Journal of
Fisheries and Aquatic Science

ISSN 1816-4927

Academic
Journals Inc.

www.academicjournals.com

Journal of Fisheries and Aquatic Science 7 (3): 205-214, 2012
ISSN 1816-4927 / DOI: 10.3923/jfas.2012.205.214
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Effect of Water Exchange to Eliminate *Vibrio* sp. During the Naupliar Development of *Artemia franciscana*

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ABSTRACT

The present study was initiated to assess the occurrence, distribution and composition of *Vibrio* sp. and to evolve management strategy in the live feed *Artemia franciscana*. The occurrence of total heterotrophic bacteria was observed in the cysts of *A. franciscana* whereas, the *Vibrio* sp. appeared only at the 16th h *A. franciscana* nauplii. In 24th h *A. franciscana* nauplii, the bacterial counts remained very high and all these indicate that the live feed organism is yet another source of bacterial entry into the hatchery system and can be a serious problem in the culture system unless remedial measures are taken. In order to reduce the bacterial counts in the larval rearing tank water, water was exchanged (100%) after 18th h of *A. franciscana* hatching. The current study also revealed that the continuous exchange of *A. franciscana* rearing tank water for an interval of every two hours will be an effective method to reduce the bacterial counts and may help to avoid subsequent disease problem.

Key words: *Artemia, Vibrio* sp., bacteria, water exchange, naupliar

INTRODUCTION

Diet is a fundamental aspect in larval culture of decapod crustaceans (Jones *et al*., 1997; Correia *et al*., 2000; Valenti and Daniels, 2000). The brine shrimp *Artemia* sp. is the most frequently used live food in the larviculture of economically important crustaceans and fishes. *Artemia* sp. is widely recognized as the best natural, storable live feed available and is used in marine finfish and crustacean hatcheries around the world because of its nutritional and operational advantages (Sorgeloos *et al*., 1986). Inspite of the advantages presented by the micro-crustacean *Artemia* sp., such as easy handling and high protein content (Emmerson, 1984), unfortunately they are reported to carry pathogens. *Artemia* sp. nauplii have been also reported as vector of pathogenic bacteria, especially *Vibrio* sp. (Gomezgil *et al*., 1994; Muroga *et al*., 1994; Verdonck *et al*., 1994). Microbiological studies have demonstrated that *Artemia* sp. cysts carry bacteria in the shell (Austin and Allen, 1982; Prieto *et al*., 1987; Garcia *et al*., 1988; Torres and Partida, 2001). Post larvae fed with *Artemia* sp. nauplii showed higher *Vibrios* sp., presumptive *V. harveyi, V. anguillarum* and *V. vulnificus* than those fed with micro encapsulated feed. Careless use of *Artemia* sp. nauplii has been shown to be responsible for the development of disease and mortality of *P. monodon* (Straub and Dioxan, 1993). Therefore, as a preventive measure all the live feeds introduced in the rearing tanks of hatchery should be rinsed thoroughly with disinfectants (Vaseeharan and Ramasamy, 2003). *Artemia* sp. nauplii are often treated in order to reduce the bacteria associated with them prior to feeding them to the larval prawn/shrimp.

Previous studies often recommend rinsing the nauplii in sterile fresh or seawater (Austin and Allen, 1982; Rodriquez *et al*., 1991), but some authors suggested that rinsing has little effect on the bacteria (Dehasque *et al*., 1991; Verdonck *et al*., 1991). Therefore, antibiotics have been used to disinfect the live feed before introducing them into the rearing system (Hatai *et al*., 1981; Yamanoi and Sugiyama, 1987; Tanasomwang and Muroga, 1989, 1992; Gomezgil *et al*., 1994). There are no satisfactory treatment methods available to control the bacteria infections possibly originating from *Artemia* sp. However, it is necessary to control the bacterial population associated with *Artemia* sp. in order to minimise the danger of infection of bacteria before their use in culture systems. Therefore, the effects of chemotherapeutants, ultraviolet radiation treatments and freezing on the *Artemia* sp. associated bacteria have been investigated to minimize the danger of bacterial infections associated with feeding live food (Hayashi *et al*., 1976; Hatai *et al*., 1981; Tabata *et al*., 1982; Yamanoi and Sugiyama, 1987; Miyakawa and Muroga, 1988; Yamanoi and Katayama, 1989). Use of antibiotics, a hypochlorite solution, an iodophor and formaldehyde have all been found to be effective in suppressing the bacterial flora of *Artemia* sp. nauplii (Gilmour *et al*., 1975; Coleman *et al*., 1980; Sumitra *et al*., 1988). Therefore, the present study was initiated to assess the occurrence, distribution and composition of *Vibrio* sp. and to evolve the management strategies the live feed *Artemia franciscana*.

MATERIALS AND METHODS

Hatching of *A. franciscana* cyst: Commercially available cysts of *Artemia franciscana* was purchased and they were disinfected by soaking for 30 min in seawater containing 0.2% sodium hypochlorite solution and then transferred and maintained in a tank filled with seawater at a temperature of 25-28°C and pH 8.0-8.5. The *Artemia* hatching tank was further illuminated at 2000 lux. Good quality cysts were found to hatch within 24 h of incubation (Vanstappen, 1996). During the process of hatching the *Artemia* cyst, samples of decapsulated cyst and also water samples were taken from the hatching tank every 4 h intervals. The samples were subjected to bacteriological study and the presence of *Vibrio* sp. was determined.

Effect of water exchange in *A. franciscana* rearing tank water: During the process of *Artemia* hatching, after 18th h, water was exchanged completely every two hours and the water samples taken before and after water exchange were analyzed for the presence of *Vibrio* sp.

Bacteriological study: Enumeration of bacteria (*Vibrio* sp.) of cyst/nauplii *A. franciscana*: The samples of cyst and nauplii were taken and the water adhering to the samples was removed by means of sterile blotting paper. The samples were weighted aseptically and homogenized in a sterile tissue homogenizer with 1 mL of autoclaved brackish water. After homogenization, the samples were serially diluted upto 10^{-6}. Autoclaved sea water was used for serial dilution. The water samples of the larval rearing tank were serially diluted upto 10^{-6}. Aliquots of 0.1 mL from each dilution were spread plated in triplicate. Two types of bacteriological media were used in the study. Nutrient agar for the enumeration of total heterotrophic bacterial count whereas, Thiosulphate Citrate Bile Salts sucrose agar (TCBS) was used for the enumeration of total viable *Vibrio* counts. After incubation the inoculated Petri plates for 24-48 h at 30-32°C, the total colony forming units (cfu)/ total viable *Vibrio* counts (cfu) were determined.

Statistical analysis: Each measurement was done in triplicate and the mean and standard deviation of the experimental results was calculated using MS-Excel.

RESULTS

Bacterial counts in the *A. franciscana* during hatching: The present study revealed the occurrence of total heterotrophic bacteria and total viable *Vibrio* counts in the *A. franciscana* during hatching in the hatchery (Fig. 1). In the dry *A. franciscana* cyst, the total heterotrophic bacterial count was 1.7±0.09 log cfu g^{-1} dry cyst while the *Vibrio* sp. was generally absent and appeared only at the 16th h in the newly hatched *A. franciscana* nauplius. The total heterotrophic bacterial count in the newly hatched *A. franciscana* nauplii remained very high 3.57±0.46 log cfu g^{-1} nauplius. In the 24th h nauplius, the total heterotrophic bacterial count was 4.07±0.22 log cfu g^{-1} nauplius and the total viable *Vibrio* count was 1.98±0.48 log cfu g^{-1} nauplius. Thus the current study has shown that the live feed *A. franciscana* given as live feed to the zoea of *Macrobrachium rosenbergii* acts as a carrier of the bacterial pathogens. *Vibrio* sp. and the total heterotrophic bacteria were very rare in the dry cysts and they get infected with *Vibrios* through the rearing water and the bacterial infection increases and builds up progressively soon after hatching.

Bacterial counts in the rearing water during the process of *A. franciscana* hatching: The total heterotrophic bacterial counts in the *A. franciscana* rearing tank water increased from 2.01±0.33 to 5.86±0.09 log cfu mL^{-1} and the total viable *Vibrio* counts increased from 2.48±0.3 to 4.68±0.35 log cfu mL^{-1} (6, 12, 16 and 18 h). This study has shown that there was a dramatic increase in the bacterial counts both in the *A. franciscana* rearing tank water as well as in the tissues of the *A. franciscana* cysts and soon after the cyst was hatched into nauplius (Fig. 2).

Bacterial counts in the 18th h *A. franciscana* nauplii after water exchange: In order to reduce the bacterial counts in the larval rearing tank water, water was exchanged (100%) after 18th h of *A. franciscana* hatching and the bacterial counts were determined in the tissues of newly hatched nauplius (Fig. 3). A marginal decrease in the bacterial counts was observed after water was exchanged. The total heterotrophic bacterial counts in the *A. franciscana* nauplii (18 h) decreased from 3.25±0.22 to 3.2±0.46 log cfu g^{-1} nauplius and the total viable *Vibrio* count decreased from 2.18±0.39 to 1.53±0.46 log cfu g^{-1} nauplius. After an interval of 2 h, the total heterotrophic bacterial count has decreased to 1.75±0.22 log cfu g^{-1} nauplius and the viable *Vibrio* counts could not be detected.

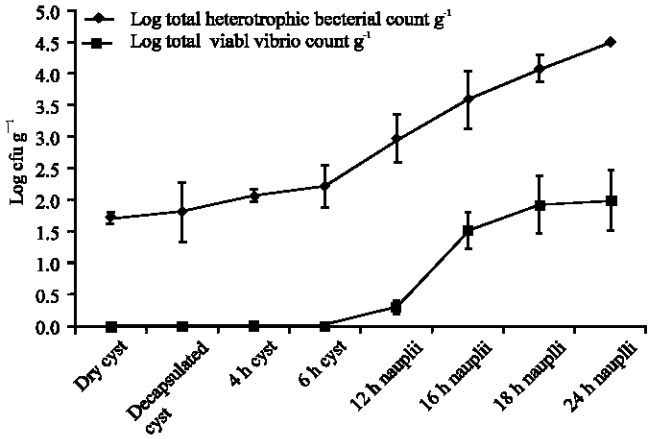

Fig. 1: Bacterial counts in *A. franciscana* During hatching

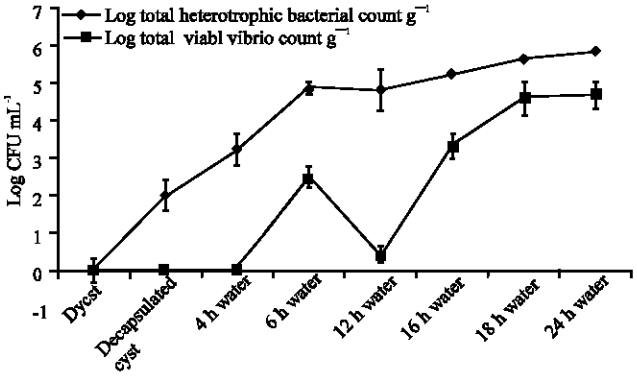

Fig. 2: Bacterial counts in *A. franciscana* sp. rearing water during hatching

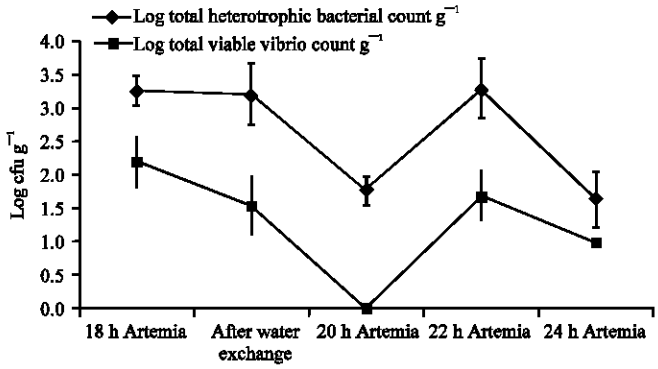

Fig. 3: Bacterial counts in 18th h *A. franciscana* nauplii after water exchange

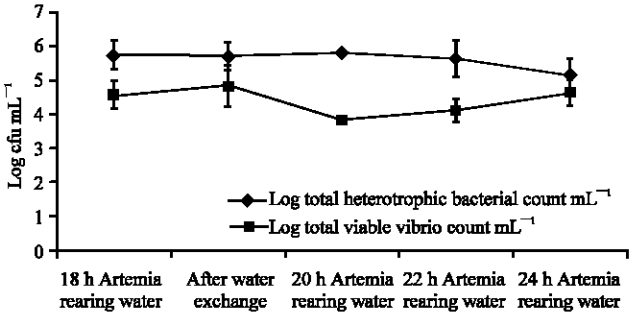

Fig. 4: Bacterial counts in 18th h *A. franciscana* rearing water after exchange

In contrast after 4 h of interval the total heterotrophic bacterial counts and the viable *Vibrio* counts had increased to 3.28±0.45 log cfu g^{-1} nauplius and 1.69±0.39 log cfu g^{-1} nauplius, respectively. But after 6 h of interval, the total heterotrophic bacterial counts had decreased to 1.63±0.42 log cfu g^{-1} nauplius and the viable *Vibrio* count was 0.97±0.09 log cfu g^{-1} nauplius.

Bacterial counts in the *A. franciscana* rearing water after water exchange: In the *A. franciscana* rearing tank water, a gradual decrease in the total heterotrophic bacterial counts was observed after water was exchanged at the 18th h of *A. franciscana* hatching (Fig. 4). The total heterotrophic bacterial counts has decreased from 5.73±0.41 to 5.71±0.41 log cfu mL^{-1} after

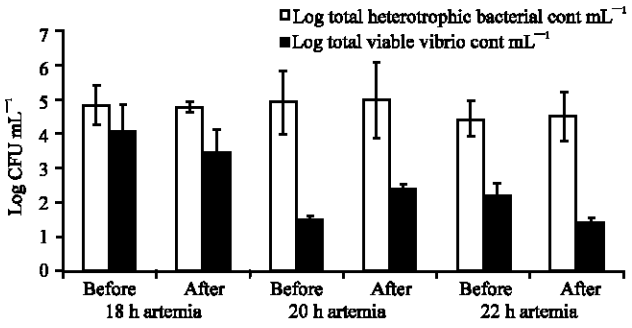

Fig. 5: Bacterial counts in 18th h *A. franciscana* before and After water exchange

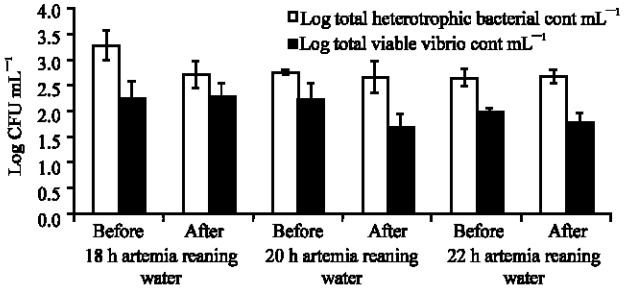

Fig. 6: Bacterial counts in 18th h *A. franciscana* rearing water before and After water exchange

2, 4 and 6 h interval whereas the *Vibrio* counts started to increase after 2 h interval from 3.82±0.09 to 4.62±0.39 log cfu mL^{-1} (4 h, 6 h). This study indicates that the effect of water exchange lasts for only 2 h in controlling the *Vibrio* sp. counts though there were significant reductions in the total heterotrophic bacterial counts (Austin and Allen, 1982).

Bacterial counts in 18th h *A. franciscana* nauplii and rearing water before and after water exchange: A marginal decrease in the bacterial counts was observed in the *Artemia* rearing water and the effect was found to last only for two hours and hence, continuous exchange of water for every two hours was carried out after 18 th h of *Artemia* hatching (Fig. 5 and 6). The total heterotrophic bacterial counts were found to decrease from 2.75±0.33 log cfu g^{-1} *Artemia* nauplius to 2.57±0.41 log cfu g^{-1} *Artemia* nauplius and the total viable *Vibrio* counts decreased from 2.3±0.46 log cfu g^{-1} *Artemia* nauplius to 0.80±0.09 log cfu g^{-1} *Artemia* nauplius. In the *Artemia* rearing tank water, the total heterotrophic bacterial counts had decreased from 5.75±0.53 to 4.65±0.23 log cfu mL^{-1} of water while the viable *Vibrio* counts had decreased from 3.95±0.54 to 3.08±0.33 log cfu mL^{-1}. The current study also revealed that the continuous exchange of *Artemia* rearing tank water for an interval of every 2 h will be an effective method to reduce the bacterial counts and may help to avoid subsequent disease problem.

DISCUSSION

The present study revealed the occurrence of total heterotrophic bacteria in the cysts of *A. franciscana* whereas, the *Vibrio* sp. appeared only at the 16th h of hatching. In 24th h *A. franciscana* nauplii the bacterial counts remained very high and all these indicate that the live feed organism *A. franciscana* is yet another source of bacterial entry into the hatchery system and

can be a serious problem in the culture system unless remedial measures are taken. Austin and Allen (1982) have reported increase of bacterial population during the rearing of *Artemia* sp. from egg to adult stage. They have shown that the total number of aerobic bacterial flora ranged from 3.8×10^3 to 8.1×10^3 Cfu/nauplius on seawater nutrient agar and 9.4×10^2 to 4.3×10^3 Cfu/nauplius on TCBS agar. Earlier studies have also reported that the heavy bacterial load associated with *Artemia* sp. nauplii (Gilmour *et al*., 1975; Austin and Allen, 1982; Gatesoupe, 1990; Tanasomwang and Muroga, 1992; Verdonck *et al*., 1991; Straub and Dioxan, 1993; Partida *et al*., 1997; Torres and Partida, 2001; *Artemia* sp. nauplii are reported to be the agent of transmission of pathogenic Vibrio sp. (Chen, 1993; Torres and Partida, 2001). *Vibrio* sp. has become dominant after 24h, probably because during hatching, *Artemia* sp. cysts are broken and a reverse organic substance, glycerol is excreted to hatching water (Sorgeloos *et al*., 1986). The microflora in *Artemia* sp. cultures consists mainly of *Vibrio* sp. In hatchery the highest bacterial numbers were found during hatching and enrichment of samples of *Artemia* sp. cysts (Austin and Allen, 1982; Igarashi *et al*., 1989; Dehasque *et al*., 1991; Verdonck *et al*., 1994). The *Vibrio* sp enumeration on TCBS gives a concentration as high as 1 to 9×10^6 bacteria growing on TCBS agar media per milliliter (Partida *et al*., 1997). The presence of bacteria especially in the *A. franciscana* feeding larval stages of *M. rosenbergii* may be related to mortality of the infected larvae. Further studies are needed to understand the time and mode of entry, pathogenesis, host pathogen interactions and dynamics. Besides, *Vibrio*, different bacterial genera have been associated with cysts of *Artemia franciscana*. Austin and Allen (1982) have found that bacteria belonging to the genus *Bacillus* sp., *Micrococcus* sp., *Staphylococcus* sp., *Erwinia* sp. and *Vibrio* sp. were reported to occur in the *Artemia* sp. (Gilmour *et al*., 1975; Austin and Allen, 1982; Verdonck *et al*., 1994). *Pseudomonas, Moraxella* and *Cytophaga* were reported to occur in the *Artemia* sp. (Tanasomwang and Muroga, 1992; Verdonck *et al*., 1991; Sahul and Balasubramanian, 2000; Torres and Partida, 2001). *Vibrio* sp. could also be introduced into the aquaculture system through the contamination of *Artemia* sp. hatching tanks operations. *V. alginolyticus, V. vulnificus, V. pelagius* and other *Vibrio* sp., were isolated from *Artemia* sp. hatching tanks and are also associated with tanks of mysis, zoea and postlarvae shrimp stages. Hatching and enrichment are performed in the same hatchery tanks without any intermediate rinsing and these have been shown to contain *Pseudomonas* sp (34%), *Moraxella* sp. (2%) and *Cytophaga* sp. (6%) besides *Vibrio* sp. (43%) (Tanasomwang and Muroga, 1992). Hatchery operations especially air supply and hatching water in the tanks may contaminate while *Artemia* nauplii present in the tank can become vector of *Vibrio* infections (Vaseeharan and Ramasamy, 2003). In contrast, studies by Partida *et al*. (1997) indicated the absence of *Vibrios* in the air supply and hatching water of the hatchery. Similar such studies are required in India to enumerate and understand the occurrence of different genera of bacteria in *Artemia* originated from various geographic regions and their capacity to survive in adverse conditions.

The present study has shown that there is a dramatic increase in the bacterial count in the *A. franciscana* rearing tank water as well as in the tissues of the *A. franciscana* cysts and soon after the cyst was hatched into nauplius. Similar observations were also reported by Lavilla-Pitogo *et al*. (1992) who has shown a higher total *Vibrio* counts to occur in both *Artemia* sp. and *Artemia* sp. rearing water. The abundance of *V. harveyi* in *Artemia* sp. rearing water (log 2.34±0.76 mL) and *Artemia* sp. nauplii (log 2.44±0.62 g) indicated that this live food source, a preferential food for the mysis and post larvae in the hatcheries studied, could have carried to tanks (Abraham and Palaniappan, 2004). This was evident from the high levels of

luminous bacteria in mysis and post larvae and their rearing waters compared to the zoea rearing tanks. A similar condition was observed in penaeid larvae due to swarming bacteria, after the introduction of *Artemia* nauplii (Igarashi *et al.*, 1989). Lavilla-Pitogo *et al.* (1992) recorded luminous bacteria upto 0.005 and 0.17% of the TVC in *Artemia* sp. nauplii and rearing water.

A marginal decrease in the bacterial counts was observed 2 h and after 6 h water exchange. In contrast, after 4 h of interval the bacterial counts has increased. Thus there is fluctuation in the bacterial counts due to water exchange. This study indicates that the effect of water exchange lasts for only two hours in controlling the *Vibrio* sp. counts though there were significant reductions in the total heterotrophic bacterial counts. The current study also revealed that the continuous exchange of *A. franciscana* rearing tank water for an interval of every two hours will be an effective method to reduce the bacterial counts and may help to avoid subsequent disease problem. This study has shown that chlorination is not effective and is not the best method to be used in the hatchery as a method to reduce the bacterial counts. Careless use of *Artemia* nauplii have been shown to be responsible for the development of disease and mortality of *P. monodon* (Gilmour *et al.*, 1975; Austin and Allen, 1982; Muroga *et al.*, 1987; Nicolas *et al.*, 1989; Gatesoupe, 1990; Straub and Dioxan, 1993). The prophylactic use of antibiotics during larval rearing, including disinfecting live feed, results in increasing the frequencies of antibiotics resistant bacteria in aquaculture systems. This is true in the present and previous investigations (Rahim *et al.*, 1984; Hjeltness *et al.*, 1987; Richards *et al.*, 1991; Gomezgil *et al.*, 1994; Karunasagar *et al.*, 1994).

CONCLUSION

A. franciscana nauplii are known to be one of the principal agents for transmission of pathogenic *Vibrio* sp. infections in hatcheries. The findings of the current study concur with previous workers and confirm that the *Vibrio* sp are always associated with *A. franciscana* nauplii. Therefore, the introduction of live feeds into the rearing tanks of hatchery need to be rinsed thoroughly so that the potentially pathogenic bacteria can be controlled or reduced before using as feed.

ACKNOWLEDGMENT

The author (A. Krishnika) thank The University of Madras for the financial support through the award of Dr. Gnanam Endownment Award.

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