

## Research Article

### Effects of four factors on *Penaeus monodon* post-larvae cannibalism

Jiang S.<sup>1,3</sup>; Zhou F.L.<sup>1</sup>; Zeng X.Y.<sup>2,3</sup>; Yang Q.B.<sup>2</sup>; Huang J.H.<sup>2</sup>; Yang L.S.<sup>2</sup>; Jiang S.G.<sup>2\*</sup>

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#### Abstract

In order to study the cannibalism behavior of *Penaeus monodon* post-larvae, the effects of four internal and external factors, such as individual size, post-larvae density (50, 80, 110, 140, 170 ind/L), food abundance and aeration, on the cannibalism rate were analyzed. The results showed that the cannibalism rate was significantly affected by individual size, post-larvae density and food abundance. When post-larval density increased from 50 ind / L to 110 ind / L, the average daily cannibalism rate increased by 358.07%. When larval density was more than 110 ind/L, cannibalism rate slightly increased. The average daily cannibalism rate decreased by 69.81% in 20 ind/mL food group than that of no-food group. The mix of individuals in different sizes could increase cannibalism rate significantly. Sufficient aeration could decrease cannibalism rate. The results indicated that insufficient food was a main reason to cause cannibalism in *Penaeus monodon* post-larvae. High stocking density and differences of individual size could induce and stimulate the cannibalism.

**Keywords:** *Penaeus monodon*, Cannibalism, Individual size, Post-larvae density, Food, Aeration

1-Shenzhen Base of South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Shenzhen 518108, P.R. China.

2-Key Lab. of South China Sea Fishery Resources Utilization, Ministry of Agriculture, South China Sea Fisheries Research Institute, Chinese Academy of Fishery Sciences, Guangzhou 510300, Guangdong, China.

3-College of Fisheries and Life Science, Shanghai Ocean University, Shanghai 201306, China

\*Corresponding author's Email: jiangsg@21cn.com

## Introduction

Due to the influence of biological nature character and environmental stress factors, lower aquatic animals produce aggression, fighting and other behaviors among similar individuals to acquire more space or resources. The most serious phenomenon of fighting behavior is the cannibalism. Cannibalism refers to the phenomenon that large (strong) individuals attack and swallow small (weak) individuals among the same individuals, resulting in injury or death of the attacked. (Dou *et al.*, 2000). The direct impact of cannibalism on the aquaculture industry is reflected in decrease in the number of seedlings cultivated per unit of water, the reduction in the survival rate of the culture, and the economic benefits of the aquaculture industry. Therefore, study the cannibalism behavior of aquatic animals can better understand the biological learning and provide reference for the development of measures to reduce cannibalism in aquaculture production. At present, there are many studies on the cannibalism of aquatic animals and most of them focused on fish (Katavić *et al.*, 1989; Polis *et al.*, 1989; Smith and Reay, 1991; Folkvord and Otterå, 1993; Hobson and Welch, 1995; Qin and Fast, 1996; Dou *et al.*, 2000). Now the current views mainly focus on two aspects for the reasons of the cannibalism: internal factors such as genetic relationships, individual size differences, feeding habits, sex and reproductive status, neurochemical factors and external factors such as food abundance, food species, living space,

light, water turbidity and so on (Li and Sun, 2013), while the main causes of cannibalism are still unclear.

The black tiger shrimp (*Penaeus monodon*, Crustacea, Decapoda, Penaeidae) is an economically and globally important marine species, and plays an important role in the aquaculture industries of southeast provinces in China (Jiang *et al.*, 2018). Cannibalism is very common in crustaceans such as *Penaeus monodon*, but the research in this field is only found in *Fenneropenaeus chinensis* (Zhang *et al.*, 2008), *Penaeus japonicus* (Chen *et al.*, 2003) and *Penaeus vannamei* (Wang *et al.*, 2015) and there are few reports on *P. monodon*. Therefore, the effects of individual size, larval density, food abundance and aeration on the cannibalism of *P. monodon* larvae were studied in order to provide reference for reducing cannibalism in the process of breeding.

## Materials and methods

### *Experimental materials*

The experiment was conducted in Shenzhen Base of South China Sea Fisheries Research Institute, Chinese Academy of Fisheries Sciences from April to May, 2018. The post-larvae of 6th day (PL6) and 10th day (PL10) of *P. monodon* “Nanhai No.2” were cultured by our research group and more than 4000 post-larvae were taken and cultured temporarily for 24 hours before the initiation of the experiment. Continuous aeration was carried out throughout the experiment. The shrimp were temporarily cultivated in water temperature of  $28.0 \pm 0.5^\circ\text{C}$ , salinity of

28 ppt, and pH of 8.0–8.3. Seawater was filtered through a sand filter, and sterilized by ultraviolet light.

### *Experimental design*

#### *Shrimp density experiment*

The experiment was carried out in a beaker with one liter volume. The PL6 and PL10 of *P. monodon* were cultivated in the densities of 50, 80, 110, 140, 170 ind per liter. Each treatment was set up with three parallel groups and the bait (commercial feed for *P. monodon*, Guangdong Haida Group) was fed once a day, and 50% water from the bottom was changed once before a sufficient amount of the bait was fed. The activity status of the post-larvae was observed and the dead individuals were removed as early as possible, and the experiment was carried out for 7 days. Individuals who were physically disabled or even separated from the head and chest can be observed by naked eyes were defined as the cannibalism individuals and the body without limb incomplete observed by microscope was considered as a natural death individual. The number of dead individual was counted separately and supplemented the shrimps to achieve the experimental design density.

#### *Bait abundance experiment*

One hundred ind PL6 and PL10 of *P. monodon* were set in a beaker of one liter, and the amount of bait was 0, 5, 10, 15, 20 ind *Artemia salina* larvae per milliliter of water. Each treatment was set up with three parallel groups. Bait abundance was observed at every 2 h

and feeding was restocked after each observation. The experiment was carried out for 4 days. Daily management and data collection were the same as density experiment.

#### *Individual specification experiment*

Fifty ind PL6 and 50 ind PL10 of *P. monodon* were set in a beaker of one liter, and the amount of bait was 0 and 20 ind *Artemia salina* larvae per milliliter of water. Each treatment was set up with three parallel groups. The cannibalism was observed at every 12 h and the experiment was carried out for 4 days. Daily management and data collection were the same as density experiment.

#### *Inflation experiment*

Fifty ind PL10 of *P. monodon* were set in a beaker of one liter, three gradients were set up for aeration, including small (S group), medium (M group) and high (H group), and three parallel groups were set for each gradient. The aeration volume was regulated by aeration valve. The amount of bait was 30 ind *Artemia salina* larvae per milliliter of water. The water surface of S group was just visible, the water surface of M group was obvious and the water surface of H group was boiling. The results were recorded every 8 hours and the experiment was carried out for 2 days. Daily management and data collection were the same as density experiment.

*Determination of cannibalism rate**Statistical analysis*

Cannibalism rate /% = number of dead shrimp by cannibalism / number of total shrimp at the beginning of experiment \*100

Daily cannibalism rate /% = number of dead shrimp in one day by cannibalism / number of total shrimp at the beginning of experiment \*100

Average cannibalism rate /% = cannibalism rate / experiment days

Results are presented as means  $\pm$  SE of three replicates. All data were subjected to one-way analysis of variance (ANOVA). When there were significant differences, the group means were further compared with Duncan's multiple-range test. All statistical analyses were performed using SPSS version 19.0 (SPSS Inc., Michigan Avenue, Chicago, IL, USA) for Windows.

**Results***Observation on cannibalism behavior of post-larvae shrimp*

During the experiment, the behavioral habits and cannibalism behavior of post-larvae shrimp were observed. It was found that the larvae preferred to support themselves with their forelimb, and their abdomen moved slowly or motionlessly. When swimming, it mainly relied on the swing by the swimming foot, and the tail fan spread to maintain the body balance and control the direction. It was common to observe post-larvae shrimp swam in abdomen upward way to backstroke, swimming foot swung frequently, foot movement was not obvious, backstroke lasted for a short time, then the body turned over to maintain a normal

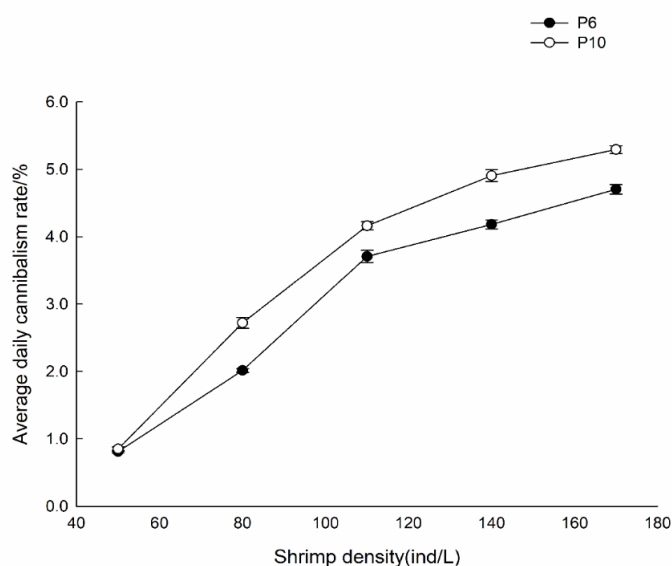
swimming posture. When the two post-larvae met during swimming, they would hold each other with their foot and grasped each other for a few times and the fight was not intense, and then one of the post-larvae bounced off. When a shrimp swam toward the other one at a distance of about 2 cm or 2 positions, one of the shrimp would suddenly hit the other one with an antenna or a rostrum, and the attacked shrimp would either retreat a few steps or bounce back quickly and rarely hit back. Sometimes one shrimp jumped onto the other and surrounded its head and chest with the foot. The shrimp below shook its body quickly or crawled forward quickly. Aggressive behavior mostly occurred between individuals with significant differences in individual specifications, generally small individuals were attacked or avoided by large individuals. When there was no cannibalism, the attack intensity of the shrimp was not strong and the duration was not long. In the presence of cannibalism, one shrimp hit the other's eye several times with rostrum, then stepped underneath to hold the other's breastplate and began to eat foot and carapace. After the death of shrimp, the phenomenon of eating from

the tail would also occur, mostly from the swimming feet, there was also the phenomenon that many shrimp scrambling to eat the dead one.

#### *Effect of post-larval density on cannibalism*

With increasing post-larval density, the average daily cannibalism rate of *P. monodon* gradually increased. When post-larval density exceeded 110 ind/L, the increase of the average daily

cannibalism rate decreased (Fig. 1). When the density of post-larvae increased from 50 ind/L to 110 ind/L, the average daily cannibalism rate increased by 358.07%. Statistical analysis showed that post-larvae density significantly affected the average daily cannibalism of post-larvae. The differences between the treatments were significant except for the difference between 140 and 170 ind/L treatments.

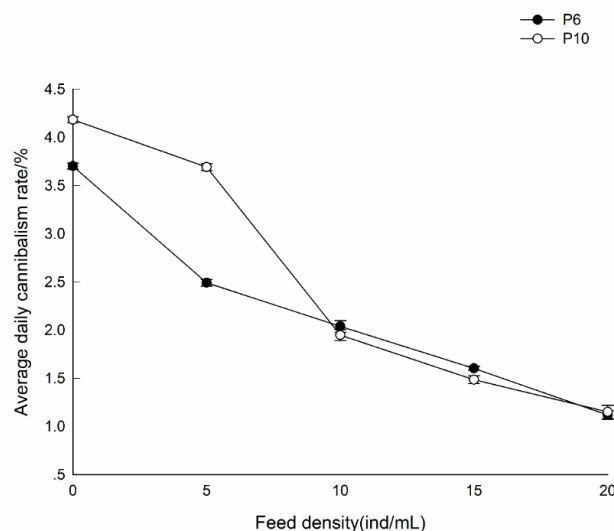


**Figure 1: Average daily cannibalism rate of *P. monodon* post-larvae in different density.**

#### *Effect of bait abundance on cannibalism*

The average daily cannibalism rate of *P. monodon* post-larvae decreased with increasing of feed abundance, and decreased by 69.81% from non-feeding to 20 ind/mL of *Artemia salina* (Fig. 2). Statistical analysis showed that the

average daily cannibalism rate of the group with food deficiency was significantly higher than that of the group with adequate food. The main reason for the increase of post-larvae cannibalism rate in *P. monodon* was the lack of food.



**Figure 2:** Average daily cannibalism rate of *P. monodon* post-larvae in different feed density.

#### *Effect of different individual specifications post-larvae polyculture on cannibalism*

The average daily cannibalism rate of post-larvae groups with different individual specifications was significantly higher than that of the groups with the same individual

specifications. The average daily cannibalism rate of bait absence groups was significantly higher than that of the bait sufficient groups. The average daily cannibalism rate of bait absence groups was 131.08% higher than the bait sufficient groups in the polyculture groups (Table 1).

**Table 1: Cannibalism results in different individual size.**

Test group	Feed amount of bait (ind/mL)	Average daily cannibalism rate/%
PL6	0	3.70±0.03 <sup>c</sup>
PL6	20	1.12±0.04 <sup>a</sup>
PL10	0	4.19±0.03 <sup>d</sup>
PL10	20	1.15±0.07 <sup>a</sup>
PL6+PL10	0	6.22±0.20 <sup>c</sup>
PL6+PL10	20	2.69±0.05 <sup>b</sup>

Notes: All values are presented as means±SD. Different superscript letters indicate significant differences among groups within each treatment.

In the bait sufficient groups, the average daily cannibalism rates of different individual specifications groups (DIS groups) were higher than those of PL6, PL10 single specification groups (SS groups) by 140.74% , 133.90%, respectively, and in the bait absence groups, the average daily cannibalism

rates of DIS groups were higher than those of the PL6 and PL10 SS groups by 67.94% and 48.57%, respectively, which indicated that individual differences of specification could promote cannibalism (Fig. 3).

### Effect of the amount of inflation on cannibalism

With the extension of time, the death by cannibalism increased gradually. The death rate of S group was the largest, and the cumulative mortality rate was 73.41% and 166.18% higher than that

of M group and B group in 48 h, respectively. Statistical analysis showed that there were significant differences in cumulative mortality of 48h between the three experimental groups, which indicated that increasing the amount of aeration in the process of seedling cultivation could reduce the cannibalism.

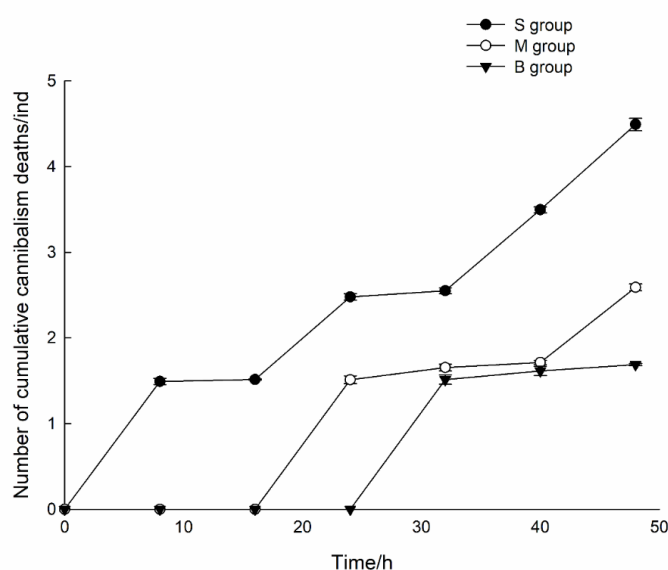


Figure 3: Number of cannibalism deaths at different aeration rates.

### Discussion

Many kinds of animals, especially aquatic animals, have the phenomenon of cannibalism, especially in their early development stage. There are many factors affecting the cannibalism of aquatic animals, such as larval density, bait abundance, individual specifications, illumination, aeration, shelter, and so on (Li and Sun, 2013). The results showed that the cannibalism rate of *P. monodon* post-larvae increased with the increase of shrimp density and the decrease of diet abundance. Individual size differences also promoted the occurrence of cannibalism. At the same time, the

cannibalism rate of the same species would be decreased to a certain extent by increasing aeration during larval culture, which is similar to the findings of many scholars on fish and other crustaceans (Moksnes *et al.*, 1998; Darias *et al.*, 2016). However, it has been reported that the cannibalism of some crabs is a potential feedback mechanism for controlling population density, which is only related to population density and not directly related to food abundance (Dutil *et al.*, 1997; Fern *et al.*, 1999). This is inconsistent with some findings that the lack of bait is the reason of cannibalism in crustaceans (Darias *et al.*, 2016). The

difference between high stocking density and individual specifications has a different view on the induction and promotion of the same kind of disability. This is contrary to view of some findings that the lack of food is the fundamental cause of cannibalism in crustaceans, and high culture density and the difference of individual specification plays a role in inducing and promoting the cannibalism (Folkvord *et al.*, 1993). No matter what kind of organism, there are growth differences between individuals. As time goes by, growth differences will inevitably lead to individual specification differences. When the difference in the individual specification is large to a certain extent, there will be a polyculture and the behavior of organisms will change (Li and Sun, 2013). In this experiment, it was found that the cannibalism of *P. monodon* post-larvae occurred more frequently among individuals with different specification differences, and the polyculture of different specification individuals aggravated the cannibalism of larvae, which can be speculated that *P. monodon* post-larvae has some discriminating mechanism for selecting the size of attacking object in cannibalism, and these results are consistent with research reported on *Chionoecetes opilio* cannibalism (Dutil *et al.*, 1997; Jr *et al.*, 2010). For this phenomenon, some findings use social order system reaction to explain: after a period of density coercion competition, the behavior of the same population is adjusted to make competition gradually relaxed, and individuals can coexist

peacefully. Individuals establish different positions in the population. These orders are established based on physical strength, individual size, and length of coexistence. After competition and elimination, a certain balance is established between surviving individuals, or form a balance of power. Nevertheless, when there are other individuals entering, the balance will be broken and the fighting will happen again. Individuals with aggressive behavior establish their dominant position in acquiring and dominating resources, and cannibalism is the embodiment of this behavior in a stressful environment (Hua *et al.*, 1998).

As an external factor, inflation has a strong influence on the behavior of *P. monodon* post-larvae. In this experiment, inflation allows the water to continually flip, resulting in a uniform distribution of bait and post-larvae, thereby increasing the chance of post-larvae contact with the bait and increasing food intake. Inflation prevents post-larvae from gathering locally, which reduces the chance of body contact and the probability of cannibalism. The attack intensity of post-larvae in inflatable state is significantly reduced, only slightly scratching or colliding with each other. Inflation can also enhance the movement behavior of post-larvae. The movement behavior of post-larvae is more active and the swing frequency of swimming feet is faster under the condition of inflation, which is consistent with previous research results. Inflation can also enhance



motor behavior of the post-larvae and motor behavior of the post-larvae is more active in the inflated state, and the swimming foot swing frequency became faster, which is consistent with previous results (Zhang *et al.*, 2008).

In conclusion, individual specifications, bait abundance and larval density significantly affected the cannibalism of *P. monodon* post-larvae, and an appropriate increase in aeration can reduce cannibalism to some extent. From the test results, *P. monodon* post-larvae showed cannibalism phenomenon more frequently in the absence of bait. Therefore, this research indicated that the lack of bait may be the main reason of cannibalism in *P. monodon* post-larvae, and high stocking density and individual specifications may induce and promote the occurrence of cannibalism. Therefore, adequate bait should be fed to the larvae in the process of seedling cultivation, and excessive density of larvae should be avoided, and polyculture of larvae at different developmental stages should be avoided, and aeration should be increased appropriately.

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### Authors' Contribution

SGJ, SJ and FLZ designed the study. QBY and JHH took part in the execution of the study. LSY, QBY, and SJ implemented the study and involved in sampling as well as testing. SJ, XYZ, FLZ, and SGJ drafted the manuscript. All the authors read and accepted the final manuscript before submission.

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