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Study on Ecological Three-dimensional Culture Model of *Procambarus clarkii* and *Rhodeus sinensis*

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Abstract: *In order to establish an ecological three-dimensional culture model of Procambarus clarkii and Rhodeus sinensis. A density gradient of 10,000 and 20,000 tails per mu was set, and the seedling mass was 5.3231±1.6827 g; 5,000 Rhodeus sinensis (1.3256±0.2126 g) were reared in a mixed manner respectively. compared with the group without Rhodeus sinensis, they were reared for a total of 130 days, and were reared for large and small from the 40th day onwards. The results showed that the average body mass of low-density shrimp group was significantly higher than that of high-density shrimp group, but there was no significant difference between the two groups. After stocking the Chinese sole, the shrimp output in the low-density group increased by 9.23 % compared with that in the control group, while that in the high-density group increased by 19.69 %. The survival rate of shrimp in high density group was 11 % higher than that in control group. Compared with the control group, the total mass of Chinese sole in the low-density group decreased by 23.3 %, and that in the high and low-density group decreased by 35.8 %. the average body mass was significantly lower than that before stocking. The results showed that the ecological polyculture model was beneficial to the growth of Procambarus clarkii population, but it would destroy the stability of the fish population.*

Keywords: *Procambarus clarkii, Cherax quadricarinatus, Rhodeus sinensis, breeding model*

1. Introduction

Procambarus clarkii and *Cherax quadricarinatus* is a large freshwater crayfish native to the southern United States and northern Mexico [1,2]. *Procambarus clarkii* is called crayfish in China. it has strong viability and has been spread to other parts of the world for natural reproduction, forming local populations. it has become a world-wide edible shrimp. Since it was introduced into China in the 1930s, it has been widely distributed in rivers, lakes, ditches, ponds and rice fields in our country, especially in the middle and lower reaches of the Yangtze river [3]. Crayfish is very popular in the market because of its rich nutrition, chewy meat and delicious taste. At present, it has become an excellent new breed of freshwater aquaculture in China's southern and northern regions. The cultivation area and output of crayfish in our country continue to grow rapidly. From 2007 to 2016, the national crayfish cultivation output increased from 265,500 tons to 989,100 tons, reaching an economic output value of 146.610 billion, with nearly 5 million employees in the entire industry chain. it is one of the most popular cultivation varieties in recent years [4].

A study on the feeding habits of *Procambarus clarkii* shows that it is a omnivorous animal that is carnivorous. It mainly feeds on animal baits such as snails, mussels, small fish and shrimps, and occasionally feeds on plants such as aquatic plants [5]. In the current method of shrimp cultivation, a large amount of animal baits such as feed and animal leftovers are mainly put in, which makes the water quality easy to pollute and induces shrimp outbreak diseases [6,7]. *Rhodeus sinensis* is a small fish in the middle and lower layers, which has the characteristics of feeding on dead animal bodies [8]. therefore, this paper will carry out ecological mixed culture of the Chinese sole and *Procambarus clarkii*, use the sole to remove the residual bait, establish an ecological breeding mode, and study the growth characteristics of shrimp and fish under this mode.

2. MATERIALS AND METHODS

2.1. Experimental Materials

The Procambarus clarkii used in the experiment came from Suzhou AoLong biotech co., ltd., and the mass of the shrimp larvae was 5.323±1.6827g. The Chinese ray fish came from the small fish living resources conservation pool in Lixiahe river, Jiangsu vocational college of agriculture, animal husbandry and science and technology, with a body weight of 1.3256±0.2126g. Mussels are *Anodontawoodiana* from Qinhu lake, Taizhou, with a body mass of 138±68.7356g. The body mass of *Bellamyaquadrata* is 2.5389±0.4415g.

2.2. Pond Conditions

The experimental pool number is 1-4 #, and the area of 1-4 # pool is 666.7m^2 with cement slope protection. The pond was surrounded by an anti-escape wall made of 0.8 m thick plastic film, which was buried under the soil for 0.3 m and the ground height was 0.5 m and was fixed with bamboo sticks. Microporous aeration equipment is installed in the pond. roots blower has a power of 2200 watts and six microporous aeration discs with a diameter of 1 m are installed in each pond. One end of the pond is provided with a water inlet pipe and the other end is provided with a drain pipe. The water depth of the experiment was controlled at 1~1.5 m. The surface of the water is covered with 1/4 of the peanuts. The water source used in the experiment is the river channel connecting the Yangtze river. the water quality meets the standard of aquaculture water. the transparency is 0.5 m, pH7.6, $NH4^+$< 0.1 mg/L, $NO2^-$< 0.05 mg /L, and the hardness is 132 mg/L based on $CaCO_3$.

2.3. Experimental Methods

On March 10, 2017, quicklime was used to disinfect the pond at a rate of 0.15 kg per square meter. On April 3, water was poured into the pond at a rate of 10 cm, and buds of *Elodea nuttallii* and *Hydrilla verticillata* were planted. On April 10, water was poured into the pond to 0.5 m. after being dissolved with 1 kg / mu of amino acid fertilizer and water paste, the whole pond was poured and inoculated with 7.5mL/m^2 of *Chlorella pyrenoidosa* with a concentration of 3.15× 10^8 cultivated in the laboratory of Jiangsu vocational college of agriculture and animal husbandry science and technology. On April 23, *Rhodeus sinensis* was put in, and 6.75 kg, or about 5,000 tails, were put in pools 1 and 3. Fry of *Procambarus clarkii* was put on April 25, and 53.2 kg, about 10,000 tails, were put on pools 1 and 2. Pool 3 and pool 4 put 106.4 kg, about 20,000 tails. On May 6, 250 kg *Bellamya quadrata* and 100 kg *Anodontawoodiana* were put into each pond.

During the experiment, the quality of shrimp was measured every 10 days. lobster commercial feed and chilled small fish were mixed and fed at a rate of 2 % of the daily feeding weight of commercial feed and 20 % of the daily feeding weight of chilled small fish. After June 5, four 20-meter-long cages will be placed in the pond at 19:00 respectively. the cages will be folded at 06:00 in the morning, shrimp will be poured out and sorted. more than 20 grams of fishing will be listed and small ones will be kept. On September 5, one-time drainage fishing was carried out to calculate the total output and survival rate and quality of *Rhodeus sinensis*.

2.4. Data Processing

All the data were sorted by excel 2010 and analyzed by SPSS19.0 statistical software. ANOVA and Duncan were compared to test the difference between the average values of the groups. P<0.05 was significant difference, p<0.05 was no significant difference.

3. RESULTS AND ANALYSIS

3.1. Determination of Growth Rate of *Procambarus clarkia*

The results of testing shrimp body quality every 10 days (Figure 1). In the 130 natural long term, the shrimp body mass showed a linear growth overall, among which the growth rate of pool 1 was higher than pool 2, pool 2 was higher than pool 3, and pool 3 was higher than pool 4. During 20 days of stocking, there was no significant difference in shrimp body mass among ponds. the number 4 pond was significantly lower than the number 1 pond and the number 2 pond from the 30th day to the 130th day (p<0.05). In the 50-130 days, except the 130 days, the shrimp body mass of pool 4 was significantly lower than that of pool 3 (p<0.05). Starting from the 80th day, the average body mass of shrimp in pool 1 was significantly higher than that in other ponds (p<0.05).

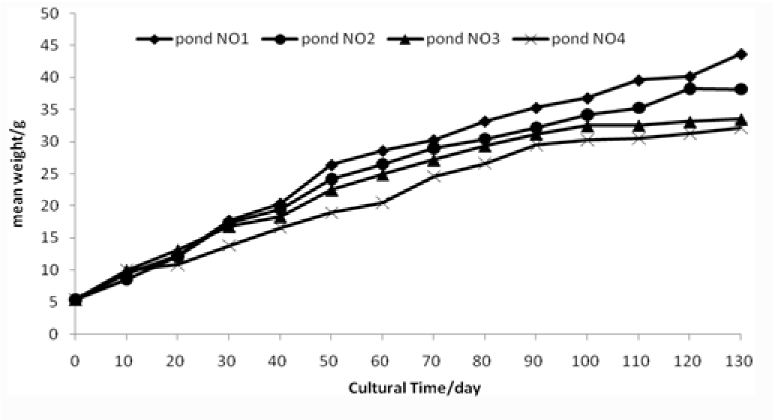

Figure1. *Average quality of Procambarus clarkii at different cultivation times*

The average body mass of *Procambarus clarkii* in different ponds during the growth cycle (Figure 2). Pond 1 is 32.74±12.27 g, pond 2 is 30.62±10.93g, pond 3 is 29.45±9.44 g, and pond 4 is 28.11±9.07 g. The average body mass of ponds 1 and 2 was significantly higher than that of ponds 3 and 4. There was no significant difference between pool 1 and pool 3 where sole was placed and pool 2 and pool 4 where sole was not placed ($p>0.05$). Pools 1 and 2 were significantly higher than pools 3 and 4 ($p<0.05$).

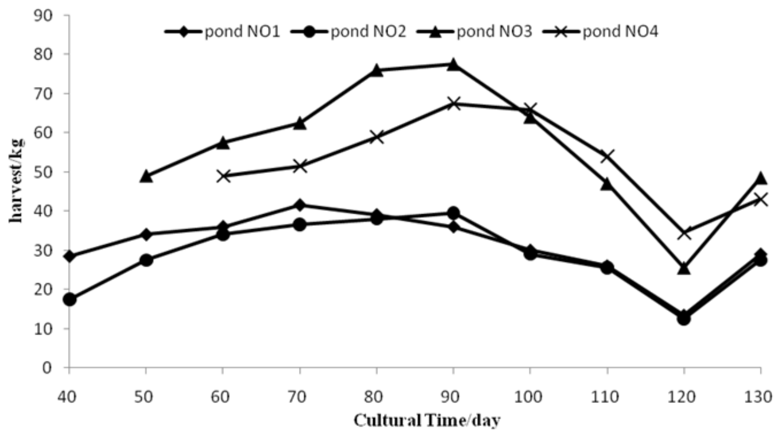

Figure2. *Average weight of Procambarus clarkii in different pond growth periods*

Judging from the growth rate, ponds 1 and 2 with a stocking density of 15 tails /m^3 grew faster than ponds 3 and 4 with a stocking density of 30 tails/m^3, which indicated that the competition for survival of concentrated ponds with a density higher than 15 tails /m^3 affected the growth rate. The growth rate of ponds No.1 and No.3 in which swimming fish were placed was faster than that of ponds No.2 and No.4, but the difference did not reach a significant level, which indicated that swimming fish had a certain role in the ecological system of intensive pond, but the effect was not obvious under the setting of this experiment.

3.2. Determination of Yield Rate of *Procambarus clarkia*

Starting from June 5, catch large and small prawns, each time catching more than 20 g of prawns. see figure 3 for the harvest. After 40 days of growth, some shrimp bodies in ponds 1 and 2 reached the market specifications of 20 grams and began fishing. among them, pond 1 caught 28.5 kg for the first time and pond 2 caught 17.5 kg. Ponds 3 and 4 have fewer large shrimps at this time. After 50 days of growth, No.3 pond caught 49 kg. After 60 days of growth, pool 4 caught 49 kg. During the fishing period, the single catch in each pond gradually increased, reaching a single catch peak on the 70th day of pond 1 cultivation, reaching a single catch peak on the 90th day of pond 2, pond 3 and pond 4 cultivation, and then the catch decreased. The catch was less on the 120th day of cultivation and was cleared to catch on the 130th day.

Looking at the growth rate of low density ponds 1 and 2 from the start of catching time, some shrimps have reached the commercial specifications since the 40th day. The higher density pools 3 and 4 reached the specifications on the 50th and 60th days respectively. Judging from the single catch, the number 3 and 4 pond with higher density were higher than the number 1 and 2 pond with lower density, among which the number 3 pond was higher than the number 4 pond 100 days before and lower than the number 4 pond afterwards. The first 80 d of pool 1 was higher than that of pool 2, and the catch was close to that of pool 2.

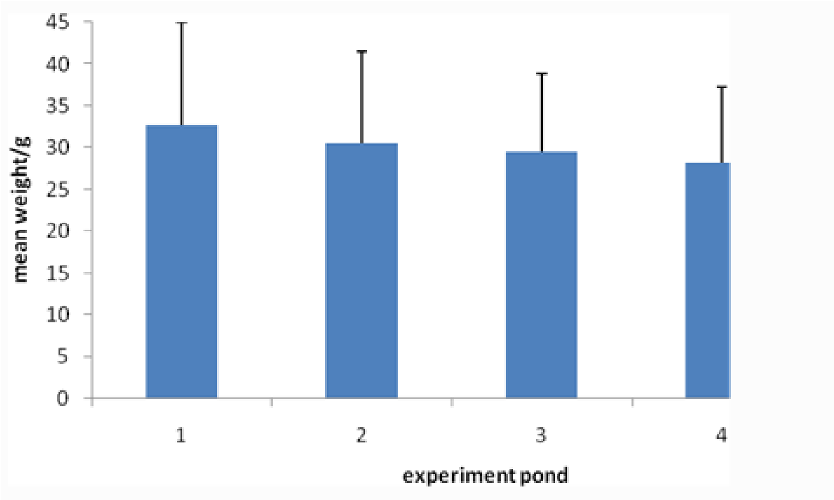

Figure3. *Weight of commercial shrimp captured at one time at different cultivation times*

The total output of shrimp in four ponds during the whole breeding cycle is shown in figure 4.313.5 kg of adult shrimp were harvested in pool 1, 287 kg of adult shrimp were harvested in pool 2, 507.5 kg of adult shrimp were harvested in pool 3, and 424 kg of adult shrimp were harvested in pool 4. The total output of shrimp in pool 3 was significantly higher than that in pool 4 (p<0.05), while that in pool 4 was significantly higher than that in pools 1 and 2 (p<0.05). There was no significant difference between pools 1 and 2 (p<0.05). Judging from the total output, the total output of ponds No. 1 and No. 3 where the sole was reared was higher than that of ponds No. 2 and No. 4 where the sole was not reared.

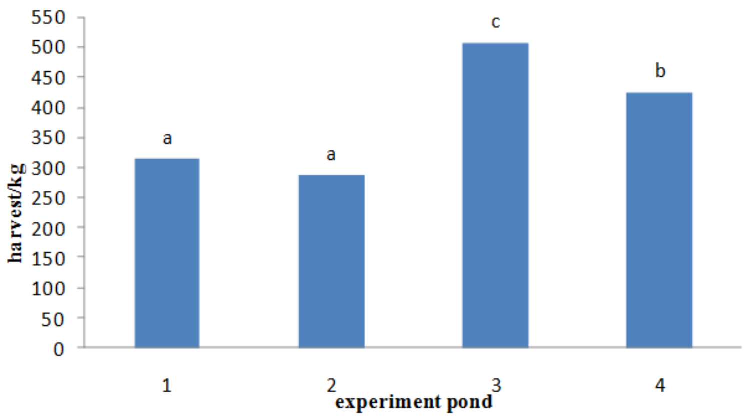

Figure4. *Total output of Procambarus clarkii in different pond growth periods*

3.3. Survival Rate of *Procambarus clarkia*

The survival rate of *Procambarus clarkii* in four ponds during the cultivation period (Figure 5). The survival rate of ponds 1 and 2 was above 90 %, with no significant difference (p>0.05). The survival rate of No.3 pond was 86 %, and that of No.4 pond was 75 %. the survival rate of No.3 pond was significantly higher than that of No.4 pond (p<0.05), but both were significantly lower than that of No.1 and No.2 ponds (p<0.05). The results showed that the higher the density, the lower the survival rate would be. There was no significant difference in survival rate between stocking sole and non-stocking sole under the condition of low density of 10,000 tails / mu, but there was a large difference in high density of 20,000 tails / mu, which indicated that mixed stocking sole was effective in improving the survival rate of *Procambarus clarkii*.

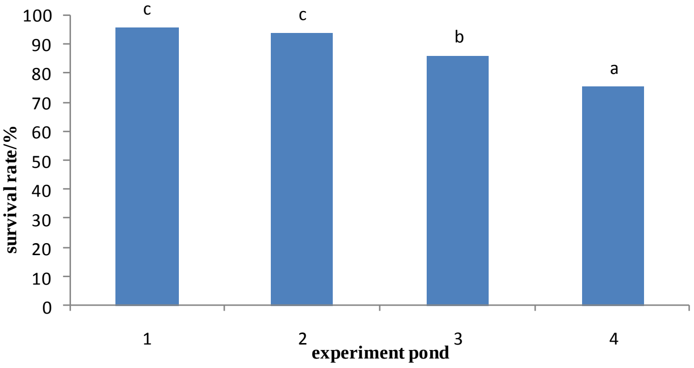

Figure5. *Survival rate of Procambarus clarkii in different ponds*

3.4. The Change of *Rhodeus sinensis* in Pond

3.4.1. Change of Total Mass of Rhodeus sinensis

See for changes in the total mass of pond sole fishes in which sole fishes are reared during the breeding period (Figure 6). Pond 1 harvested 5.18 kg of sole, a decrease of 23.3%, pond 3 harvested 4.33 kg, a decrease of 35.8%. All of them were significantly lower than the weight of 6.75 kg when stocking ($p<0.05$). Pool 3 was significantly lower than that of pool 1 ($p<0.05$). The results showed that stocking *Rhodeus sinensis* would lose a part of the fish, and the higher the shrimp density, the greater the amount of fish species lost.

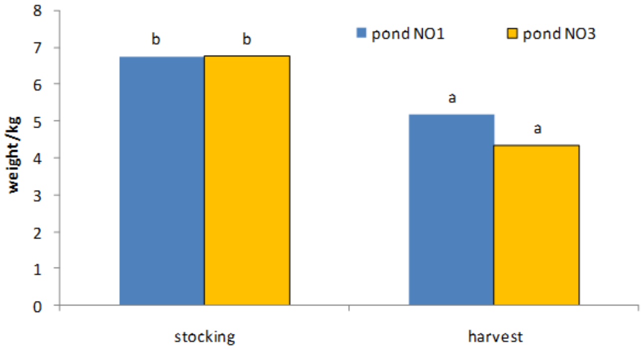

Figure6. *Total mass change of sole in different ponds*

3.4.2. Change in Quantity of Rhodeus sinensis

Changes in the number of *Rhodeus sinensis* in different ponds (Figure7). Pond 1 harvested 6094 rays, an increase of 20.4%, pond 3 harvested 4655 rays, a decrease of 9.08 %. Pond number 1 was significantly higher than that of stocking ($p<0.05$). There was no significant difference in the harvest quantity and stocking quantity of pool 3 ($p>0.05$). Many new small fish were found when the sole was harvested. The results showed that the sole can reproduce normally and maintain a certain population density in the *Procambarus clarkii* culture pond.

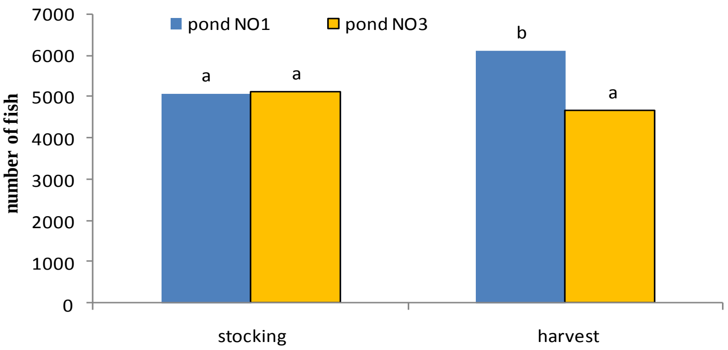

Figure7. *Changes in the number of Rhodeus sinensis in different ponds*

3.4.3. Changes in average body mass of Rhodeus sinensis

See figure 8 for the change of the average body mass of pond sole fish that reared sole fish during the breeding period. The average body mass of pond 1 was 0.85 g and pond 3 was 0.93 g.. All of them were significantly lower than the body mass when stocking ($p<0.05$). The results showed that stocking sole in *Procambarus clarkii* culture pond would reduce the individual size of the population. It may be that large individuals are eaten by shrimp in the process of spawning and living in the bottom layer, while the larvae can easily survive in the surface layer of water after they leave the mussel.

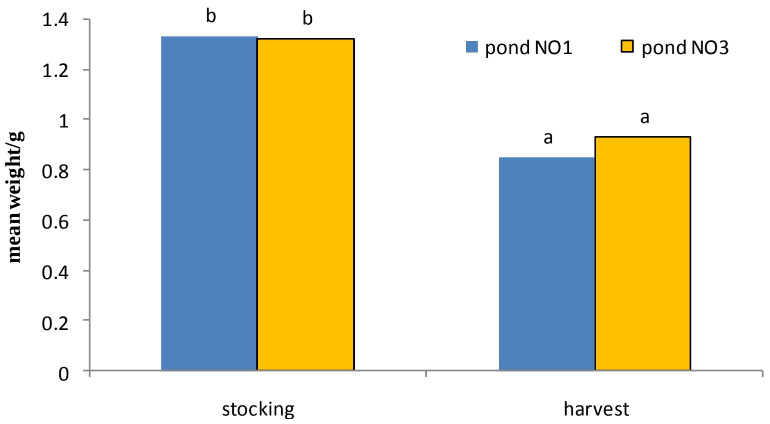

Figure8. *Average body weight change of sole in different ponds*

4. DISCUSSION

At present, *Procambarus clarkii* is very popular in domestic breeding, with an output value of over 100 billion yuan, forming a huge industry. The main breeding objects are *Procambarus clarkii* native to America and *Procambarus clarkii* native to Australia [4]. The breeding modes of *Procambarus clarkii* mainly include pond culture [9], shrimp and crab mixed culture [10], shrimp and mandarin fish mixed culture [11] and rice field culture [12], etc. Among them, the shrimp and crab mixed culture mode planted a large number of aquatic plants [13], with thinner water quality and cleaner shrimp body surface. Other cultivation modes will release fertilizer, farm manure and other fertilizer and water, and shrimp shells, appendages and gills are easy to bear ciliates, causing shrimp to become dirty [14]. Animal leftovers, fresh ice fish and the like are often used as auxiliary baits in the breeding process. In this kind of culture environment, there are many residual baits and excrement in the water body, the ammonia nitrogen, nitrite and total bacteria in the water body are all high, the water body is easy to lack oxygen, and shrimp disease outbreaks are often induced.

Rhodeus sinensis is a small fish in the middle and lower layers and has the characteristics of feeding on dead animal bodies. During reproduction, the sole needs to inject eggs into the gill cavity of the mussel through a long spawning tube. after the sperm eggs are fertilized in the gill cavity, the fertilized eggs leave the mussel after developing into seedlings in the gill cavity of the mussel [15]. Mussels live mainly by filtering organic particles suspended in water bodies such as algae, bacterial biofilms, small protozoa, etc. therefore, mussels have the function of purifying water quality [16].

In order to solve the problem that there are many residual baits and water quality is easy to pollute in the process of *Procambarus clarkii* cultivation, this study established an ecological three-dimensional cultivation model of cynomolgus carinata by using the characteristics of the residual baits eaten by Chinese sole and the characteristics of the filtered and purified water quality of freshwater mussels. The scope of this experiment shows that after stocking Chinese sole in the *Procambarus clarkii* pond system, the yield of low density group increased 9.23% and that of high density group increased 19.69%. The survival rate of the high-density group increased by 11% after stocking Chinese sole, but had little effect on the average body mass of *Procambarus clarkii*. the results showed that stocking Chinese sole in *Procambarus clarkii* culture pond could significantly improve its yield and survival rate. After stocking sole in *Procambarus clarkii* pond, the number of sole will decrease, the population will experience miniaturization, and the greater the density, the more obvious the trend will be. During the experiment, the phenomenon of *Procambarus clarkii* catching injuries and feeding on sole was often observed, which indicated that mixed culture with *Procambarus clarkii* would cause damage to the sole population.

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