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Effects of Shelter on the Hatching, Immune Performance, and Profitability of the Ovigerous Red Swamp Crayfish *Procambarus clarkii* under High Stocking Density

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Abstract: To develop the intensive breeding technology of the seed of the red swamp crayfish *Procambarus clarkii*, the survival rates, hatching effects (hatching rate, incubation level, and number of juveniles), and immune performance of ovigerous *P. clarkia* as well as economic benefits are evaluated under different shelter conditions under a high stocking density in this study. The experimental design includes three different forms of shelter treatments (D1: experiment without any shelters; D2: experiment with closed shelters; D3: experiment with open shelters), each with three replicates. The results show that the concentration of the total antioxidant capacity (T-AOC) and activities of phenoloxidase (PO), catalase (CAT), and acid phosphatase (ACP) in the D3 treatment are higher than those in the D1 treatment (all $p < 0.05$), with the highest concentrations of total antioxidant capacity (T-AOC) and malondialdehyde (MDA) and the highest activities of phenoloxidase (PO), superoxide dismutase (SOD), catalase (CAT), acid phosphatase (ACP), and alkaline phosphatase (AKP) among the treatments being present in the ovigerous *P. clarkii* in the D3 treatment. The hatching rates of the three treatments vary from 69.51% to 94.28%, with the highest rate found in the D3 treatment and the lowest in the D1 treatment, but there is no significant difference among them ($p > 0.05$). The highest incubation level (ind.·m^{-2}) and the highest number of juveniles (ind.·m^{-2}) among treatments are found in the D3 treatment, with the incubation level (ind.·m^{-2}) in the D3 treatment being significantly higher than that in the D1 treatment ($p < 0.05$). The benefit–cost ratios (BCRs) of the D2 and D3 treatments remain significantly higher than that of the D1 treatment when *P. clarkii* prices change (all $p < 0.05$). Our results indicate that a high stocking density habitat with open shelters could effectively improve the hatching and immune performance of ovigerous *P. clarkii*. Our findings are relevant for the indoor aquaculture management of ovigerous *P. clarkii*.

Keywords: open shelter; closed shelter; embryo hatching; broodstock and juvenile management; *Procambarus clarkii*

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1. Introduction

The red swamp crayfish *Procambarus clarkii* (Girard, 1852), originating from south-central United States and northeastern Mexico, has become the most produced freshwater crustacean species in the world due to its rapid growth and high nutritional and commercial value [1,2]. As the top-ranking country in aquaculture, China's *P. clarkii* production had a commercial value of CNY 71 billion and the country produced 2,161,903 tons of *P. clarkii* in 2019, accounting for 62.21% of worldwide freshwater crustacean aquaculture production [1,3].

At present, *P. clarkii* seedlings mainly originate from the self-propagation and self-breeding of *P. clarkii* populations in ponds and paddy fields [4,5]. This breeding method

can satisfactorily meet the seedling needs of the majority of farmers; nevertheless, *P. clarkii* seedlings produced in this way have some shortcomings, such as a wide disparity in individual size, unstable quality, high mortality after transportation, price decline caused by concentrated supply, etc. [6], seriously affecting the sustainable development of the crayfish industry. It is therefore critical to develop intensive breeding technology for *P. clarkii* to remove the technical barrier restricting the sustainable development of *P. clarkii*.

The intensive breeding technology of *P. clarkii* involves high-quality parent selection; parent transport; parent rearing; parent mating and spawning; fertilized egg hatching; and seed rearing. The hatching effect of fertilized eggs directly affects seed yields. Previous studies have indicated that the incubation of crustacean fertilized eggs is closely related to abiotic factors such as temperature, food, light, waterbody eutrophication, salinity, shelter, and stocking density [7–14]. It is well established that stocking density is the key factor in determining profitability [15,16]. However, the negative effects of high stocking density are reflected in crayfish health, food demand, and aquaculture water quality. For example, a high stocking density can increase the feed conversion ratio and nutrient load in water, thereby reducing the immune and digestive performance of crayfish [17–19]. A high stocking density can also increase intraspecific aggression, leading to the high incidence of residual limbs and a high mortality rate [20–22]. However, some studies have indicated that shelters could provide a suitable habitat for *P. clarkii* and effectively reduce the incidence of cannibalism, thereby improving the survival rate [23,24]. To improve the yield and profitability of the crayfish industry, suitable habitats must be created to mitigate the negative effects caused by a high stocking density. Thus, the selection of suitable shelter is a prerequisite for the implementation of high stocking density aquaculture.

Shelter has been widely used in the crayfish industry [23–26], providing a habitat for molting and survival during the growth stage of seedlings. As per their utility in the growth stage of seedlings, shelters that reduce intraspecific attacks and increase the survival of *P. clarkii* can also be used during the breeding stage. Aquatic plant species such as *Elodea nuttallii*, *Vallisneria natans*, and *Eichhornia crassipes*, which have been widely used in aquaculture [27–29], can provide effective shelters for crustaceans. Industrial products such as plastic pipes and artificial plants can also provide shelters for crustaceans [24,30]. During the process of the intensive maternal incubation of *P. clarkii* eggs, aquaculture farmers have attempted to use *Eichhornia crassipes* to provide a sheltered area for *P. clarkii* [29]. However, a high density of aquatic plants decreases the amount of dissolved oxygen in the water at night, affecting the survival of crustaceans [31]. In addition, *P. clarkii* can only utilize a small sheltered area provided by *E. crassipes* as its poor swimming ability prevents it from using the water's middle area [32]. A few studies have compared the hatching effects of ovigerous *P. clarkii* using asbestos tiles, bricks, and PVC pipes as shelters [9,33]. These shelters were too artificially influenced, resulting in significant variations in hatching effects and hindering the specialized production of hatching facilities. In addition, both closed and open shelters can affect *P. clarkii* reproduction, but the effects of using these shelters have not been evaluated. Thus, exploring shelters and developing a stable and effective shelter are essential to achieving the intensive maternal incubation of *P. clarkii*.

We hypothesize that open and closed vertical shelters in hatching systems can effectively improve the hatching performance of ovigerous *P. clarkii* under high stocking density conditions. In this study, two shelter facilities are designed to compare the effects of shelters on the survival, hatching, nonspecific immune ability, and economic benefit of *P. clarkii*. It is hoped that the present study develops *P. clarkii* reproductive performance using artificial shelters in high-density conditions and provides practical information on the indoor cultivation of ovigerous *P. clarkii*.

2. Materials and Methods

2.1. Experimental Materials and Design

The experiment was conducted from 13 October to 7 December 2019, at the Reproduction Center of *P. clarkii*, Huanggang, Hubei Province, China. Nine plastic tanks

(0.85 m × 0.85 m × 0.75 m, L:W:H) were filled with 400 L water collected from a nearby reservoir. The stocking density for this study was determined to be quadruple the maximum feasible stocking density, which was originated from stocking densities of 11 to 21 ind.·m^{-2} for breeding ovigerous *P. clarkii* in existing research [29]. Ovigerous *P. clarkii* (initial body weight: 18.84 ± 0.42 g, initial body length: 78.96 ± 0.63 mm, initial egg number: 259 ± 18 eggs, and initial HSI: 4.65 ± 0.15%) were obtained from the same *P. clarkii* reproduction center and were stocked in three treatments with a high stocking density of 84 ind.·m^{-2} (D1: experiment without any shelters, D2: experiment with closed shelters, and D3: experiment with open shelters), each with three replicates. Two closed shelters were set in each plastic tank in the D2 treatment, and two open shelters were set in each plastic tank in the D3 treatment. The structures of the two multilayer shelters are shown in Figure 1. The closed shelter was a closed vertical box net (0.7 m × 0.25 m × 0.5 m, L:W:H), which was divided into four layers (0.7 m × 0.25 m × 0.1 m, L:W:H). The height between each layer was 10 cm, and the bottom layer was 10 cm away from the bottom of the tank. The side of the box net was covered by a 0.5 cm polyethylene mesh, and the bottom of each layer was covered by a 1 cm polyethylene mesh with a layer of 5 mm soft glass laid above the bottom mesh. A retractable opening was left in the center of the side of each layer for placing or removing ovigerous *P. clarkii*. Six ovigerous *P. clarkii* individuals were placed in each layer. The open shelter was a four-layer structure (0.7 m × 0.25 m × 0.5 m, L:W:H) composed of bricks and perforated plastic plates. The height between each layer was 10 cm, and the bottom layer was 10 cm away from the bottom of the tank. Each layer had plastic plates as the top and bottom plates (0.7 m × 0.25 m × 0.01 m, L:W:H), which were supported by two bricks (0.20 m × 0.03 m × 0.1 m, L:W:H).

During the experiment, *P. clarkii* were fed to satiation twice daily with a 30% protein commercial feed (Guangdong Haida Feed Co., Ltd., Guangzhou, China). A microporous oxygenation system was installed at the bottom of each plastic tank to ensure a sufficient dissolved oxygen level. Water was exchanged every 3 days, and the exchange amount was 1/3 of the water volume. At the beginning of the experiment and on the 25th day of the experiment, the concentrations of total nitrogen (TN; mg L^{-1}), total phosphorus (TP; mg L^{-1}), ammonia nitrogen (NH_4^+-N; mg L^{-1}), nitrate (NO_3^--N; mg L^{-1}), nitrite (NO_2^--N; mg L^{-1}), and chemical oxygen demand (COD_{Mn}; mg L^{-1}) were measured using a standard method [34], and the turbidity (TUR; NTU) was measured by a turbidimeter (HACH 2100Q, USA). During the experiment, pH and dissolved oxygen (DO; mg L^{-1}) were measured by a YSI ProPlus meter (Thermo Fisher Scientific Company, USA) every day, and the water temperature (WT; °C) was automatically measured by a thermometer (HOBO UA-001-64, USA) every 8 h.

2.2. Sample Collection

At the beginning of the experiment, 30 ovigerous *P. clarkii* individuals were randomly selected from the temporary rearing female *P. clarkii*, and the growth parameters and the egg number were measured. Determination of growth parameters included body length (L), weight (W), hepatopancreas weight (HW), and hepatosomatic index (HSI).

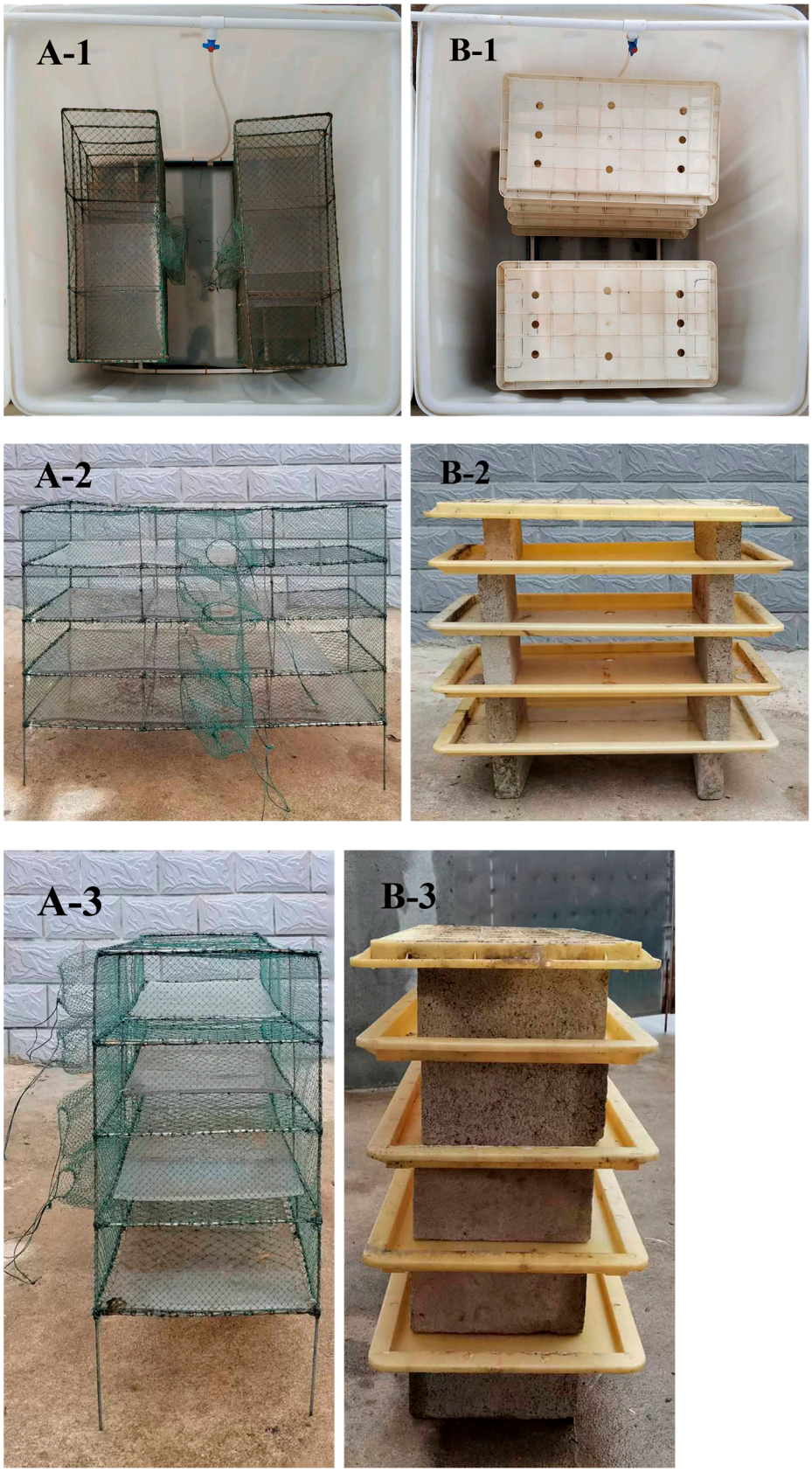

Figure 1. Top view image of the closed shelter (**A-1**) and the open shelter (**B-1**); front view image of the closed shelter (**A-2**) and the open shelter (**B-2**); lateral view image of the closed shelter (**A-3**) and the open shelter (**B-3**).

On the 25th day of the experiment, all ovigerous *P. clarkii* had hatched. The number of ovigerous *P. clarkii* in each plastic box was counted, and 10 ovigerous *P. clarkii* individuals were randomly sampled from each treatment to record the incubation levels of each individual. Then, the incubation levels per unit area (that is the incubation level (ind.·m^{-2}), abbreviated as "incubation numbers") and the hatching rate were calculated. Hemolymph and hepatopancreas were collected from ovigerous *P. clarkii* samples for nonspecific immune parameter determination. An ovigerous *P. clarkii* sample was anesthetized in an ice bath for 10 min, and hemolymph from the cardiac sinus of the sample was collected using syringes and immediately centrifuged at 9000 rpm and 4 °C for 20 min [35]. Subsequently, the hepatopancreas was aseptically collected, homogenized in saline at a ratio of 1:9 (hepatic sample weight:saline volume), and centrifuged [35]. After centrifugation, the supernatant was frozen in liquid nitrogen and stored at 80 °C for determination. The experiment ended after 55 days, the number of juveniles was recorded, and the juvenile quantity per unit area (that is, the juvenile quantity (ind.·m^{-2}) abbreviated as "juvenile numbers") was calculated. Parameters were calculated as follows:

$$\text{HSI (\%)} = (\text{hepatopancreas weight}/\text{weight}) \times 100\%$$

$$\text{Survival rate (\%)} = \text{final ovigerous } \textit{P. clarkii} \text{ numbers}/\text{initial ovigerous } \textit{P. clarkii} \text{ numbers} \times 100\%$$

$$\text{Incubation level (ind.·m}^{-2}\text{)} = \text{the number of } \textit{P. clarkii} \text{ larvae}/\text{bottom area of the plastic tank}$$

$$\text{Hatching rate (\%)} = \text{the number of } \textit{P. clarkii} \text{ larvae}/\text{egg number} \times 100\%$$

$$\text{Juvenile quantity (ind.·m}^{-2}\text{)} = \text{the number of } \textit{P. clarkii} \text{ juveniles}/\text{bottom area of the plastic tank}$$

where the number of *P. clarkii* larvae refers to the number of first instar larvae attached to the abdomen of *P. clarkii* and egg number refers to the number of eggs attached to the abdomen of *P. clarkii*.

2.3. Nonspecific Immune Parameter Determination

The total protein (NO. MM-9227B); the concentrations of total antioxidant capacity (T-AOC, NO. MM-91115O1) and malondialdehyde (MDA, NO. MM-90004O1); the activities of superoxide dismutase (SOD, NO. MM-0740O1), catalase (CAT, NO. MM-0741O1), acid phosphatase (ACP, NO. MM-1443O1), and alkaline phosphatase (AKP, NO. MM-91119O1) in the hepatopancreas; and the activity of phenoloxidase (PO, NO. LB5509B) in the hemolymph of ovigerous *P. clarkii* were determined by enzyme-linked immunosorbent assay (ELISA) kits (Jiangsu Meimian Industrial Co., Ltd.), and then the samples were measured by a microplate reader (Rayto, RT-6100, China). Specific experimental procedures were given by Luo and Xu [36,37].

2.4. Profitability Analysis

The profitability of breeding ovigerous *P. clarkii* in different treatments was measured by gross profit and benefit–cost ratio (BCR) [38], since gross profit refers to sale income minus product cost, which is the real measure of profitability, and the benefit–cost ratio (BCR) is the ratio of income and cost used to compare benefit per unit of cost. In addition, sensitivity analysis was used to evaluate the relationship between production, fixed costs, variable costs, and sale price per unit. In this experiment, the price of juvenile and adult *P. clarkii*, which represents the price most easily affected by the market, was selected for the sensitivity analysis to determine the relationship between variable parameters and expected profits [39]. The profitability (profitability refers to the benefit-cost ratio) at the new value of variable cost was calculated at 5%, 10%, 15%, and 20% of the variation range in variable parameters (both positive and negative) by keeping other inputs constant. Parameters were calculated as follows:

$$\text{Gross profit (RMB)} = \text{income} - \text{total cost}$$

Benefit-cost ratio (BCR) = total income/total cost

2.5. Statistical Analysis

Descriptive statistics are presented as the mean ± standard error (SE). First, we used the Shapiro test to test for normality and the Levene test to test for homogeneity of variance. For parameters (pH, TN, TP, NH_4^+-N, NO_2^--N, COD_{Mn}, TUR, HSI, survival rate, hatching rate, incubation numbers, and immune parameters) that met normality and homogeneity of variance, one-way ANOVA was used to test the difference among treatments implemented in R using the package "stats" [40]. Where applicable, a post hoc multiple comparison test (Tukey test) was used to determine specific differences among treatments implemented in the R package "emmeans" [41]. For parameters (WT, DO, NO_3^--N, the juvenile numbers, gross profit, and BCR) that did not meet normality and homogeneity of variance, multiple nonparametric, one-way ANOVAs (Kruskal–Wallis tests) were used to test the difference among treatments also implemented in the R package "coin" [42], and a two-way nonparametric ANOVA (Scheirer–Ray–Hare test) was used to test the BCR parameter difference among treatments in the sensitivity analysis implemented in the R package "rcompanion" [43]. If the difference was significant, pairwise comparisons were performed using the Kramer (Nemenyi) test with Tukey–Dist approximation [44] in the R package "PMCMRplus" [45]. Logarithmic transformation was applied to all percentage data before testing. Statistical differences were considered significant at $p < 0.05$. All data analyses and figures were performed in R Version 4.1.0 (R Core Team, 2019).

3. Results

3.1. Water Quality

The water temperature was suitable for ovigerous *P. clarkii* during the experiment, with an average water temperature of 20.18 ± 0.15 °C and no significant difference among treatments (Kruskal–Wallis test, $\chi^2 = 2.36$, $p = 0.31$; Table 1). The dissolved oxygen concentrations were high, ranging from 8.13 to 8.43 mg L^{-1}, with no significant difference among treatments (Kruskal–Wallis test, $\chi^2 = 5.84$, $p = 0.054$). After 25 days of the experiment, the concentration of nutrients such as TN and NH_4^+-N, NO_3^--N and COD_{Mn} in the plastic boxes significantly increased, but there was no significant difference in TN, NH_4^+-N, NO_3^--N among treatments (one-way ANOVA, TN: $F = 6.24$, $p = 0.03$; NH_4^+-N: $F = 10.21$, $p = 0.01$; Kruskal–Wallis test, NO_3^--N: $\chi^2 = 8.16$, $p = 0.04$). The COD_{Mn} was significantly different among treatments (one-way ANOVA, TUR: $F = 57.17$, $p = 0.00$). The COD_{Mn} in the D1 treatment was significantly higher than that in the D3 treatment (Tukey test: $p = 0.03$).

Table 1. Water quality parameters (mean ± SE) at the beginning of the experiment and during the experiment with three different treatments. The WT, DO, and pH values were observed for 55 days. The TN, TP, NH_4^+-N, NO_2^--N, NO_3^--N, COD_{Mn}, and TUR values were observed over 25 days. Different letters within the columns indicate significant differences among different treatments ($p < 0.05$).

Water Quality Parameters	At the Beginning of the Experiment	During the Experiment with Three Treatments		
		D1	D2	D3
WT (°C)		20.45 ± 0.24	19.99 ± 0.22	20.10 ± 0.29
DO (mg L^{-1})		8.14 ± 0.14	8.43 ± 0.13	8.13 ± 0.12
pH		8.14 ± 0.02	8.07 ± 0.02	8.13 ± 0.03
TN (mg L^{-1})	2.18 ± 0.29 ^{a}	6.33 ± 1.09 ^{b}	5.19 ± 0.09 ^{b}	6.75 ± 0.22 ^{b}
TP (mg L^{-1})	0.34 ± 0.02	0.46 ± 0.11	0.65 ± 0.03	0.46 ± 0.02
NH_4^+-N (mg L^{-1})	0.43 ± 0.01 ^{a}	1.58 ± 0.37 ^{b}	1.24 ± 0.10 ^{ab}	1.39 ± 0.09 ^{b}
NO_2^--N (mg L^{-1})	−0.00 ± 0.00	0.53 ± 0.15	0.19 ± 0.07	0.71 ± 0.21
NO_3^--N (mg L^{-1})	0.09 ± 0.00 ^{a}	1.36 ± 0.56 ^{ab}	1.99 ± 0.02 ^{ab}	2.39 ± 0.17 ^{b}
COD_{Mn} (mg L^{-1})	8.29 ± 2.38 ^{a}	27.10 ± 0.63 ^{c}	22.88 ± 1.95 ^{bc}	18.80 ± 0.35 ^{b}
TUR (NTU)	4.71 ± 0.45	10.08 ± 2.66	8.14 ± 0.84	10.03 ± 3.24

3.2. Survival and HSI

The survival rate of *P. clarkii* in the D3 treatment was higher than that in the D1 and D2 treatments, but there was no significant variation among treatments (one-way ANOVA: $F = 1.62$, $p = 0.27$; Figure 2). The HSI of *P. clarkii* after 25 days of the experiment was lower than that of ovigerous *P. clarkii* at the beginning of the experiment. The HSI of *P. clarkii* did not significantly differ among treatments (D1 and D2, D3) (one-way ANOVA: $F = 0.38$, $p = 0.69$; Figure 3).

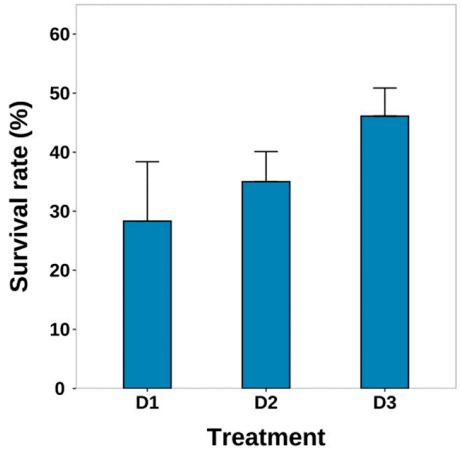

Figure 2. Survival rate (%) of ovigerous *P. clarkii* in the three different treatments after 25 days.

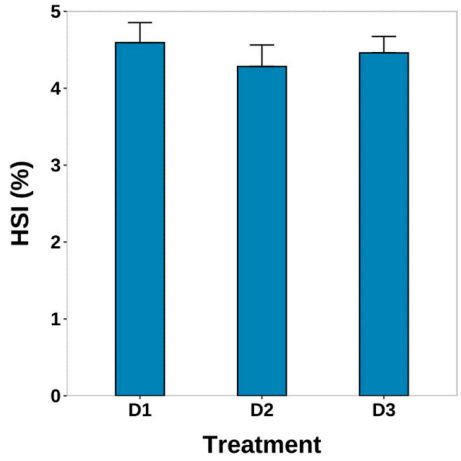

Figure 3. HSI (%) of ovigerous *P. clarkii* in the three different treatments after 25 days.

3.3. Nonspecific Immune Condition

The highest T-AOC, MDA concentration, and the highest SOD, CAT, ACP, AKP, PO activities of ovigerous *P. clarkii* were observed in the D3 treatment. Although there were no significant differences in MDA concentration and SOD, AKP activities of ovigerous *P. clarkii* among different treatments (one-way ANOVA, MDA: $F = 3.39$, $p = 0.06$; SOD: $F = 2.24$, $p = 0.14$; AKP: $F = 3.13$, $p = 0.07$; Figure 4), there were significant differences in T-AOC, CAT, ACP, PO activities of ovigerous *P. clarkii* (one-way ANOVA, T-AOC: $F = 4.34$, $p = 0.03$; CAT: $F = 18.43$, $p = 0.00$; ACP: $F = 11.96$, $p = 0.00$; PO: $F = 11.89$, $p = 0.00$; Figure 4). The concentration of T-AOC of ovigerous *P. clarkii* in the D3 treatment was higher than that in the D2 treatment (Tukey test: $p = 0.70$) and significantly higher than that in the D1 treatment (Tukey test: $p = 0.03$). In addition, the activities of CAT, ACP, PO of ovigerous *P. clarkii* in the D3 treatment were significantly higher than those in the other treatments (D1 and D2) (Tukey test: all $p < 0.05$).

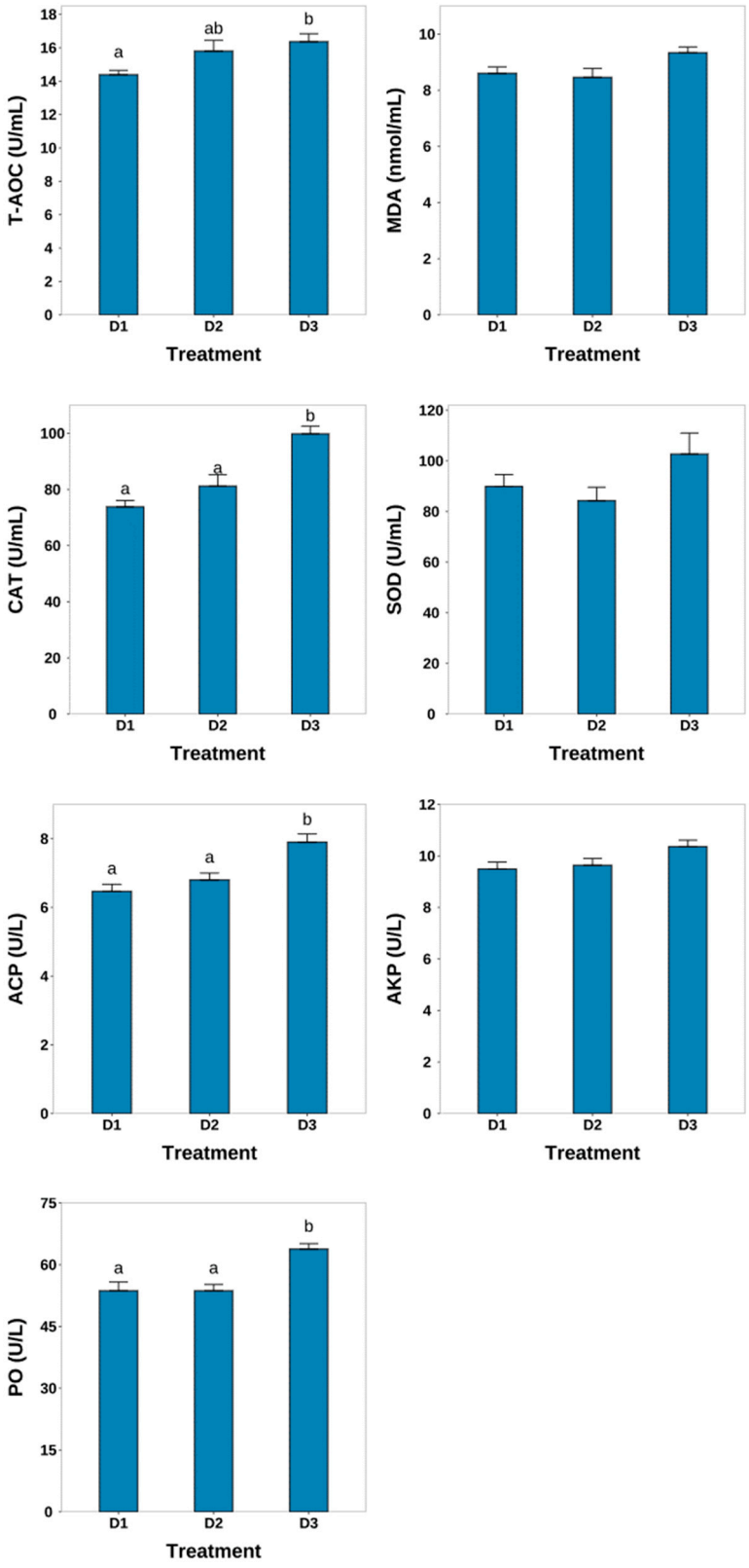

Figure 4. The concentrations of T-AOC and MDA and the activities of CAT, SOD, ACP, AKP, and PO in ovigerous *P. clarkii* at different shelters after 25 days. Different lowercase letters indicate significant differences ($p < 0.05$) among shelter treatments.

3.4. Hatching Condition

The hatching rate of ovigerous *P. clarkii* ranged from 69.51% to 94.28% among the three treatments (Figure 5). There was a fact that the hatching rate and the incubation numbers in the D3 treatment were higher than those in the D1 and D2 treatments, with the D1 treatment having the lowest hatching rate and incubation numbers. No significant difference existed in the hatching rate of *P. clarkii* among treatments (one-way ANOVA: $F = 3.31$, $p = 0.052$). However, there was a significant difference in the incubation numbers of *P. clarkii* among treatments (one-way ANOVA: $F = 5.16$, $p = 0.049$; Figure 6), and the incubation numbers in the D3 treatment were significantly higher than that in the D1 treatment (Tukey test: $p = 0.045$). The juvenile numbers in the D3 treatment were higher than that in the D1 and D2 treatments, although no significant difference was found compared with the D1 and D2 treatments (Kruskal–Wallis test: $\chi^2 = 2.76$, $p = 0.25$; Figure 7).

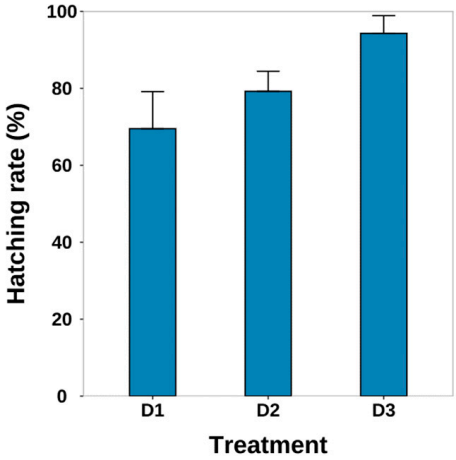

Figure 5. Hatching rate (%) of ovigerous *P. clarkii* in different shelters after 25 days.

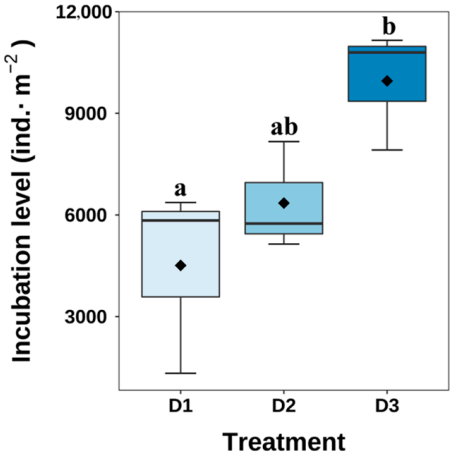

Figure 6. Incubation levels (ind.·m^{-2}) of ovigerous *P. clarkii* in the three different treatments after 25 days. The diamond symbol inside the box represents the mean. Different lowercase letters indicate significant differences ($p < 0.05$) among shelter treatments.

3.5. Profitability Analysis

There was no significant difference in the gross profit and the benefit-cost ratio (BCR) among treatments (Kruskal–Wallis test, gross profit: $\chi^2 = 2.76$, $p = 0.25$; BCR: $\chi^2 = 2.76$, $p = 0.25$; Table 2); the gross profit and the BCR in the treatments with the multilayer shelters (D2 and D3) were higher than those in the treatment without the shelter (D1), and the highest gross profit and BCR was in D3 treatment.

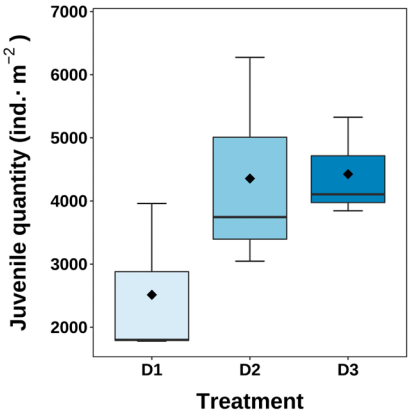

Figure 7. Number of juvenile (ind.·m^{-2}) ovigerous *P. clarkii* in the three different treatments after 55 days. The diamond symbol inside the box represents the mean.

Table 2. The economic benefits (gross profit and BCR; mean ± SE) of breeding *P. clarkii* at different shelter treatments.

Treatment		*P. clarkii*	Quantity	RMB. Unit^{-1}	Cost (RMB)	Gross Profit (RMB. m^{-2})	BCR (Ratio. m^{-2})
D1	Income	Juvenile	1816 ind.	0.15. ind.$^{-1}$	272.42	275.24 ± 108.48	5.13 ± 1.48
	Expenditure	Adult	0.83 kg	70. kg^{-1}	58.13		
		Feed	5.14 kg	3. kg^{-1}	15.42		
D2	Income	Juvenile	3146 ind.	0.15. ind.$^{-1}$	471.97	548.99 ± 147.10	8.67 ± 1.95
	Expenditure	Adult	0.83 kg	70. kg^{-1}	58.13		
		Feed	5.73 kg	3. kg^{-1}	17.19		
D3	Income	Juvenile	3197 ind.	0.15. ind.$^{-1}$	479.60	559.46 ± 68.53	8.80 ± 0.91
	Expenditure	Adult	0.83 kg	70. kg^{-1}	58.13		
		Feed	5.76 kg	3. kg^{-1}	17.27		

Sensitivity analysis demonstrated that there was no significant difference in the BCR obtained for 5%, 10%, 15%, and 20% changes (both positive and negative changes) in the *P. clarkii* price (Scheirer–Ray–Hare: $H = 17.84$, $p = 0.33$), but there was a significant difference in the BCR of different treatments after changing the *P. clarkii* price (Scheirer–Ray–Hare: $H = 50.19$, $p = 0.000$), and the interaction between the two conditions was not significant (Scheirer–Ray–Hare: $H = 0.44$, $p = 1.00$; Table 3). When other conditions remained unchanged, the BCR of different treatments increased or decreased by 5% for every 5% fluctuation of the juvenile *P. clarkii* price. However, the adult *P. clarkii* price fluctuated by 5%, and the corresponding BCR changing range of the different treatments was inconsistent. If the price of adult *P. clarkii* decreased by 5%, the BCRs of the D1, D2, and D3 treatments increased by 4.04%, 4.05%, and 4.06%, respectively. If the price of adult *P. clarkii* decreased by 20%, the BCRs of the different treatments increased by more than 10%; the BCRs of the D1, D2, and D3 treatments increased by 18.69%, 18.29%, and 18.29%, respectively. Similarly, if the price of adult *P. clarkii* increased by 20%, the BCRs of different treatments decreased by more than 10%; the BCRs of the D1, D2, and D3 treatments decreased by −13.71%, −13.34%, and −13.32%, respectively. Under the conditions of various changes in the prices of juvenile and adult *P. clarkii*, the BCR of the D1 treatment was significantly lower than that of the D2 and D3 treatments (Tukey–Kramer (Nemenyi) test: all $p < 0.000$), and the difference between the last two was not significant (Tukey–Kramer (Nemenyi) test: $p = 0.38$).

Table 3. The sensitivity analysis of the BCR (mean ± SE) to variations in the juvenile *P. clarkii* price and adult *P. clarkii* price.

Scenario	BCR (Ratio. m^{-2})		
	D1	D2	D3
Business as usual	5.13 ± 1.48	8.67 ± 1.95	8.80 ± 0.91
If juvenile price reduced by 5%	4.87 ± 1.40	8.24 ± 1.86	8.36 ± 0.86
If juvenile price reduced by 10%	4.61 ± 1.33	7.81 ± 1.76	7.92 ± 0.82
If juvenile price reduced by 15%	4.36 ± 1.25	7.37 ± 1.66	7.48 ± 0.77
If juvenile price reduced by 20%	4.10 ± 1.18	6.94 ± 1.56	7.04 ± 0.73
If juvenile price increased by 5%	5.38 ± 1.55	9.11 ± 2.05	9.24 ± 0.95
If juvenile price increased by 10%	5.64 ± 1.62	9.54 ± 2.15	9.68 ± 1.00
If juvenile price increased by 15%	5.90 ± 1.70	9.97 ± 2.25	10.12 ± 1.05
If juvenile price increased by 20%	6.15 ± 1.77	10.41 ± 2.34	10.57 ± 1.09
If adult price reduced by 5%	5.34 ± 1.54	9.02 ± 2.03	9.16 ± 0.95
If adult price reduced by 10%	5.57 ± 1.60	9.40 ± 2.12	9.54 ± 0.98
If adult price reduced by 15%	5.82 ± 1.67	9.81 ± 2.21	9.96 ± 1.03
If adult price reduced by 20%	6.09 ± 1.75	10.26 ± 2.31	10.41 ± 1.07
If adult price increased by 5%	4.93 ± 1.42	8.35 ± 1.88	8.48 ± 0.88
If adult price increased by 10%	4.75 ± 1.37	8.05 ± 1.81	8.17 ± 0.84
If adult price increased by 15%	4.58 ± 1.32	7.77 ± 1.75	7.89 ± 0.81
If adult price increased by 20%	4.43 ± 1.27	7.51 ± 1.69	7.63 ± 0.79

4. Discussion

4.1. Survival

The choice of shelter, which can mitigate the negative effects caused by a high stocking density, determines profitability in the intensive breeding of *P. clarkii* seedlings. We bred ovigerous *P. clarkii* under similar water temperatures and dissolved oxygen conditions with high stocking densities without shelters and with open shelters and closed shelters. As the experiment progressed, dead ovigerous *P. clarkii* appeared in different treatments, which may have been due to the high energy consumption of ovigerous *P. clarkii* and high-density stress during hatching. The following results were observed: (1) High energy consumption resulted in increased mortality [46]. The HSI of *P. clarkii* after hatching was lower than that of ovigerous *P. clarkii* at the beginning of the hatching period, which was related to energy storage and loss [47], indicating that ovigerous *P. clarkii* consumed substantial amounts of energy for reproductive output. (2) High-density stress resulted in increased intraspecific competition and mortality [21,22].

The survival rate of ovigerous *P. clarkii* in the open shelter (D3) was highest among the high stocking density treatments, which was related to shelter structure. The multilayer shelters in the study provided a larger habitat. The closed multilayer area of the group-rearing *P. clarkii* was too small and confined, which resulted in a low survival rate due to negative behavior among individuals in the limited area [48]. However, the open multilayer area could be interconnected, wherein *P. clarkii* freely moved. The open shelter with interconnected areas created a complex and variable habitat and reduced intraspecific competition among *P. clarkii*. Similar results were found in a study by Corkum LD, in which habitat complexity significantly reduced *Orconectes propinquus* intraspecific aggression and increased food consumption [49]. Thus, the intraspecific competition of *P. clarkii* in the open shelter had less of an impact on the survival rate.

4.2. Nonspecific Immune Condition

High stocking density impairs the growth and metabolism of crustaceans by affecting their immune systems [50,51]. This should be instigated by the production of reactive oxygen species (ROS). Higher ROS concentrations increase the risk of DNA damage, lipid peroxidation, and protein denaturation. This suggests that when an organism is stimulated, this affects the immune system and may even cause cellular damage and metabolic inhibition [52]. Thus, monitoring the activities of immune-related enzymes is

a useful method to evaluate the health of *P. clarkii*. The levels of the determined immune factors of ovigerous *P. clarkii* in the D3 treatment were highest among the treatments, in which the activities of PO, CAT, and ACP were significantly higher than those in the other treatments (D1 and D2) and the concentration of T-AOC was significantly higher than that in the D1 treatment. The effects of high stocking density treatment with the open shelter (D3) on the immune enzymes of ovigerous *P. clarkii* could be explained by the fact that the D1 and D2 treatments produced higher environmental stress than did the D3 treatment, and the immune systems of ovigerous *P. clarkii* in the D1 and D2 treatments were damaged following excessive stress, resulting in their inability to respond with high immunological levels.

PO, CAT, and ACP are important aspects of the crustacean immune system and are responsible for eliminating harmful substances in crustaceans caused by external stimuli [53–55]. Thus, the concentration of T-AOC and the activities of immune enzymes such as PO, CAT, and ACP in crustaceans were increased under stress [56,57]. However, it has been reported that immunological levels in crustaceans decrease under excessive stress: (1) A weakened immune response was demonstrated in crustaceans under poor water quality conditions [58]; (2) *Euastacus armatus* was immunosuppressed under captive stress and exhibited declining PO activity [59]; and (3) a significant declining trend in the activities of CAT and ACP was demonstrated in *P. clarkii* and *Palaemonetes sinensis* under excessive density stress [18,60]. A high stocking density increased TN and NH_4^+-N, NO_3^--N, and COD_{Mn} levels in this study, with significantly higher COD_{Mn} levels exhibited in the D1 treatment than in the D3 treatment. The ovigerous *P. clarkii* resided in an unsheltered treatment (D1), only moving at the bottom of the box and in turn stimulating the release of organic matter from sediment. The behavior of *P. clarkii* indirectly increased the COD_{Mn} levels in a manner similar to that induced by river resuspension [61]. The ovigerous *P. clarkii* in the D2 and D3 treatments were able to live in the shelters and in turn disturb sediment less frequently. These results indicate that the D1 treatment was associated with poor water quality and excessive density stress, which reduced immunity levels compared with those of the D2 and D3 treatments. The closed shelter treatment (D2) was subjected to captive stress compared with the open shelter treatment (D3). Considering that *P. clarkii* exhibits anxiety-like characteristics [62], we believe that confinement stress could directly induce psychological stress and competitive stress in *P. clarkii* [63] and indirectly modulate the activity of immune cells in *P. clarkii* [64,65]. In addition, the survival rates, hatching rates, and incubation levels of ovigerous *P. clarkii* in the D3 treatment were higher than those in the D1 and D2 treatments. Thus, we concluded that the ovigerous *P. clarkii* in the D3 treatment responded with high immunity levels under the high stocking density condition, whereas the ovigerous *P. clarkii* in the D1 and D2 treatments had impaired immune systems due to environmental stress, excessive density stress, and confinement stress and could not respond with high levels of immunity. It has been reported that fertilized eggs are rich in polyunsaturated fatty acids, which can act as ROS substrates [66]. However, the embryo also has immune capacity [67], and its immune capacity is not affected when oxidative stress occurs in the mother [68]. Therefore, density stress primarily affected the health status of ovigerous *P. clarkii*, and the open shelter was advantageous for maintaining the immune system stability of ovigerous *P. clarkii*.

4.3. Hatching Conditions

Different hatching conditions affect the hatching rate of *P. clarkii*. It has been demonstrated that the hatching rate of *P. clarkii* is relatively high in a salinity range of 0–4 practical salinity units (psu) [69]. Abnormalities and the death of all eggs have been observed at high temperatures above 29 °C, whereas no embryo abnormalities were observed below 25 °C [10]. In this study, the hatching rate under different shelters ranged from 69.51% to 94.28%. The hatching rate of ovigerous *P. clarkii* in the open shelter treatment (D3) was highest, but there were no significant differences among the treatments. Existing research has shown that as stocking density increases, hatching success decreases [70]. However, multi-

layer shelter treatments with high stocking density, especially an open shelter treatment with a 94.28% hatch rate, could effectively reduce egg loss due to density stress. Density stress also led to changes in incubation levels. The incubation levels in the open shelter treatment (D3) were higher than those in the closed shelter treatment (D2) and significantly higher than those in the D1 treatment without shelter, clearly demonstrating the advantage of the shelter, which is suitable for the hatching of *P. clarkii* in the breeding period.

Newly hatched *P. clarkii* juveniles need to develop in the maternal abdomen until they are able to freely move, which enhances the viability and evolutionary potential of the offspring [71]. At present, no research has reported the time when the juveniles of *P. clarkii* are separated from their mothers. It is necessary for juvenile breeding to synchronize with the mother during the primary juvenile breeding period. The open shelter treatment (D3) in this study exhibited the highest number of juveniles, but there was no significant difference between the treatments. This result indicates that an open shelter favors the survival of juvenile *P. clarkii* under an appropriate high density. However, size variation within groups increases with extended culturing times, and dominant individuals exclude smaller individuals from food resources [72]. High intraspecific competition among juveniles reared in an environment with excessive density stress (caused by high incubation levels such as in the D3 treatment) results in a decreased number of juveniles [73]. Thus, the balance between the impact of space size and stocking density on the mother and juveniles should be further researched.

4.4. Profitability Analysis

The economic benefit of the open shelter treatment (D3) was higher than that of the other treatments, but there was no significant difference between the treatments. The monthly price of *P. clarkii* significantly changed, which affected the farm's profitability. Thus, the price of juvenile and adult *P. clarkii* was selected for a sensitivity analysis. When the yield and feed cost obtained from the study were known, we could predict the effects of juvenile and adult *P. clarkii* variable prices on the economic benefits among different shelter treatments [38]. The sensitivity analysis found that farmers benefited most from higher juvenile *P. clarkii* prices and were hurt more by lower juvenile *P. clarkii* prices than they were from adult *P. clarkii* price changes. In addition, the BCR in the D1 treatment remained significantly lower than those in the D2 and D3 treatments, regardless of variation in *P. clarkii* prices. Based on these results, we concluded that the use of shelter at a high stocking density resulted in higher economic benefits.

5. Conclusions

Under high stocking density conditions, the health status (the indicators of which include immunological level and HSI) of ovigerous *P. clarkii* negatively changed, which affected their survival rate and hatching rate. However, shelter reduced the risk of density stress and increased the survival rate, hatching rate, number of juveniles, and profitability of *P. clarkii*. Notably, the open shelter treatment, which created a complex habitat structure for the *P. clarkii*, demonstrated a significant positive effect on the immune performance and incubation levels of breeding *P. clarkii* under a high stocking density. The present study provides practical information on the indoor cultivation of ovigerous *P. clarkii*.

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