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## Short Communication

# Water Pretreatment Affects the Bacteria Communities in *Penaeus monodon* Larvae Culture

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**Authors' Contributions**
**LQ conceived and designed the study. CZ, JF, HX, QY and ZM performed the experiments. CZ wrote the paper. JGQ reviewed and edited the manuscript.**

**Key words**
**Water pretreatment, UV, Formalin, Bacteria communities, *Penaeus monodon*.**

## ABSTRACT

This study examined the effects of UV and formalin treatment on the survival of *Penaeus monodon* larvae and bacteria communities in the rearing tanks. The survival of *P. monodon* in the 36 h experimental period was not significantly affected by the water pretreatment ($P > 0.05$). However, the bacteria communities were significantly affected by water pretreatment ($P < 0.05$). Heterotrophic bacteria and vibro counts in the formalin treatment were significantly lower than UV treatment and untreated group ($P < 0.05$). Based on the results obtained in the present study, we suggest that formalin treatment could be a better away to control bacteria in *P. monodon* larvae culture.

Black tiger shrimp, *Penaeus monodon* is an important aquaculture species with high economic value in China and also in other countries (Chen *et al*., 2016; Chaiyapechara *et al*., 2012; Duan *et al*., 2014). In *P. monodon* culture, bacteria exist and play an important role in nutrient cycle in the marine ecosystem (Yu *et al*., 1995). For example, the heterotrophic bacteria could decompose organic matters into inorganic substances and act as feed for organisms at a higher trophic level (Yu *et al*., 1995). Vibriosis is the most predominant bacterial disease causing mass mortalities of cultured shrimp worldwide (Adams, 1991; Lavilla-Pitogo *et al*., 1998). Vibriosis in giant tiger shrimp (*P. monodon*) is commonly caused by several different vibrios such as *Vibrio harveyi*, *Vibrio parahaemolyticus*, and *Vibrio alginolyticus*. Among them, the Gram-negative bacterium *V. harveyi* is the most virulent and prevalent pathogen of larval and grow-out shrimp culture (Lavilla-Pitogo *et al*., 1990; Jiravanichpaisal *et al*., 1994; Karunasagar *et al*., 1994; Leaño *et al*., 1998). Which has caused significant losses in the aquaculture industry worldwide (Defoirdt and Sorgeloos, 2012). However, the poor understanding on the role of bacteria in water quality management has resulted in diseases outbreak related to the dominance of heterotrophic bacteria in *P. mondon* aquaculture and caused severely mortality economic loss. Therefore, bacterial control is essential for maintenance of water quality to avoid disease outbreak. Formalin and ultraviolet treatments have been used in fish aquaculture to reduce bacteria loads (Kasai *et al*., 2002; Gieseker *et al*., 2006). However, bacterial control with these treatments in shrimp culture is rare. Therefore, this study explored the effects of formalin and ultraviolet treatments on the bacteria flora in rearing water and the survival of *Penaeus monodon*. Results from the present study would provide valuable information on the bacteria control in the *Penaeus monodon* hatchery.

### *Materials and methods*

The healthy nauplii of *Penaeus monodon* were collected from the same cohort in a commercial hatchery in Shenzhen. The nauplii were stocked at a density of 80 ind. $mL^{-1}$ in nine 500 L fiberglass tanks at the zoea stage. During the experimental period, no feed was added into the rearing tanks, and the experiment of nauplius culture lasted 36 h. The temperature was maintained at 30°C during the experimental periods.

Two treatments *viz*., UV and formalin treatment were involved in this study. Treatment 1 used the seawater treated with ultraviolet (The UV light source was caused

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by a 30 W, 254 nm wavelength UV lamp, and the intensity of the light was not lower than 90μW/cm$^2$ in the present study, which was measured using an ultraviolet radiometer (model UVX-25, UNP, CA)), and treatment 2 used the seawater treated with formalin (12 ppm), while the untreated seawater was used as the control.

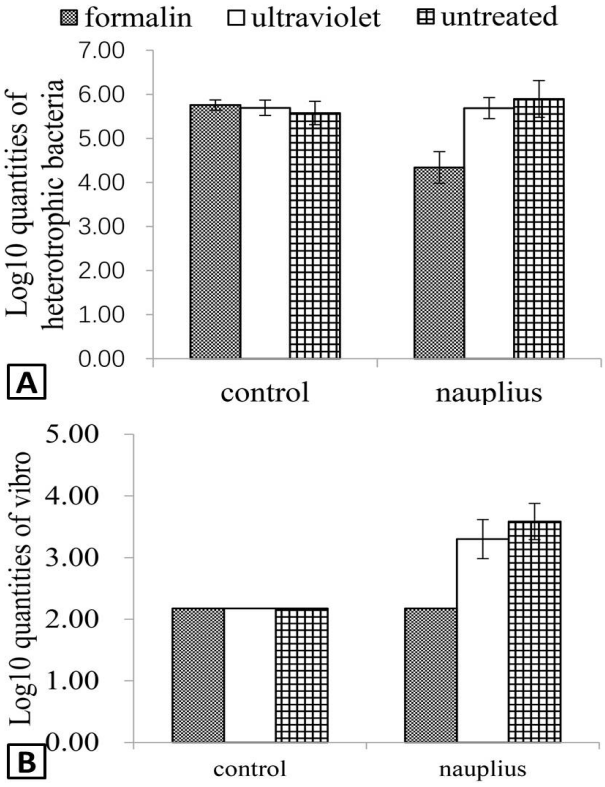


Fig. 1. The number of heterothrophic bacteria (A) and vibro bacteria (B) in the seawater treated with formalin or ultraviolet. Control, without rearing nauplii; Nauplius, with nauplii.

During the culture period, the seawater properties (temperature, dissolved oxygen, pH, salinity, ammonia nitrogen, nitro nitrogen, nitroso nitrogen and total phosphorus) were monitored by a multi-parameter water quality instrument (556MPS, YSI Incorporated, Ohio, USA), and the water parameters were within the optimum range for the shrimp nauplii. The numbers of bacteria (heterotrophic bacteria and vibrios) in the seawater in each treatment were counted, and the taxonomy of bacteria were identified using 16s rDNA analysis. Heterotrophic bacteria were investigated with 2216E plate, and vibrios were investigated with TCBS plate. The sampling method and protocol were as follows: the water sample from each treatment was collected in triplicate before nauplii were stocked into the rearing tanks, and after the experiment was completed. Water samples were kept in sterilized flasks and polysorbate was added into each water sample to reach the final concentration of 5 μg/mL, and the each sample was shaken for 30 min. Then use the 4.5 mL sterilized seawater diluted to $10^{-1}$, $10^{-2}$, $10^{-3}$ and $10^{0}$, $10^{-1}$, $10^{-2}$ concentrations for 2216E and TCBS plates, respectively. The 0.1 mL seawater sample was coated in two replicates.

The counting for TCBS and 2216E plates was conducted after 2 and 4 days at 28°C, respectively, in order to quantify the concentration of heterotrophic bacteria and vibrio in the seawater. The number of bacterial colonies in each diluted sample was from 30 to 300. Bacterial culture medium was made up 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 bacteria culture medium was sterilized at 121°C for 20 min before being transferred on a plate containing 15-20 mL bacteria culture medium. The water samples were filtered through the 0.45 μm membrane pressured by a vacuum pump. Each water sample was filtered on two membranes and the filtered membrane was folded and stored into a sterilized centrifuge tube.

Data were analyzed using SPSS version 18.0 (SPSS Inc., Chicago, IL, USA). All data were analyzed 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 of $< 0.05$ was considered statistically significant.

*Results and discussion*

In this study, the number of heterotrophic bacteria was not significantly different before nauplii were stocked into the rearing tanks ($P > 0.05$, Fig. 1A). However, upon completion of the experiment, the number of heterothrophic bacteria in the formalin treatment was significantly lower than that in the UV treatment and the control ($P < 0.05$, Fig. 1A). The number of heterothrophic bacteria was not significantly different between the UV treatment and the control ($P > 0.05$). In this study, the number of vibro was not significantly different before nauplii were added into the rearing tanks ($P > 0.05$, Fig. 1B). At the end of the experiment, the number of vibro was significantly affected by the experimental treatment ($P < 0.05$, Fig. 1B). The lowest number of vibro was observed in the formalin treatment, and the highest number of vibro was observed in UV treatment and untreated seawater (Fig. 1B). The number of vibro was not significantly different between UV treatment and untreated seawater ($P > 0.05$). Upon the 36 h experiment period, the survival of *P. monodon* was not significantly different between treatments ($P > 0.05$, Fig. 2).

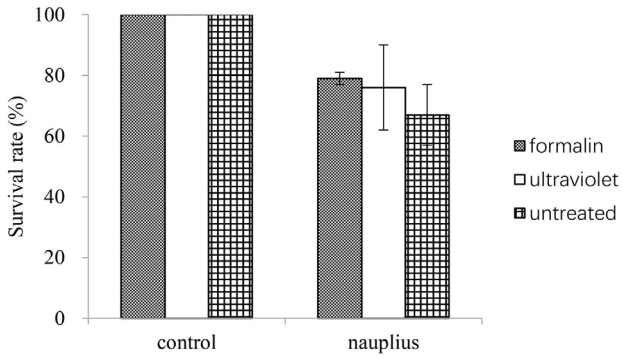


Fig. 2. The survival rate of nauplii treated with formalin and ultraviolet. Control: without rearing nauplii, Nauplius: with nauplii.

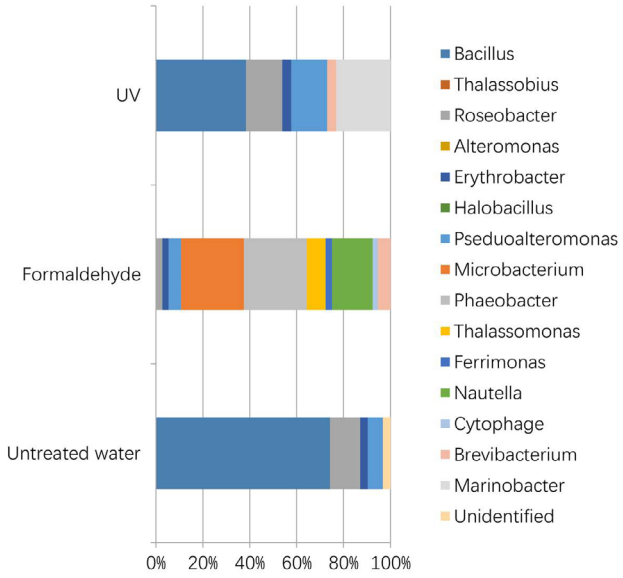


Fig. 3. Microbial communities in the rearing environment.

The microbial communities in the rearing environment were significantly affected by the experimental treatments ($P < 0.05$). In the untreated group, the microbial community included 74.2% *Bacillus* sp., 12.9% *Roseobacter* sp. and 6.5% *Pseduoalteromon* sp. (Fig. 3). In contrast, in the UV treatment, *Bacillus* sp. accounted for 27% and *Roseobacter* sp. accounted for 10.8% ($P < 0.05$). While the percentage of *Pseduoalteromon* sp. increased from 6% to 15% in the UV treatment. In the formalin treatment, the percentage of *Bacillus* sp. was reduced from 63% to 0%, while the percentage of *Pseduoalteromon* sp. was not significantly different from the control ($P > 0.05$, Fig. 3).

Water pretreatment has been considered as an essential procedure in marine aquaculture (Bly *et al*., 1993; Harlioglu, 2017). Evidence has indicated that the formalin treatment can reduce mortality of rainbow trout (Gieseker *et al*., 2006). Upto present, information on the effects of water pretreatment on the survival of *P. monodon* and bacteria communities is rare. In the present study, the final survival of *P. monodon* was not significantly affected by the water treatment. However, the microbial communities in the rearing environment were significantly affected by water pretreatment. Both heterotrophic bacteria and vibro were reduced in the water pretreated with formalin. By comparing bacteria counts, formalin was recommended as an efficient way to control the bacteria in larval *P. monodon* rearing environment.

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*Statement of conflict of interest*

Authors have declared no conflict of interest.

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