

## Analysis of mortality rate of *Penaeus vannamei* larvae transferred from large culture ponds to small culture tanks

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### Abstract

In this study, we investigated the related factors that affect mortality rate during the transfer of *Penaeus vannamei* larvae from large culture pond to small culture tanks. The results showed that the average survival time of *P. vannamei* under different conditions was about 10 days. The results of *Vibrio* detection showed that *P. vannamei* from shrimp farms might be *Vibrio* carriers. During the culture of *P. vannamei*, water qualities (temperature, salinity, dissolved oxygen, pH, ammonia nitrogen, nitrite, etc.), pathogens, stocking densities, physiological conditions and the operation techniques could affect the survival of *P. vannamei*.

**Keywords:** *penaeus vannamei*; small tanks culture; mortality rate

### Introduction

In the past decades, *P. vannamei* farming industry in China has developed rapidly. According to the data released by the Food and Agriculture Organization of the United Nations, the total production of shrimp in China in 2018 was 2.05 million tons, among which the output of *P. vannamei* was 1.76 million tons (85.79%). However, there are still some problems in the pond culture of *P. vannamei*.

The large culture ponds are easily affected by external environments. For example, the weather could affect the dissolved oxygen content in the culture ponds; the convection could bring the organic matters, such as feces, residual diets, ammonia, hydrogen sulfide, pathogenic microorganisms, from the sediments into the water; rainfall could affect the salinity, pH<sup>[1]</sup> and water quality, resulting in the stress response of *P. vannamei*.

Disease restricts the development of *P. vannamei* aquaculture. One of the prevention methods is to cut off the path of pathogen transmission, but there are difficulties to conduct such management in large ponds. On the other hand, farmers usually use excessive amounts of antibiotics in shrimp ponds. The abuse of these drugs not only caused drug residues in *P. vannamei*, wastewater and pond sediment, which seriously threatened the health of consumers and polluted the environment<sup>[2,3]</sup>.

In addition, the utilization rate of nutrients in large ponds

culture is relatively low. Previous studies has shown that about 59.5% of N and 79.6% of P in feeds are not utilized, and most of them were discharged into adjacent ecosystems<sup>[4]</sup>.

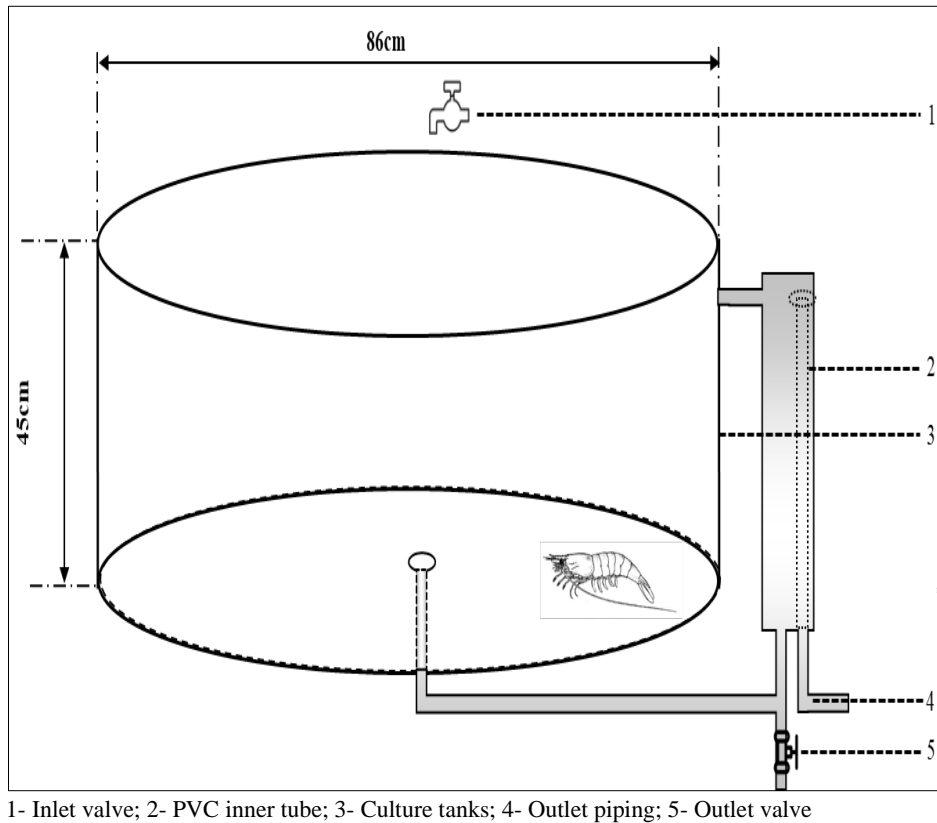
*P. vannamei* aquaculture has become a high-risk industry due to its large scale and high costs. Compared with the conditions of large ponds, those of indoor small culture tanks are easy to control. In addition, the costs of small culture are relatively low for farmers. However, there are some differences in water quality, aquaculture density, physical factors, etc. between indoor small culture tanks and outdoor aquaculture ponds, and how they affect the survival of *P. vannamei* remains unknown.

Thus, the mortality rates during the transfer of *P. vannamei* larvae from large culture ponds to small culture tanks were examined in the present study, with the aim to explore a more environmentally friendly and low-cost culture method of *P. vannamei* with indoor small tanks.

### Materials and Methods

#### Experimental Materials

*P. vannamei* larvae were purchased from farms such as Haiou Island, Jiangmen and Zhongshan, Guangdong Province. The body length and the body weight was 0.5-1.5 cm and 0.007-0.024 g. The bottom diameter and height of the laboratory's small culture tanks was 86 cm and 45 cm (Figure 1).



1- Inlet valve; 2- PVC inner tube; 3- Culture tanks; 4- Outlet piping; 5- Outlet valve

**Fig 1:** The drawing of small culture tanks for *P. vannamei*.

#### Laboratory-experiment of *P. vannamei* culture

##### Experiment setting of laboratory-experiment of *P. vannamei* culture

We experimentized three indoor *P. vannamei* culture experiments, which were denoted as experiment A, experiment B, and experiment C. One week before the start of the experiment, all the culture tanks were cleaned up and disinfected with 10 ppm potassium permanganate solution, and rinsed with tap water.

**Experiment A:** We purchased 4,000 shrimp larvae from a shrimp farm in Haiou Island, Panyu District, Guangzhou City, and transported them to the laboratory by bus in an oxygenated transport bag with ice. First, we put the oxygenated transport bag in the laboratory small culture tanks and stand for 15-20 min to make the water temperature of the oxygenated transport bag the same as tanks. Then, we opened the mouth of the oxygenated transport bag so that the water in the culture tanks slowly flowed into the oxygenated transport bag, gently lifted the end of the oxygenated transport bag to wait for the *P. vannamei* larvae to swim into the culture tanks. We have set up 6 culture tanks, the stocking densities of each tank was 500 shrimp /tank, the size were 0.5-1.5 cm.

**Experiment B:** We purchased 5,000 shrimp larvae from a shrimp farm in Zhongshan and transported them to the laboratory by taxi in an oxygenated transport bag with ice. The shrimp stocking process was similar to Experiment A. We have set up 6 culture tanks, the stocking densities of each tank was 200 shrimp /tank, the size were 0.5-1.0 cm.

**Experiment C:** We purchased 2,000 shrimp larvae from a shrimp farm in Jiangmen and transported them to the laboratory by taxi in an oxygenated transport bag with ice.

The shrimp stocking process was similar to Experiment A. 6 culture tanks were set, and the stocking density of each tank was 100 shrimp /tank.

#### Daily management

Artificial seawater which was prepared by adding artificial sea salt to tap water (aerated one week in advance to remove Cl<sup>-</sup> in tap water) was used, and the salinities was set according to the salinities of shrimp farm pond water.

The feed used in the breeding process was Rongchuan Feed 2# for *P. vannamei*. Shrimps were fed four times daily<sup>[5]</sup>, 7:00 am, 12:00 am, 17:00 pm, and 22:00 pm. Each feeding amount is 8% of the shrimp body weight in the tanks. Residual diets were removed with a siphon after feeding for 1 hour.

Water was partial exchanged every day. No fishery medicine was used during the whole culture process.

#### Vibrio detection

*Vibrio* in water and feed was detected by TCBS method. In the detection of *Vibrio* in the water, 0.1 ml water sample was put on the medium with a pipette and evenly coated with a disposable aseptic coating rod, the petri dish was covered and laid upside down for 1-2 hours, then the petri dish was incubated at temperature 20-25 °C for 2 days. The number of *Vibrio* was calculated as follows:

$$Y=X \times 10 \text{ (item/ml)},$$

Y is the number of *Vibrio*, and X is the number of colonies in the medium. The feed was put in distilled water, mixed evenly, and filtered; the filtrate was collected to detect *Vibrio* with the method mentioned above.

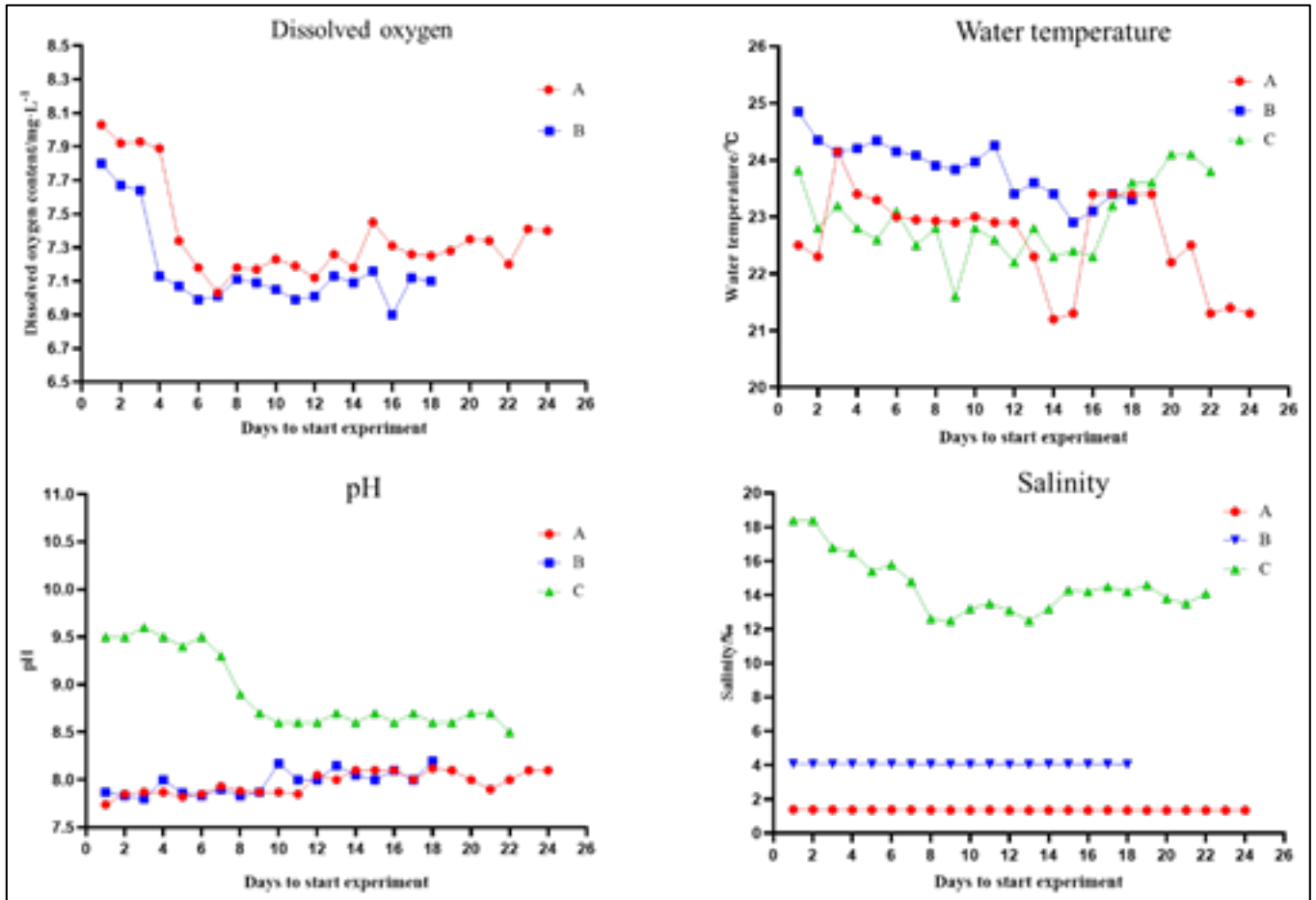
#### Results

##### Water quality and *Vibrio* detection

The temperature, dissolved oxygen, pH, and salinity of each

group during the experiment were shown in Figure 2. In Experiment A, the water temperature, dissolved oxygen, pH, and salinity were  $22.90 \pm 0.69^\circ\text{C}$ ,  $7.49 \pm 0.43 \text{ mg/L}$ ,  $7.89 \pm 0.14$ , and  $1.36 \pm 0.02 \text{ ‰}$ , respectively. In Experiment B, the water temperature, dissolved oxygen, pH, and salinity were

$24.06 \pm 0.48^\circ\text{C}$ ,  $7.26 \pm 0.38 \text{ mg/L}$ ,  $7.92 \pm 0.12$ , and  $4.06 \pm 0.01 \text{ ‰}$ , respectively. In Experiment C, the water temperature, pH, and salinity were  $22.85 \pm 0.59^\circ\text{C}$ ,  $9.08 \pm 0.42$ , and  $15.11 \pm 2.05 \text{ ‰}$ , respectively.

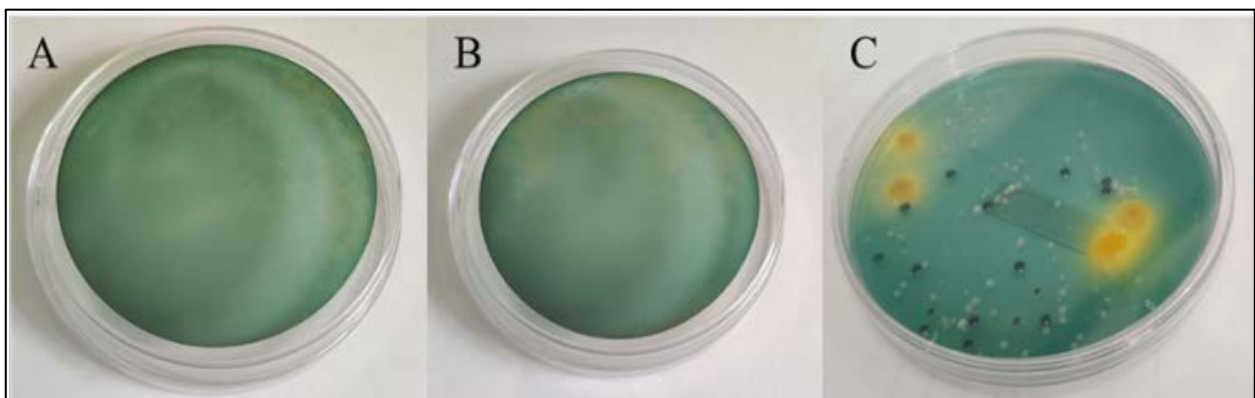


A, B and C represent different experimental groups.

**Fig 2:** Water quality of different experimental groups.

*Vibrio* detection results showed that there was no *Vibrio* in feed samples and water samples before cultivation, the presence of *Vibrio* was detected in the water samples during

the culture process, and the total number of *Vibrio* was 200 item/mL (Figure 3).



A represent the sample of feed filtrate, B represent the water sample before the start of culture, C represent the water sample during the culture process.

**Fig 3:** *Vibrio* detection.

**Survival time**

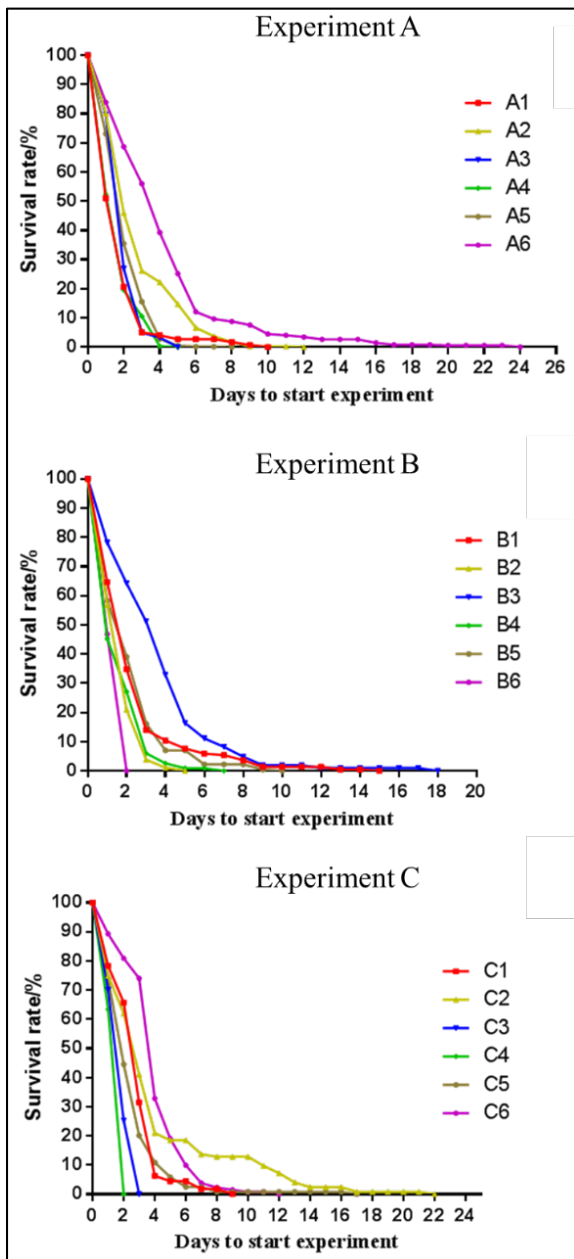
The survival time of *P. vannamei* in the tanks ranged from 2 to 24 days (Table 2). There was no significant differences

between stocking densities ( $P > 0.05$ ).

**Table 1:** Survival time of *P. vannamei* in experimental tanks.

	A	B	C
1	10	15	9
2	12	5	22
3	5	18	3
4	5	7	2
5	8	10	17
6	24	2	12
Average Value	10.7	9.5	10.8
Standard Deviation	7.09	6.10	7.83

After the *P. vannamei* larvae were transferred to the culture tanks, the survival rate decreased rapidly from day 1 to day 5, and was relatively low on day 6- day10 (Figure 4).



**Fig 4:** Survival rate of *P. vannamei* during the culture.

**Behavior Record**

On the first day, *P. vannamei* larvae swam into the culture tank from the oxygenated transport bag within 1-10 minutes, and stayed on the bottom of the tank within 1-2 hours; some *P. vannamei* larvae moved along the tank wall at the bottom

of the breeding tank, and some floated on the surface water and moved along the tank wall. The next day, some *P. vannamei* larvae did a “8” shaped rotation movement in the water, and the abnormal movement was common throughout the experiment. When some *P. vannamei* larvae lied at the bottom of the tank, and other larvae nibbled at the dead shrimps. Some *P. vannamei* larvae started feeding behavior within 1-3 minutes after the feed was put into the tanks. When feeding, *P. vannamei* larvae swam to the feed and used the ambulatory legs and antennals to hold the feed particles and send the food into the mouth. Sometimes two shrimps ate one feed together.

**Observation of dead shrimp**

Dead shrimps were found while cleaning the residual feed. On the fourth day of culture, some sloughed shrimps floated on the surface of water in some tanks. The color of the dead shrimps are milky, reddish, or transparent color, the sand veins were not obvious, the carapaces were soft, and some dead shrimps’ carapaces were partly lossed (Figure 5). In addition, microscopic observation showed that the liver and pancreas of the dead shrimp were mostly tan or milky, and there was almost no content in the intestine. Small red spots and "snowflake" black stripes on the carapaces of some dead shrimps were found.



**Fig 5:** Status of dead shrimp.

**Discussion**

Studies have shown that *P. vannamei* can live in the condition of 18-35 °C [6], salinity from 0 ‰ to 12 ‰ [7], and the dissolved oxygen content over 3 mg/L [8]. In this experiment, the water temperature was 21-25 °C during the entire cultivation period the salinities were controlled in the range of 1-2‰, 3-5‰, and 12-15‰ according to the water salinities of the shrimp farms, and the dissolved oxygen content was controlled above 7 mg/L, which met the water quality conditions in the above study.

Aquatic pathogens, e.g., *Vibrio*, have an important effect on the survival of *P. vannamei*. In the experiment, the *Vibrio* bacteria might be derived from shrimps or shrimp farm water. Muscle trauma infection of *P. vannamei* plays a key role in the pathogenesis of *Vibrio*, and the infected muscles the shrimp were white and soft [9], which was similar to the shrimp disease observed in the present study. Combined with the cannibalism phenomenon of *P. vannamei* under high-density farming conditions, we believed that the damage of the shrimp body caused by the cannibalism increased the possibility of trauma infection by *Vibrio*, which lead to the death of *P. vannamei*. However, increasing the temperature of cultured water could significantly increase the resistance of *P. vannamei* to pathogens [10]. The influence of the culture density on the survival rate of *P. vannamei* is conditional. Only when the culture density reached a certain threshold, the culture density might have a



restrictive effect on the survival rate of *P. vannamei* [11]. Under high-density farming conditions, *P. vannamei* can cannibalize [12]. As the culture density increases, the living space might inevitably decrease, exacerbating the competition for living space and resources.

*P. vannamei* has a low survival rate in some specific physiological process, such as molting. In the process of molting, the shrimps are vulnerable to physical and viral attacks. It should pay an attention to protect the shrimps during molting.

The culturing operation affect the growth of *P. vannamei*. After cleaning the culture tanks and filling tap water, we used only one week for oxygenation, properly not enough to remove harmful gases in the water. During the transfer of *P. vannamei* from the transport bag to the culture tank, the temperature adaptation time in our experiment was 15-20 min, which was quite different from the 1-2 hours mentioned in previous studies, which might affect the growth of *P. vannamei*. There was some difference in the composition of the natural seawater in the shrimp farm and the artificial seawater, which might cause a stress reaction in *P. vannamei*. In addition, the long-distance transportation process might also affect the health status of *P. vannamei*.

### Conclusions

During the process of rearing *P. vannamei* larvae from large ponds to small-scale breeding tanks, many factors, such as stocking density, temperature, salinity, pH, compositional differences between natural seawater and artificial seawater, pathogens, and operation techniques, can affect the survival rates. Controlling the stocking density can not only reduce the competition of *P. vannamei* larvae, but also indirectly increase the resistance of *P. vannamei* larvae to the pathogenic bacteria such as *Vibrio*. Raising the temperature of the culture water can effectively increase the resistance of *P. vannamei* to certain viruses. In addition, it is critical to reduce the stress response during the cultivation of *P. vannamei*.

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