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Short Communication

Dynamic Changes in Bacteria and Water Quality and their Relationship with Survival Rate during *Penaeus monodon* Larva Culture

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ABSTRACT

Although shrimp larvae are susceptible to many pathogens and water quality and diseases affect commercial shrimp production, the relation between these factors are less studied. Therefore, we aimed to investigate the effect of bacterial dynamics and water quality factors on *Penaeus monodon* larvae. We recorded the survival rates, water quality (total ammonia, nitrite, and nitrate concentration), heterotrophic bacteria, and vibrios during different *P. monodon* stages (nauplii, zoea, mysis stage, postlarvae). In ponds 1, 2, 3 and 4, the survival rates were significantly lower but the nitrite concentration and vibrio numbers (before mysis stage) were considerably higher than those in other ponds ($P < 0.05$), indicating that nitrite and vibrios influenced *P. monodon* survival during larva culture. Nitrite and nitrate concentrations increased from day 6, and increased dramatically by day 12. Total ammonia concentration increased continuously from day 1 to 15. Heterotrophic bacteria increased slowly in the nauplii and zoea stages, grew rapidly in the mysis stage, and remained almost stable and reduced slightly in postlarvae. In most ponds, vibrio numbers did not change significantly in the nauplii and zoea stages, but increased from the mysis stage and were $>10^{-4}$ cfu/mL, which potentially cause shrimp disease. Therefore, we suggest that the mysis stage is the key time when water quality and bacteria increase rapidly, and precautions should be taken to improve water quality and inhibit vibrio growth.

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Authors' Contributions
CZ and SF designed the study, drafted the manuscript, and performed the statistical analysis. LQ performed manuscript editing and review.

Key words
***Penaeus monodon*, Larva culture, Heterotrophic bacteria, Vibrios, Survival rate, Water quality.**

Black tiger shrimp *Penaeus monodon* is an important shrimp species with high economic value in China and other countries (Sengprasert *et al*., 2015; Wilson *et al*., 2015; Chen *et al*., 2016). Although, advanced techniques for industrial seedling rearing have been developed recently, Shrimps in the larval stages, especially from the zoea to postlarvae periods, show low immunity and are susceptible to many pathogens, resulting in mass mortalities in a shrimp hatchery (Vandenberghe *et al*., 1999). Most diseases of cultured shrimp are caused by viral or bacterial infections (Lightner and Redman, 1998). Many elements such as water quality, number and structure of bacterial community influence the survival and quality of larvae. Nitrogenous wastes derived from excrement, food, and dead body of the shrimp, are major concerns in shrimp production (Montoya *et al*., 2002). Accumulation of nitrogenous waste and its derivatives, such as ammonia (NH_3) and nitrite (NO_2^-), poses a threat to the environment and can predispose fish to infestation by parasites and pathogens owing to reduced immunity (Liao and Mayo, 1974; Wickins, 1976; Barman *et al*., 2015). As basic decomposers, microorganisms directly or indirectly decompose faeces, residual feeds, and organic matter, thus purifying the water and maintaining the balance of pathogens during mariculture.

Heterotrophic bacteria (such as *Bacillus*, *Lactobacillus*, and photosynthetic bacteria) not only improve water quality (Weisse and MacIsaac, 2000), but also regulate the intestinal microbiota and increase host resistance to pathogenic bacteria (Luisvillaseñor *et al*., 2013). Conversely, some pathogenic bacteria such as the *Vibrio* species frequently infect shrimp during aquaculture, and the outburst of bacterial pathogens in aquaculture systems is a complex phenomenon causing substantial industrial loss (Jeney and Jeney, 1995; Prayitno and Latchford, 1995; Irie *et al*., 2005). Thus, bacterial composition in larva culture strongly influences the growth, immunity, and disease resistance in larvae.

Aquatic ecosystems are extremely complex systems that maintain a balance between water quality, pathogens, and aquatic organisms. Water quality and diseases are the main factors that affect commercial shrimp production. Currently, the influence of bacterial dynamics and water quality on survival rate are rarely studied in industrial

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shrimp seedling rearing, and the interactive relationship between these factors remains unclear. Therefore, in this study, bacterial dynamics, water quality changes, and survival rate was investigated in real time to identify the key influential factors and develop an effective approach for *P. monodon* larva culture.

Materials and methods

We collected healthy *P. monodon* nauplii of the same cohort from a commercial hatchery in Shenzhen. The nauplii were stocked at a density of 80 ind./mL in 13 (marked number 1-13) 10 m^3 concrete ponds. The temperature, dissolved oxygen, pH, and salinity were monitored using a multi-parameter water quality instrument (556MPS, YSI Incorporated, Ohio, USA), and the water parameters were within the optimum range. The survival rates in different stages of *P. monodon* (nauplii, zoea, mysis, and postlarvae) were recorded. Sample was randomly collected from five spots of each pond using a specialized sampling bottle, and the survival rate was estimated by counting numbers of shrimp in 1 L water samples.

Bacteria were counted using 2216E plates (for heterotrophic bacteria) or Thiosulfate-citrate-bile salts-sucrose (TCBS) plates (for vibrios). The sampling method and protocol were as follows: water sample from each pond was collected in triplicate, the water samples were transferred to sterilized flasks and polysorbate was added into each water sample to obtain a final concentration of 5 µg/mL, and each sample was shaken for 30 min. Then, 4.5 mL sterilized seawater was diluted to 10^{-1}, 10^{-2}, 10^{-3}, and 10^{0}, 10^{-1}, 10^{-2} concentrations for 2216E and TCBS plates, respectively, and 0.1 mL seawater sample was plated in two replicates. The TCBS and 2216E plates were incubated at 28ºC for 2 and 4 days, respectively, and the bacterial colonies were counted to quantify the concentration of heterotrophic bacteria and vibrio in seawater. The number of bacterial colonies in each diluted sample was from 30 to 300. Bacterial culture medium consisted of 2216E marine agar, 5 g peptone, 1 g yeast powder, 0.1 g ferric citrate, 15-20 g powdered agar, 1000 mL seawater (pH 7.2–7.5). The bacterial culture medium was sterilized at 121ºC for 20 min, and 15–20 mL of sterilized medium was transferred to each plate. The water samples were filtered through a 0.45 µm membrane pressured by a vacuum pump. Each water sample was filtered through two membranes, and the filtered membrane was folded and stored in a sterilized centrifuge tube.

Total ammonia (NH_3-Nt), nitrite (NO_2^-) and nitrate (NO_3^-) concentrations of each pond were measured every 3 days, using the hypobromite oxidation method, naphthylethylenediamine photometric method (GB 17378.4-2007), and ultraviolet spectrophotometry (SL84-94), respectively.

Data were analysed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). All data were analysed for normality by probability plots and for homogeneity of variances by Levene's test. One-way analysis of variance (ANOVA) was used to determine the significance of each parameter among different treatments. If the effect was significant, the ANOVA was followed by Tukey's test. The P-value < 0.05 was considered statistically significant.

Results and discussion

The NO_2^- and NO_3^- concentrations increased from day 6, and increased dramatically on day 12 of *P. monodon* larva culture. The NO_2^- concentrations in ponds 1–4 were significantly higher than those in other ponds after day 12 (Fig. 1A). The NO_3^-concentration in ponds 1, 2, 4, and 8 were considerably higher than those in other ponds (Fig. 1B). The survival rates in ponds 1–4 ponds were significantly lower than those in other ponds (P<0.05, Fig. 2). These results suggest that NO_2^- and NO_3^- concentrations are important factors that influence survival rate during *P. monodon* larva culture. The NH_3-Nt concentration increased continuously from day 1 to 12 (Fig. 1C), but there was no obvious correlation with survival rate. Ammonia and nitrite toxicity has been demonstrated in fish, molluscs, and crustaceans, and ammonia is considered to cause damage to the central nervous system (Wright, 1995). In penaeid shrimp, ammonia may affect acid-base

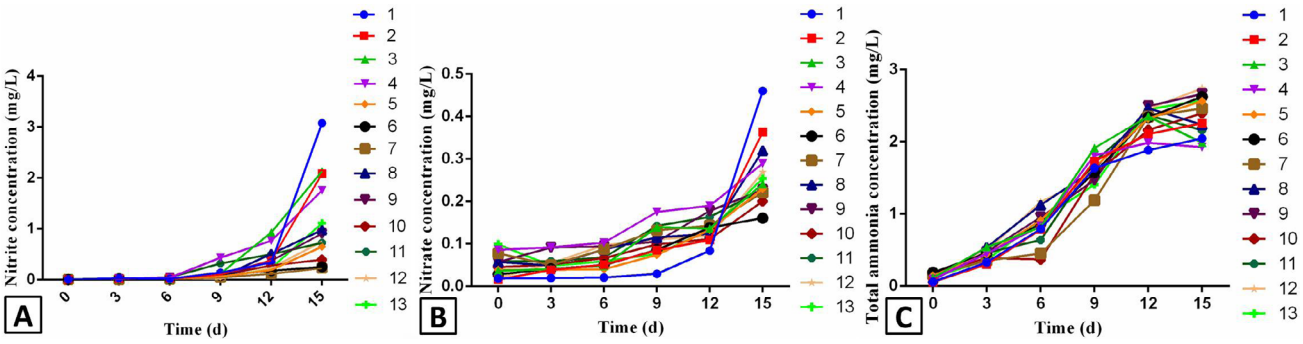

Fig. 1. Dynamic change in nitrite concentration (A), nitrate concentration (B) and the total ammonia concentration (C) in ponds 1–13 during *Penaeus monodon* larva culture.

balance, haemolymph osmolarity, nitrogen metabolism, respiration, and growth rates and may enhance molting (Chen and Kou, 1992; Chen and Lai, 1992; Chen and Cheng, 1993). Nitrite in the water can enter the bloodstream of aquatic animals, resulting in reversible formation of methaemoglobin, which cannot transport oxygen to tissues (Boyd and Tucker, 1998). Moreover, ammonia and nitrite can predispose fish to infestation by parasites and pathogens by reducing immunity. Our result showed that NO_2^- and NO_3^- concentrations increased from day 6, which corresponds to the mysis stage. Hence, it is important to reduce NO_2^- and NH_3-Nt concentrations before the mysis stage via precautionary measures such as addition of a sorbent or oxidant of NO_2^- and NH_3^-, or use of probiotics.

The graph showing the dynamics of heterotrophic bacteria during *P. monodon* larva culture is shown in Figure 3A. The number of heterotrophic bacteria increased slowly in the nauplii and zoea stages, increased rapidly in the mysis stage, and remained almost stable or reduced slightly in postlarvae (Fig. 3A). Thus, the mysis stage is a key time when heterotrophic bacteria, NO_3^-, NO_2^-, and NH_3-Nt increased rapidly. However, although NO_2^- and NH_3-Nt accumulated rapidly, heterotrophic bacteria did not increase ceaselessly. This may be related to the C/N ratio of water; many studies showed that increasing the C/N ratio can induce a shift in the biofloc community from photoautotroph or chemoautotroph to heterotroph-dominated communities (Avnimelech, 1999; Xu *et al*., 2016). This transformation strongly influences water quality and biofloc biomass production (Ebeling *et al*., 2006). Therefore, survival rate can be improved by both using a carbon source and probiotics. Number of vibrios did not change significantly in nauplii and zoea stages but started to increase in the mysis stage. After that, the number of vibrios in most ponds was considerably higher than 10^{-4} cfu/mL, which potentiallycaused disease in shrimps (Fig. 3B).

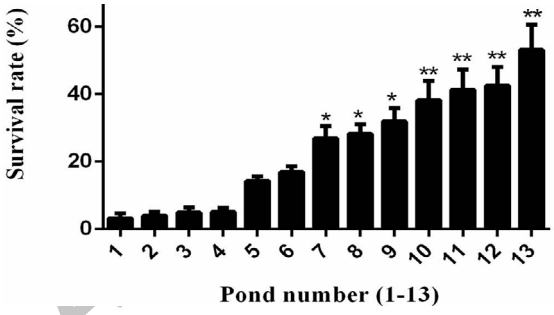

Fig. 2. Survival rates of *Penaeus monodon* in ponds 1–13. Bars represent the mean ± SD (n = 3). * and ** indicate statistically significant differences ($P < 0.05$).

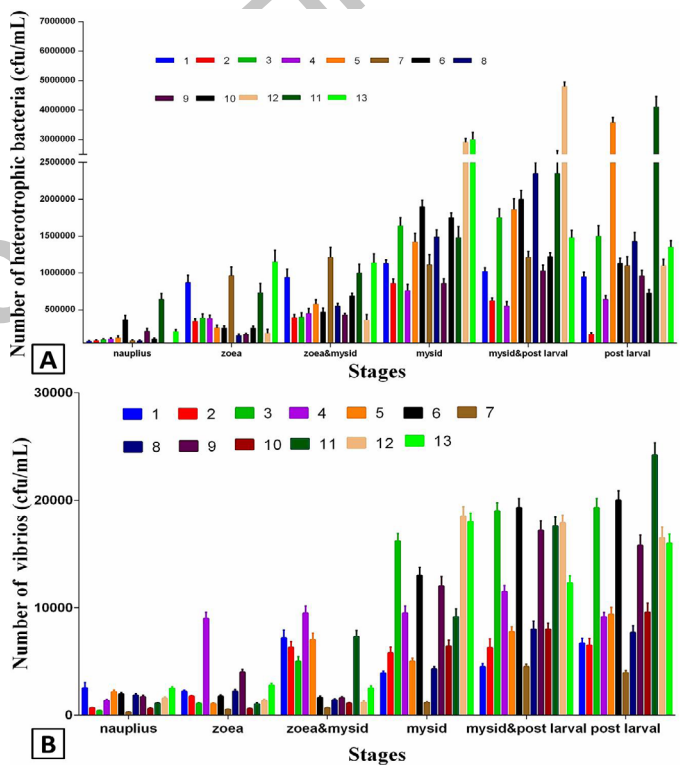

Fig. 3. Number of heterotrophic bacteria (A) and vibrios (B) in the different seedling stages of *Penaeus monodon*. Different colours represent the 13 different ponds. Each bar represents the mean ± SD (n = 3).

Notably, before the mysis stage, the number of vibrios in ponds 1–4 was considerably higher than that in other ponds. The corresponding low survival rate in ponds 1–4 (Fig. 2), indicates that vibrios may be an important factor in reducing the survival rate during larva culture. Therefore, to avoid mass mortality in the shrimp larva culture, it is necessary to use probiotic bacteria (such as *Bacillus*, *Lactobacillus*, and *Rhodopseudomonas capsulata*) to inhibit vibrio growth (Shen *et al*., 2007; Boonthai *et al*., 2011; Zokaeifar *et al*., 2014).

Conclusions

Both water quality and bacterial community can directly influence the growth and survival of *P. monodon* larvae, and bacteria play an important role in regulating water quality. Therefore, understanding the dynamic relationship between water quality and bacterial community is important for successful *P. monodon* larva culture. The results of the present study show that NO_2^- and vibrios strongly influence the survival of *P. monodon* larvae. Mysis stage is a key time when bacteria, NO_2^-, NO_3^-, and NH_3-Nt increased rapidly. Therefore, increasing the C/N ratio and using probiotics before the mysis stage may be beneficial to regulate water quality and inhibit vibrio growth. Furthermore, we speculated that the mysis stage is the key time when water quality and bacteria increase rapidly, and precautions should be taken to improve water quality and inhibit vibrio growth. Nevertheless, the present study is an initial and fundamental work, there is still much experiments should be done in the next work to clarify the dynamic changes in bacteria and water quality and their relationship with survival rate during *Penaeus monodon* larva culture.

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Statement of conflict of interest

Authors have declared no conflict of interest.

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