt}, \quad (3)$$

in which:  $N_t$  = number of prawns inside the tank at time  $t$  (in days). This equation was determined for each replicate and used to estimate the daily larvae quantity inside the tanks.

The individual use of prey (nauplii larval day) was obtained by dividing the total number of nauplii consumed per day by the number of larvae in the tank on that day. The number of *Artemia* nauplii per postlarvae (PL) was obtained by dividing the total number of nauplii used per tank during the entire culture by the number of PLs produced.

#### 2.4. Survival, Metamorphosis Rate, Productivity and Dry Weight

The end of the experiment occurred 21, 19, 20, 20 and 20 days after stocking in blocks I, II, III, IV and V, respectively. All larvae and postlarvae (PL) were collected and counted individually. The variables determined were the survival of the larvae and postlarvae (%), metamorphosis rate in postlarvae (% PL), productivity in postlarvae (PL L<sup>-1</sup>) and postlarvae dry weight (mg).

To determine the postlarvae dry weight, PL samples from all replicates in each treatment were taken, rinsed in distilled water, dried on filter paper and transferred to aluminum cartridges at predetermined weights. The cartridges containing the PL were dried (60 °C) for 24 h and kept inside the desiccator for at least two hours. Then, the cartridges were weighed on an analytical scale (Mettler Toledo AT21, accuracy 1 µg). Ten replicates for each tank were weighted.

To express the relationship between the stocking density and productivity, an exponential Equation (4) was adjusted:

$$P = P_{max} \left[ 1 - e^{-k(D-D_0)} \right], \quad (4)$$

where  $P$  = the productivity of postlarvae L<sup>-1</sup>,  $P_{max}$  = the maximum average productivity of postlarvae L<sup>-1</sup> that can be obtained in this system,  $e$  = base of natural logarithms,  $D$  = the stocking density,  $K$  and  $D_0$  = constants.

#### 2.5. Statistical Analyses

The larval stage index, larval condition index, nauplii *Artemia* used by tank (nauplii mL<sup>-1</sup> day<sup>-1</sup>), nauplii *Artemia* used by larvae and postlarvae (nauplii larvae<sup>-1</sup> and nauplii postlarvae<sup>-1</sup>), survival, metamorphosis rate, productivity (PL L<sup>-1</sup>) and weight were expressed as the mean ± standard deviation. Percentage data were normalized via arcsine transformation before statistical analyses. All data were subjected to normality (Cramer–von Mises) and variance (Levene’s homoscedasticity test) tests. As no deviations were observed, data were subjected to an analysis of variance (one-way ANOVA). When significant differences ( $p < 0.05$ ) were detected, treatment means were compared by the Tukey–Kramer test. All the statistical analyses were performed based on Sheskin [28], using a Statistical Analysis System (SAS Institute, Inc., Cary, NC, USA, version 9.0). The regression between stocking density and postlarvae productivity was determined using Excel® Version 2401/2023 (Microsoft Corporation, Redmond, WA, USA).

### 3. Results

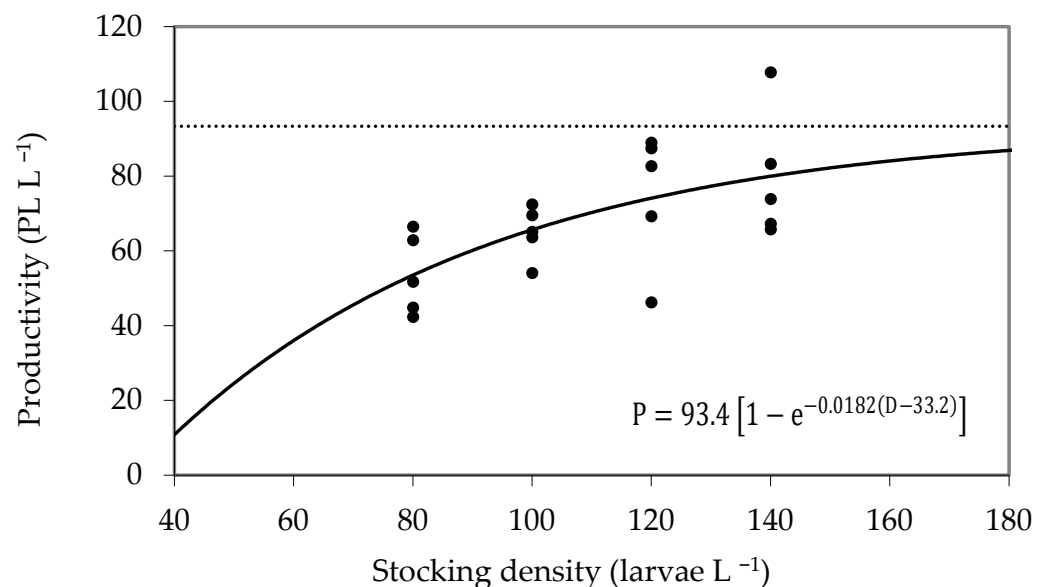
The intensification of the *M. amazonicum* hatchery did not affect the larval development or quality ( $p > 0.05$ ). The LSI did not differ among the treatments with different stocking densities during the rearing cycle (Table 2). The larval condition index (LCI) showed

random variation throughout the rearing cycle above 1.6 and did not differ among stocking densities. The mean LCIs were  $1.70 \pm 0.12$ ,  $1.71 \pm 0.09$ ,  $1.72 \pm 0.09$  and  $1.70 \pm 0.09$  for the 80, 100, 120 and 140 larvae  $L^{-1}$  treatments, respectively.

**Table 2.** Larval stage indices (means  $\pm$  standard deviations) for larviculture of *M. amazonicum* at different stocking densities. CV = coefficient of variation.

Rearing Time (Days)	Stocking Density (Larvae $L^{-1}$ )				F-Value	p-Value	CV (%)
	80	100	120	140			
2	$1.1 \pm 0.1$	$1.4 \pm 0.4$	$1.3 \pm 0.5$	$1.1 \pm 0.0$	0.54	0.66	27.5
4	$2.8 \pm 0.2$	$2.8 \pm 0.5$	$2.7 \pm 0.1$	$2.7 \pm 0.5$	0.04	0.98	14.2
6	$4.5 \pm 0.3$	$4.4 \pm 0.4$	$4.7 \pm 0.2$	$4.5 \pm 0.2$	0.64	0.60	6.2
8	$5.5 \pm 0.3$	$5.2 \pm 0.2$	$5.4 \pm 0.4$	$5.5 \pm 0.3$	0.86	0.49	6.0
10	$6.3 \pm 1.0$	$6.5 \pm 0.6$	$6.8 \pm 0.4$	$6.6 \pm 0.2$	0.40	0.75	9.6
12	$7.9 \pm 0.3$	$7.6 \pm 0.2$	$8.0 \pm 0.3$	$7.8 \pm 0.5$	0.76	0.54	4.7
14	$8.4 \pm 0.2$	$8.3 \pm 0.3$	$8.3 \pm 0.4$	$8.3 \pm 0.2$	0.18	0.91	3.35
16	$8.6 \pm 0.3$	$8.6 \pm 0.1$	$8.7 \pm 0.4$	$8.5 \pm 0.3$	0.28	0.83	3.8
18	$8.7 \pm 0.2$	$9.0 \pm 0.1$	$8.6 \pm 0.2$	$8.6 \pm 0.5$	0.52	0.68	2.8

The average daily *Artemia* use ranged from 2 to 9 nauplii  $mL^{-1}$  and did not differ ( $p > 0.05$ ) among the treatments. However, the amount of *Artemia* used per larvae was lower at high densities ( $p < 0.05$ ), and the *Artemia* used per postlarvae produced was greater at a density of 80 larvae  $L^{-1}$  ( $p < 0.05$ ) (Table 3). Survival and metamorphosis rates were not affected by culture intensification (Table 4). The productivity (PL  $L^{-1}$ ) was lower for the 80 larvae  $L^{-1}$  density than for the other stocking densities ( $p < 0.05$ ) (Table 4). The postlarval dry weight was not significantly different among the tested stocking densities ( $p > 0.05$ ) (Table 4). The productivity of postlarvae  $L^{-1}$  curve as a function of the stocking density showed that the average maximum productivity reached in this system was 93 PL  $L^{-1}$  (Figure 1).



**Figure 1.** Relationships between stocking density and productivity of *Macrobrachium amazonicum* hatchery in recirculating aquaculture system. PL = postlarvae. The dotted line represents the asymptotic maximum productivity, which is estimated by the exponential model.

**Table 3.** Means  $\pm$  standard deviations of the daily consumption of *Artemia*, measured in nauplii per milliliter (mL) of water and in nauplii per larvae, and the total number of nauplii ingested per larvae during culture to reach the postlarvae (PL) stage.

Rearing Time (Days)	Stocking Densities (Larvae L <sup>-1</sup> )							
	80		100		120		140	
	Nauplii mL <sup>-1</sup>	Nauplii Larvae <sup>-1</sup>	Nauplii mL <sup>-1</sup>	Nauplii Larvae <sup>-1</sup>	Nauplii mL <sup>-1</sup>	Nauplii Larvae <sup>-1</sup>	Nauplii mL <sup>-1</sup>	Nauplii Larvae <sup>-1</sup>
2	1.6 $\pm$ 2.3	20 $\pm$ 29	1.3 $\pm$ 1.1	14 $\pm$ 11	1.9 $\pm$ 2.1	16 $\pm$ 19	3.0 $\pm$ 1.0	22 $\pm$ 7
3	3.6 $\pm$ 0.7	46 $\pm$ 14	1.8 $\pm$ 2.7	19 $\pm$ 29	3.7 $\pm$ 1.1	32 $\pm$ 10	4.3 $\pm$ 1.8	32 $\pm$ 14
4	4.5 $\pm$ 1.3	59 $\pm$ 26	4.6 $\pm$ 1.0	49 $\pm$ 11	5.0 $\pm$ 1.2	45 $\pm$ 11	4.2 $\pm$ 1.2	32 $\pm$ 9
5	5.0 $\pm$ 1.5	66 $\pm$ 15	5.1 $\pm$ 1.5	55 $\pm$ 16	5.7 $\pm$ 1.8	52 $\pm$ 15	5.5 $\pm$ 1.3	42 $\pm$ 10
6	5.1 $\pm$ 0.8	68 $\pm$ 8	5.0 $\pm$ 1.8	55 $\pm$ 20	5.5 $\pm$ 0.8	52 $\pm$ 12	5.7 $\pm$ 1.7	45 $\pm$ 13
7	5.9 $\pm$ 1.0	79 $\pm$ 13	5.1 $\pm$ 1.1	57 $\pm$ 12	6.4 $\pm$ 1.7	61 $\pm$ 18	5.8 $\pm$ 1.6	47 $\pm$ 14
8	4.9 $\pm$ 1.9	67 $\pm$ 27	5.5 $\pm$ 0.6	63 $\pm$ 6	6.0 $\pm$ 2.7	57 $\pm$ 24	5.2 $\pm$ 0.9	42 $\pm$ 8
9	5.4 $\pm$ 2.1	74 $\pm$ 23	4.3 $\pm$ 1.9	49 $\pm$ 21	6.0 $\pm$ 1.5	58 $\pm$ 10	6.1 $\pm$ 0.9	50 $\pm$ 7
10	5.7 $\pm$ 2.9	80 $\pm$ 31	6.0 $\pm$ 1.6	71 $\pm$ 17	6.1 $\pm$ 0.9	61 $\pm$ 7	6.9 $\pm$ 1.9	57 $\pm$ 16
11	6.3 $\pm$ 4.5	91 $\pm$ 53	6.4 $\pm$ 1.3	77 $\pm$ 18	5.6 $\pm$ 1.2	57 $\pm$ 12	5.2 $\pm$ 1.1	44 $\pm$ 9
12	5.1 $\pm$ 2.3	75 $\pm$ 59	5.1 $\pm$ 2.3	62 $\pm$ 29	4.8 $\pm$ 1.1	51 $\pm$ 16	5.5 $\pm$ 0.7	47 $\pm$ 7
13	3.9 $\pm$ 0.8	56 $\pm$ 16	5.9 $\pm$ 3.1	74 $\pm$ 41	3.9 $\pm$ 1.8	42 $\pm$ 20	6.6 $\pm$ 2.3	57 $\pm$ 20
14	4.3 $\pm$ 1.9	63 $\pm$ 23	5.0 $\pm$ 1.3	63 $\pm$ 18	5.3 $\pm$ 2.4	60 $\pm$ 36	6.1 $\pm$ 2.2	54 $\pm$ 19
15	5.3 $\pm$ 2.0	79 $\pm$ 27	5.0 $\pm$ 1.4	65 $\pm$ 19	5.4 $\pm$ 2.5	59 $\pm$ 27	6.8 $\pm$ 1.9	60 $\pm$ 15
16	7.3 $\pm$ 5.0	110 $\pm$ 59	6.5 $\pm$ 1.6	85 $\pm$ 21	7.4 $\pm$ 2.5	80 $\pm$ 21	7.3 $\pm$ 3.0	67 $\pm$ 29
17	7.2 $\pm$ 1.6	108 $\pm$ 25	7.7 $\pm$ 1.6	101 $\pm$ 17	7.6 $\pm$ 1.6	88 $\pm$ 30	8.7 $\pm$ 1.9	80 $\pm$ 15
18	6.5 $\pm$ 2.0		9.4 $\pm$ 2.5		8.6 $\pm$ 2.7		6.1 $\pm$ 0.6	
Nauplii PL <sup>-1</sup>	1559 $\pm$ 497 a		1237 $\pm$ 185 b		1230 $\pm$ 423 b		1206 $\pm$ 261 b	

Letters indicate significant differences ( $p < 0.05$ ) among treatments in the same line by an ANOVA followed by the Tukey's test.

**Table 4.** Variables of production (mean  $\pm$  standard deviations) obtained in the larviculture of *M. amazonicum* at different stocking densities. PL = postlarvae.

Variables	Stocking Densities (Larvae L <sup>-1</sup> )			
	80	100	120	140
Survival (%)	80 $\pm$ 5	72 $\pm$ 6	72 $\pm$ 15	75 $\pm$ 5
Metamorphosis rate (%)	67 $\pm$ 13	65 $\pm$ 7	62 $\pm$ 15	57 $\pm$ 12
Productivity (PL L <sup>-1</sup> )	54 $\pm$ 11 a	65 $\pm$ 7 ab	75 $\pm$ 18 b	80 $\pm$ 17 b
PL dry weight (mg)	1.29 $\pm$ 0.10	1.21 $\pm$ 0.20	1.15 $\pm$ 0.20	1.23 $\pm$ 0.30

Letters indicate significant differences ( $p < 0.05$ ) among treatments in the same line by an ANOVA followed by the Tukey's test.

#### 4. Discussion

Intensifying the *M. amazonicum* hatchery up to 140 larvae L<sup>-1</sup> in a simple RAS did not affect the water quality or larval development, well-being, growth or survival. On the other hand, the intensification increased productivity, while decreasing the quantity of *Artemia* used to produce each postlarvae. Consequently, it improved the efficiency of the rearing system. Producing more PL per unit of water, using the same infrastructure may reduce production costs and increase profitability and sustainability because it optimizes the use of resources.

Water variables were kept within the range recommended for *M. amazonicum* larvae at all stocking densities [3,6,29]. Therefore, intensifying the *M. amazonicum* hatchery up to 140 larvae L<sup>-1</sup> does not interfere with the water quality in an RAS comprised of crushed shell bed biofilters, dimensioned at 25% of the total rearing tank volume. This simple system was effective in converting ammonia and nitrite to nitrate and maintaining the temperature, dissolved oxygen, salinity and pH suitable for the culture of *M. amazonicum*.

Larvae presented similar development indices when cultured from 80 to 140 individuals  $L^{-1}$  throughout the rearing cycle. In addition, the media larval condition index obtained in this study was greater than 1.7 at all stocking densities. Therefore, larvae reared at stocking densities up to 140 larvae  $L^{-1}$  exhibit suitable development and satisfactory larval conditions, suggesting that larvae welfare was effective in accordance with McKay et al., 2023 [30].

The survival and metamorphosis rates were similar for stocking up to 140 larvae  $L^{-1}$ . Barreto and Soares [31] reported that no correlation was observed between the stocking density and survival or metamorphosis when *M. amazonicum* is stocked from 10 to 75 larvae  $L^{-1}$ . However, a negative correlation between the larvae stocking density and survival was found for the prawn *M. rosenbergii* [16,17,32] and for fish larvae production systems [33]. The authors attribute the survival reduction at higher stocking densities to cannibalism. In other studies, *M. rosenbergii* larvae cannibalism was related to the stocking density and feeding regime [16,34,35]. Authors recommend that the increase in stocking density must be concomitant with an increase in feeding supply. Nhan et al. [16] suggest that increasing the stocking density to 200 larvae per liter and the feeding frequency to three times a day led to the highest production efficiency of *M. rosenbergii*. Following this management, productivity was 48 PL  $L^{-1}$ , and the consumption of *Artemia* per postlarvae produced was 7100 nauplii. David et al. [17] recommend stocking hatchery tanks with 80 to 100 larvae  $L^{-1}$  to ensure an optimal production of about 50 PL  $mL^{-1}$ . Therefore, the performance of *M. amazonicum* obtained in the present study was higher than that obtained for *M. rosenbergii* in previous studies.

In the present study, larvae were fed “ad libitum”; therefore, the feed was not limiting. There was no significant difference in the consumption of *Artemia*, measured as nauplii  $mL^{-1}$ , among the different stocking densities throughout the rearing cycle. However, nauplii consumption per larvae to reach the stage of postlarvae was higher at 80 larvae  $L^{-1}$  than in higher densities. Thus, larvae stocked at higher densities have a lower predator–prey relationships. According to Barros and Valenti [36], the predator–prey relationship influences consumption because it is associated with the highest number of encounter opportunities. These findings were consistent with those of Maciel et al. [26] for *M. amazonicum*, Gomes et al. [37] for *Macrobrachium equidens* and David et al. [17] for *M. rosenbergii*, respectively.

The highest feeding of *Artemia* at the lowest density did not improve the larval development, survival, metamorphosis rate or final dry weight. Therefore, the efficiency of feed use increased at densities from 100 larvae  $L^{-1}$  on. This suggests the occurrence of superfluous feeding in *M. amazonicum* larvae similar to *M. rosenbergii* (see David et al. [17]). The intensification of the *M. amazonicum* hatchery optimized the *Artemia* cyst use by ~20%. This may represent a substantial expense reduction because *Artemia* is up to 24% of variable costs in freshwater prawn hatcheries [18].

Agonistic behavior and cannibalism were not quantified, but they were observed especially at high densities (120 and 140 larvae  $L^{-1}$ ) during the last days of culture. This is a common behavior in *Macrobrachium* species, as evidenced by Coyle [16,38], and may have occurred due to the competition for space and a lack of shelters. However, the increase in cannibalism was not enough to decrease survival at the highest stocking densities. Cannibalism in a *Macrobrachium amazonicum* hatchery was also reported by Araujo and Valenti [39] at a density of 80 larvae  $L^{-1}$ . Therefore, this behavior does not seem to depend on the farming density.

The maximum mean productivity found in this study was 80 PL  $L^{-1}$  when the stocking density was 140 larvae  $L^{-1}$ . Experimental studies on *M. amazonicum* hatcheries showed mean productivities of 70–75 PL  $L^{-1}$  [26], 64 PL  $L^{-1}$  [40] and 59 PL  $L^{-1}$  [41], for a stocking density of 80 larvae  $L^{-1}$  cultured during ~20 days, like in the present study. The most farmed freshwater prawn in the world is the *Macrobrachium rosenbergii*. New ref. [42] reported that commercial hatcheries of this species, operating in RASs, showed a mean productivity of 50 PL  $L^{-1}$ . Nhan et al. [16] observed a productivity of 48 PL  $L^{-1}$ , stocking

200 larvae L<sup>-1</sup>. David et al. [17] stocked 80 to 140 larvae L<sup>-1</sup> of *M. rosenbergii* and obtained a productivity from 42 to 52 PL L<sup>-1</sup>. The cycle of larviculture is about 22 to 35 days [17,42]. These data show that an *M. amazonicum* hatchery operating in an RAS may be more productive than the hatcheries of *M. rosenbergii*, which have the solid hatchery technologies used in different countries [8].

The model adjusted showed that the potential maximum mean productivity in the studied system was approximately 93 PL L<sup>-1</sup>. Productivity stabilizes around this value even with increasing stocking density. A similar study conducted with *M. rosenbergii* showed the maximum theoretical mean productivity of 51 PL L<sup>-1</sup> [17]. Changes in the production system design or feeding regime may alter the carrying capacity of the system and therefore affect the potential maximum productivity. Nevertheless, no limitation from the feed or water quality was observed in the maximum stocking density tested in the present study as well as in the study of David et al. [17], which was 140 larvae L<sup>-1</sup>. Therefore, the results suggest that these limits are due to the intrinsic characteristics of the species and probably that the space is the principal limiting factor.

## 5. Conclusions

In conclusion, an *M. amazonicum* hatchery may be intensified by at least 140 larvae L<sup>-1</sup> using simple RASs. Productivity may reach around 93 PL L<sup>-1</sup> in about 20 days of culture. The use of *Artemia* nauplii per PL produced is reduced by ~20% as the culture is intensified. Intensification may increase the profitability and sustainability of the system because more PL is produced using the same quantity of water, space, energy and *Artemia*. These experimental results should be confirmed in large commercial tanks. Sustainability and economic studies should be performed to determine the best level of intensification for a hatchery of *M. amazonicum*.

## 6. Patents

This study partially supports the *Macrobrachium amazonicum* hatchery technology patented on the Brazilian patent basis #BR 10 2019 027248 1; 19 December 2019.

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**Data Availability Statement:** Data are contained within the article.

**Conflicts of Interest:** Laurindo André Rodrigues and Janaina Mitsue Kimpara are employed at the Brazilian Agricultural Research Corporation (Embrapa). The authors declare that the conflict of interest did not influence the content and results of the study. The other authors declare no conflicts of interest.



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