

Effect of new aeration technology on the bacteriology of shrimp ponds growing *Penaeus monodon*

Sheetal V. Karekar, R. A. Sreepada, Resha R. Shirodkar, Shantanu Kulkarni, Praveen Kumar, P. A. Loka Bharathi, Ashjorn Bergheim¹, Christian Vogelsang²

Aquaculture Laboratory, National Institute of Oceanography, Goa - 403004, India

¹RF-Rogaland Research, Stavanger, Norway

²Norwegian Institute for Water Research (NIVA), Oslo, Norway

Abstract

The present study was conducted to evaluate the effects of a HOBAS aeration technology developed by HOBAS Water Engineering in Norway on shrimp ponds in India. Two ponds (P1 and P2) having an area of ~ 0.6 ha located at Alvekodi, coastal Karnataka (India) were chosen for the study. In one of the ponds, (P1), four aerators were placed in each corner to facilitate aeration and water circulation. To stress the environment in an aerated pond, shrimps were stocked @ 12 PL m⁻², while the control pond (P2) had a stocking density of 5 PL m⁻². The aim of the study was to evaluate the changes occurring in different bacterial communities, particularly the disease causing bacteria in response to aeration during the cultivation of tiger shrimp, *Penaeus monodon*.

In HOBAS aerated pond (P1) showed more stable DO levels and the pond sediment was healthier with no visual black sulphur deposits. However, the DO levels (measured at 30 cm above the sediment) were not significantly different ($P > 0.05$) in aerated and control ponds. Further, the bacterial communities in both rearing water and sediment in the aerated pond turned to be very similar to the bacterial communities found in the control pond in spite of more than two-fold higher stocking density in the former. No significant differences between bacterial abundance in rearing water and pond sediment was observed ($P > 0.05$). Heterotrophic bacterial counts were in the range of 10³ to 10⁴ ml⁻¹ or g⁻¹ and were higher by 1-2 orders of magnitude than other forms of bacteria. Abundance of nitrifiers and denitrifiers did not vary much in both the ponds. Sulphate reducing bacteria (SRB) were in the range of 10² ml⁻¹ or g⁻¹ and the counts reduced in the aerated ponds as the cycle proceeded. Potentially disease causing bacteria such as *Vibrio* spp., *Salmonella* sp., *Shigella* sp., and *Staphylococcus* sp. etc were within the threshold levels. Luminous *Vibrios* capable of causing *Vibriosis* were below detectable levels. From a bacteriology point of view, the new HOBAS aeration technology appeared to be helpful in maintaining a disease free environment while sustaining higher yields with optimum food conversion ratio (FCR) compared to the control pond.

Keywords: Aeration technology, Bacteriology, Shrimp ponds, *Penaeus monodon*, FCR

1. Introduction

Shrimp aquaculture has intensified due to excellent market value, lack of the availability of water and land resources and competition from other user community of natural resources. Intensification of farming practices can have an adverse affect on the health of the farm as a result of stressed conditions, which may result in the outbreak of disease (Bachere, 2000; Otta et al., 2003). Environmental management strategies have to be applied in order to minimize the impacts of shrimp aquaculture and to improve water quality conditions. Apart from increasing the overall yield of the product, emphasis should also be on preserving coastal environments and achieve eco-friendly production.

In intensive culture systems, aeration plays a pivotal role in maintaining optimal pond water and sediment quality. Poor water quality in the pond leads to the stress the environment, which in turn will make the cultured species more susceptible to diseases. When pathogen populations are low, shrimp's defenses are normally capable of preventing disease, but when stressed by poor water quality coupled with high stocking density, shrimps fall prey to "shell-loving" bacteria, fungi and viruses. Hence there is a need of a system, which will help manage these conditions. The obvious role of aeration systems in ponds is to supply oxygen to the cultured species, and to facilitate microbial transformations of nitrogen or organic carbon degradation. Continual aeration can also help to reduce the metabolite diffusion of ammonia, carbon dioxide and other dissolved gases across the water-air interface into the atmosphere. Constant circulation also maintains the higher concentration of suspended solids, which hastens the rate of oxidation and decomposition of waste organic matter (Avnimelech et al., 1992).

Aeration thus helps to maintain stable water quality conditions and to renew the dissolved nutrients that sustain healthy algal blooms in their extensive and semi-intensive ponds. This process introduces freshly oxygenated water and helps flush out wastes. They are used at night and early in the morning when DO levels are at minimum. Low DO concentrations also increase the toxicity of ammonia to shrimp. Gases such as ammonia can impact aquatic life by acute and chronic toxicity as well as ammonia oxidation, which reduces dissolved oxygen levels. Ammonia in shrimp ponds causes high mortality rates and can also cause low growth rates in shrimp. Therefore, there is a direct relationship between the oxygen concentrations and exchanges of toxic elements occurring in the ponds, as well as the healthy state of the pond water and sediment and the physiological status of aquatic organisms reared in the ponds.

2. Materials and methods

2.1 HOBAS aeration technology

There are different types of aerators, which have been used in the recent past and are mentioned for their principle, use and performance (Boyd, 1998) in aquaculture practices. HOBAS technology developed by HOBAS Water Engineering, Norway mechanically mixes air and water in order to replace oxygen deficits, and to flush the excreted, potentially toxic, carbon dioxide and ammonia. The pump also creates horizontal physical water current in the pond when sufficient numbers of aerators are employed and implementation is done correctly. The core unit consists of a pump, a valve, an air inlet hose and a specially adjusted raft for vertical implementation in the pond. While the pump is running, the partial vacuum in the pump house (submerged in water at the lower part of the venturi) cause air to be pulled into the intake port where air is dispersed into the water (as shown below). Onshore, a valve is used to regulate the amount of air being introduced into the impeller.

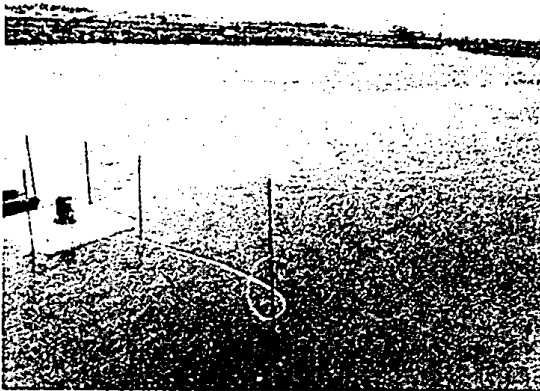


Fig. 1 System for dispersal of air into the water

2.2 Shrimp ponds and farm management

The shrimp ponds chosen for this study are located close to the Kumta town in the Karnataka state ~ 180 km south of Goa. (India). The farm covers a total area of 7 ha where the reservoir and farming area

constitute 1.5 ha (Fig 2a).

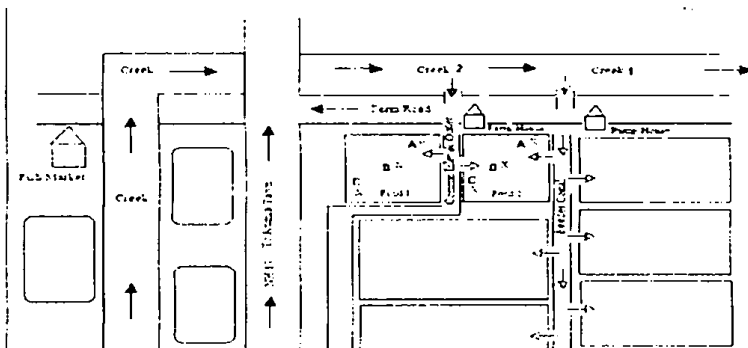
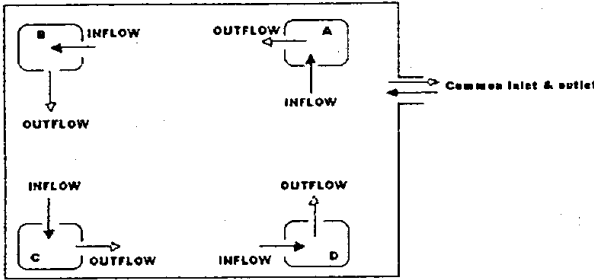


Fig. 2 (a) Adjacent infrastructure -Experimental cum demonstration farm and (b) Positioning of aerators in experimental pond (P1)

Two ponds (P1 and P2) having an area of ~ 0.6 ha were selected to evaluate the effect of HOBAS aeration technology on the bacteriology of shrimp ponds. To stress the environment in an aerated pond,



shrimps were stocked @ 12 PL m², while the control pond (P2) had a stocking density of 5 PL m². Four aerators were placed in each corner of P1 in such a way to facilitate aeration and complete water circulation (Fig. 2b)

2.3 Samples and sampling

Samples of rearing water and pond sediment were collected one day prior to transfer of post-larvae (0-DOC) and thereafter at fortnightly intervals after stocking up to 135-DOC between April 2004 and August 2004 at three pre-determined sampling locations in two ponds.

A Niskin bottle sampler was used to collect water samples in ponds (20 cm above bottom) and the creek water. Sampling time was between 08.00 and 09.00 h. The samples were transferred aseptically into sterile BOD bottles. The sediment samples were collected with the help of a hand held acrylic core (400 mm length, 40 mm dia.) from the same positions and were transferred immediately into clean polythene bags. All samples were preserved onto ice in an insulated icebox and transported to the main laboratory.

Water quality parameters such as temperature, salinity and pH were measured in the field at fortnightly intervals at sampling locations (Fig. 2a) with the help of centigrade thermometer, Refractometer (Atago, Japan), calibrated pH meter (Lab-India, PHAN), respectively. Dissolved oxygen (DO) content was estimated by Winkler's method (Parsons et al., 1984). Sediment pH was measured with calibrated pH meter (Lab-India, PHAN) and organic matter was estimated following the method described by El Wakeel and Riley (1956). Nutrient and chlorophyll analysis was also done using standard methods described in (Parsons et al., 1984).

Bacterial groups such as Heterotrophs, Vibrios, Coliforms, *Salmonella-Shigella* like organisms, Nitrifiers, Denitrifiers, Sulphate reducing bacteria (SRB) were enumerated in both the aerated pond (P1) and control pond (P2). An aliquot of 100 ml of pond water samples were directly spread plated onto all these media. For isolation of sediment bacterial groups, 10 g of wet sample were diluted in 90 ml of 50 %

sterile seawater (SSW). Appropriate dilutions were carried out and 100 ml were plated onto media. The bacterial counts in rearing water and sediment were expressed as cfu ml⁻¹ and cfu g⁻¹ of dry weight, respectively.

To analyze the heterotrophic bacterial composition colonies were selected based on morphological characters and pigmentation from nutrient agar containing 20-200 colonies. The heterotrophic cultures were then identified using the key schemes for identification of marine organisms by Oliver (1982). Different groups of bacteria with culture conditions are given in Table 1.

Table 1. Different groups of bacteria with culture conditions

Genera	Media	Inoculum	Growth conditions
Heterotrophs	Nutrient agar	0.1 ml	RT for 48 h
Vibrios	Thiosulphate Citrate Bile-salt agar (TCBS)	0.1 ml	RT for 48 h
Coliforms	Hichrome agar	0.1 ml	RT for 48 h
<i>Salmonella-Shigella</i> like organisms	Xylose Lysine decarboxylase agar (XLD)	0.1 ml	RT for 48 h
Nitrifiers	Mineral media	0.1 ml	RT for 7-10 days
Denitrifiers	Denitrifying media	2 ml	RT for 7-10 days
Sulphate reducing bacteria (SRB)	SRB medium	3ml	RT for 7-10 days

Representative *Vibrio* isolates from TCBS were selected and purified on NA plates. Identification of *Vibrio* was based on their morphological, physiological and biochemical characteristics as described by Ruangpan and Kitao (1991) and identified up to the species level wherever possible by using keys as described by (Holt *et al.*, 1994).

3. Results

In the present study, the stocking density was 12 PL m⁻² in the aerated pond (P1) whereas in the control pond (P2), it was 5 PL m⁻². Previous study carried out in the same ponds suggested that a stocking density > 5 Post Larvae (PL) m⁻² were not sustainable without use of aeration. Due to the higher stocking density in the aerated pond, one would have expected higher bacterial counts as the organic load is higher. However, in the present study the bacterial counts in the aerated and the control ponds did not vary significantly ($P > 0.05$).

The heterotrophic load of aerated ponds was similar to that of control pond. The *Vibrio* counts were in the range of 10^3 ml^{-1} in water and 10^3 g^{-1} in sediment and within threshold limits. Luminous disease causing organisms were below detectable levels. The Coliforms and *Salmonella*-*Shigella* like organisms were in the range of 10^2 in both pond water and sediment.

The nitrifiers, denitrifiers and SRB, which play an important role in maintaining the sediment quality, were present in acceptable numbers and were found to be in the range of 10^2 - 10^3 in water and sediment.

Physico-chemical parameters were similar in both ponds and fell within the acceptable range for rearing *P. monodon* (Chien, 1992). Temperature ranged between 26 °C and 33 °C. pH values varied between 7.1 and 8.5 in water and 5.3 to 7.2 in the sediment. DO values ranged from 4.8 to 8.0 mg l^{-1} . Salinity levels ranged from 8.1 to 41.9 ppt. The variation in measured environmental parameters in aerated and control ponds are presented in Table 2.

Table 2. Physico-chemical characteristics of rearing water and pond sediment in aerated and control ponds during the cultivation of *Penaeus monodon* in modified extensive shrimp ponds in southwestern coastal India

Parameters	Aerated pond	Control pond
Temperature (°C)	27-35	27-35
pH	7.11-8.32	7.16-8.50
Sediment (Eh)	62.80-112.77	54.60-94.03
Salinity (ppt)	9.40-41.89	8.10-41.89
DO (mg l^{-1})	4.88-6.78	4.76- 8.01
BOD (mg l^{-1})	0.26-3.05	1.16-3.73
COD (mg l^{-1})	10-87	14-93
Nitrate (imol l^{-1})	0.30-2.72	0.33-0.98
Nitrite ($\mu\text{mol l}^{-1}$)	0.11-0.53	0.08-0.74
Ammonia (imol l^{-1})	0.10-2.0	0.31-4.76
Phosphate (imol l^{-1})	0.13-3.97	0.72-3.91
POC ($\mu\text{g C l}^{-1}$)	3.41-18.63	3.85-12.56
H_2S (ig l^{-1})	0.43-1.29	0.38-1.37
Chlorophyll <i>a</i> (mg m^{-3})	16.02-50.19	8.01-57.85
Phaeophytin (mg m^{-3})	12.64-100.57	24.63-92.31
Organic Carbon (%)	1.42-5.42	1-5.22

The qualitative analysis of Vibrios showed the dominance of *V. fluvialis* and *V. metschnikovii* in water and sediment samples, these Vibrios are not disease causing but are usually present as the normal flora. Disease causing Vibrios such as *V. parahaemolyticus* and *V. harveyi* were present below detectable levels. The vibrios were present below the threshold disease causing limits. Among the heterotrophic group, it showed the dominance of *Staphylococcus*, *Peptococcus*, *Micrococcus* in the Gram-positive bacteria group and *Vibrio*, *Enterobacter*, *Pseudomonas* in the Gram-negative group.

4. Discussion

The HOBAS aeration technology (Drengstig *et al.*, 2004) used in this study helped maintain all the key water quality parameters like temperature, salinity, pH, dissolved oxygen *etc.* quite stable and within the acceptable limits in the experimental pond. When the stocking densities are high it usually leads to the increased accumulation of toxic gases like CO₂, NH₃, H₂S *etc.*, which can have adverse effects on the cultured species if not kept under limits. These toxic gases can cause stress conditions and lead to culmination of disease in the cultured species. The aeration systems used here helped maintain all these toxic gases under control and thus helped in maintaining the water and sediment quality.

The bacteriology of pond sediment is largely driven by a combination of three dominant factors, namely input of pelleted feed, ambient temperature and limiting levels of oxygen. Dissolved oxygen is a critical factor which helps maintain all the parameters stable. Stressed environmental conditions lead to both decrease in the diversities of the microbial community and an increase in the numbers of functionally specific groups. Outbreaks of disease in shrimp ponds are strongly related to the significant increase in the proportion of potentially pathogenic *Vibrio* community in the rearing water. The *Vibrio* loads were found to be below threshold levels *i.e.* 10⁴ ml⁻¹ and the *Vibrio* species found to be most dominant in the water and sediment were *V. fluvialis* and *V. metschnikovii* were not the most pathogenic. Pathogenic species like *V. parahaemolyticus* and *V. harveyi* were found to be present below detectable levels. Quantitative and qualitative data of heterotrophic bacteria revealed that the ponds were well managed from environmental point of view. The counts were fairly low and shrimps did not show any signs of major disease during the production cycle.

With the use of HOBAS aeration system, the pond is well managed in spite of the higher stocking densities. All the key water quality parameters are within optimum limits (Table 3) and there is no rise in the levels of toxic gases in water or sediment of the rearing pond. The

overall growth (Table 4) was good and there were no signs of major disease in the cultured shrimps.

Table 3. Changes in bacterial groups in pond water and sediment in aerated and control ponds with response to aeration

Bacterial groups	Control water	Aerated water	Control sediment	Aerated sediment
Heterotrophs	10^4 gradual	10^4 $\uparrow 10^3-10^4$ after aeration	10^3 cyclic- 2peaks	10^3 $\uparrow 10^3-10^6$ after aeration S>W
Total <i>Vibrio</i>	10^3 cyclic- 2peaks	10^2 $\uparrow 10^2-10^3$ after aeration	10^2 \downarrow in mid cycle 10^2-10^1	10^2 $\downarrow 10^3-10^1$ after aeration
Nitrifiers	10^3 cyclic- 2peaks	10^2 $\downarrow 10^2-10^1$ after aeration	10^2 cyclic- 2peaks	10^3 $\downarrow 10^3-10^1$ after aeration
NRB	10^1 $\uparrow 10^1-10^2$ from 6 th sampling	10^1 slight \uparrow after aeration	10^1 $\uparrow 10^1-10^2$ after 6 th sampling	10^2 slight - after aeration
SRB	10^1 $\uparrow 10^1-10^2$ in 4 th sampling	10^1 similar pattern in W & S	10^1 cyclic- 2peaks	10^1 no change after aeration
Total Coliforms	10^3 cyclic- 2peaks	10^3 slightly \uparrow after aeration	10^4 \downarrow with sampling	10^2 $\downarrow 10^3-10^1$ after aeration
<i>Salmonella</i> - <i>Shigella</i> like organisms	10^3 cyclic- 2peaks	10^3 $\uparrow 10^2-10^3$ after aeration	10^3 $\downarrow 10^2-10^1$ after 2 nd sampling	10^3 $\downarrow 10^2-10^1$ after aeration

\uparrow Increase \downarrow Decrease

Table 4. Details of production and FCR (Food Conversion Ratio) in aerated and control ponds

Area (ha)	Pond type	Stocking density (No. m ⁻²)	Production (kg ha ⁻¹)	Mean weight (g)	Mean length (mm)	FCR
0.65	HOBAS Aeration	12	2,400	30.00	157.4	1.34
0.60	Non-aerated (control)	5	1,191.67	33.78	163.0	1.56

5. Conclusion

The results of the study show that the pond is well managed in spite of the higher stocking densities with the HOBAS aeration system and all key water quality parameters lie within optimum limits.

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