Bacteria of Sanitary Significance in Brackish Water Aquaculture Farms and Shrimps

RAKESH KUMAR and P.K. SURENDRAN
Central Institute of Fisheries Technology
P.O. Matsyapuri, Cochin - 682 029, India

Microbial parameters *viz.*, *Escherichia coli*, coliforms, vibrios and sulphite reducing clostridia of brackish water aquaculture farms and shrimps along with physico-chemical parameters have been investigated. The study covers the brackish water farms of Ernakulum District (Kerala, India). There were visible variations in the pH, dissolved oxygen, salinity and light penetration in the farm water. Total bacterial count of water, sediment, and the sample of shrimps varied among different farms. Total plate count (TPC) for shrimp was highest followed by sediment and water. Counts for vibrios, coliforms, *E. coli* and sulphite reducing clostridia (MPN) in sediment samples also showed the same trend. Counts for vibrios, coliforms, *E. coli*, and sulphite reducing clostridia in prawn samples were higher than in the water and sediment samples. There was a seasonal variation in the bacterial counts as TPC, *E. coli* and total coliform counts were higher during monsoon season while the vibrio count was higher during the post-monsoon season. Salinity was maximum during pre-monsoon and post-monsoon, whereas it was less during monsoon period. Dissolved oxygen was 8 $mg.l^{-1}$ during monsoon period, whereas it was less during pre-monsoon and post-monsoon period.

Key words : ***Escherichia coli*, coliform, vibrios, sulphite reducing clostridia physicochemical parameters, aquaculture**

Shrimp production through aquaculture has reached 97,100 t. during 2000-2001 and has earned Rs. 3620 crores from exports which is 86% of total shrimp exports. Brackish water aquaculture has a major role in farmed shrimp production. Because of the growing awareness among the consumers regarding food safety, there is need to have more information on pathogenic organisms in shrimp and farm water. The microbial and physicochemical parameters i.e., temperature, pH, dissolved oxygen (DO), light penetration and salinity has a great role in the total productivity of the aquaculture farm. DO is one of the most important parameters in water quality assessment and reflects the physical and biological processes prevailing in the pond. Its presence is essential to maintain the higher number of the phytoplankton and zooplanktons which are major nutrient food for shrimps. Organisms have specific requirement for dissoled oxygen, e.g., game fish requires at least 5 $mg.l^{-1}$ DO whereas coarse fish and crustaceans require minimum 2 $mg.l^{-1}$ dissolved oxygen in water (Trivedi & Goel, 1984). Most chemical and biological reactions occur at a narrow range of pH. In pond, pH changes diurnally and seasonally due to the variation in the photosynthetic activity and the increases in pH due to the consumption of the CO_2 in the process. Temperature controls the major biological reaction in the organisms in water. It controls the other parameters of water. Salinity also changes the microbial flora of the water bodies.

It is observed that environment reflects on the microflora associated with the fish (Horsley, 1971; Shewan, 1962). Even for the same fish species significant variations in the microflora under different culture methods have been obsersed (Nedoluha & Westhoff, 1997). The culure practices such as manuring and feeding techniques with livestock waste or agricultural byproducts (Toor *et al.*, 1991) give a high possibility for contamination of the farm and shrimp.

The microbiology of farmed shrimp has been studied extensively in India and abroad (Nayyarahamed *et al.*, 1995; Budisusilowati & Haryani, 1995; Surendran *et al.*, 1994; Twiddy & Reilly, 1995). In this study the microbiological parameters with special emphasis on pathogens and physico-chemical parameters of 20 different brackish water farms located in Ernakulam District (Kerala) has been made.

Material and Methods

The brackish water farms selected for the study were from Ernakulum District (Kerala, India). The samples were collected from 20 brackish water farms. Golden Creek farm from Kannamaly was chosen for studies on seasonal variation. Samples were collected during pre-monsoon, monsoon, and post-monsoon period. Water samples were collected in sterile bottles and sediment samples scooped out from ponds and collected in sterile polythene bags. Shrimp samples were collected in sterile polythene bags and brought to the laboratory immediatly. Microbial analysis was carried out as per the USFDA methods (2001). Total plate count was determined by pour plate method using tryptone glucose agar and incubated at 37°C for 48 h. Total coliform was determined by VRBGA (Violet Red Bile Glucose Agar) and *E. coli* was detected by Tergitol-7 (T-7) agar and further tests were performed as per USFDA (2001). Vibrios were determined by using selective Thiosuphate Citrate Bile Salt Sucrose (TCBS) agar and further identification as per the USFDA method (2001). Sulphite reducing clostridia was determined by MPN method using DRCM (Differential Reinforced Clostridia Medium).

Physico-chemical parameters i.e., temperature, salinity, dissoved oxygen and pH of farm water were determined as per the standard methods. Light penetration was determined by Secchi disc method (Trivedi & Goel, 1984).

Results and Discussion

TPC of the farm water ranged from 10^3 to $10^6.g^{-1}$. Similar microbial loads were reported from culture ponds in Indonesia and Thialand (Putro *et al.*, 1990; Rattagool *et al.*, 1990). TPC of sediment samples were in the range of 10^5-$10^7.g^{-1}$. Higher bacterial count in sediment could be due to higher nutrient inputs in the farms. It was observed that TPC of water and sediment were always lower than that of the prawn samples and the same trend was observed in *E. coli*, total coliform and vibrios (Table 1). TPC of whole shrimp ranged from 10^6 to $10^7.g^{-1}$. High bacterial counts of the shrimp samples could be due to the shrimp gut which had higher bacterial load as compared to other body parts (Lobrerra *et al.*, 1990; Surendran *et al.*, 2000).

Table 1. Microbial parameters of farms

Sample	TPC	*E. coli*	Total coliform	Vibrio	Sulphite reducing Clostridia
Water	$2.8x10^3$-$9.6x10^6$ $cfu.ml^{-1}$	2-20 $cfu.ml^{-1}$	54-95 $cfu.ml^{-1}$	12-$2.05x10^2$ $cfu.ml^{-1}$	45-$450.100ml^{-1}$
Sediment	$2.5x10^5$-$2.6x10^7$ $cfu.g^{-1}$	28-$2x10^2$ $cfu.g^{-1}$	50-$5.3x10^3$ $cfu.g^{-1}$	$2.4x10^3$-$8.4x10^3$ $cfu.g^{-1}$	45 to $110.g^{-1}$
Prawn	$5.3x10^6.g^{-1}$-$1.1x10^7$ $cfu.g^{-1}$	36 to $3x10^2$ $cfu.g^{-1}$	69 to $1.02x10^4$ $cfu.g^{-1}$	$3.1x10^3$-$3.6x10^4$ $cfu.g^{-1}$	0 to $>140.g^{-1}$

E. coli from water, sediment and shrimp samples were in the range of 0-$20.g^{-1}$, 10^1-$10^2.g^{-1}$ and 10^1-$10^2.g^{-1}$, respectively. Total coliform in all these samples were in higher range as shown in Table1. The higher counts of *E.coli* and coliforms in sediment and prawn samples indicate that sediment and shrimp gut harbour *E.coli* and coliforms. Vibrio count of water, sediment and shrimp were in the same range. Highest count was in shrimp followed by sediment and water. Sulphite reducing clostridia of farm water sample ranged from 45 to > 450 MPN.100ml^{-1} and for sediment, from 45 to 110.g^{-1}. In the case of shrimp samples, the range was from 0 to 140.g^{-1}. Sulphite reducing clostridia could not be detected from 3 prawn samples by MPN method.

The various physicochemical parameters of the farm water are shown in Table 2. Temperature ranged from 28 to 34°C, pH 6.4 to 8.3, salinity 3.2 to10.2 ppt, and light penetration was in between 22-48 cm. These parameters vary with location of farm, type of pond soil and seasons.

Table 2. Physicochemical parameters of farms

Parameter	Range
Temperature, °C	28-34
Dissolved oxygen, mg.l^{-1}	6.0-9.2
pH	6.4-8.3
Light penetration (secchi disc), cm	22-48
Salinity, ppt*	3.2-10.2

* Parts per thousand

Season had a great role in the physicochemical parameters as well as microbial flora, particulary indicator and pathogenic organisms. As shown in Table 3, during monsoon period salinity and tempearture were 5.2 ppt and 29°C, respectively. During pre-monsoon period salinity of farm water was 10.64 ppt and during post-monsoon again it reached to 7.3 ppt. During monsoon season, DO of farm water recorded high values and salinity was low because of the flow of rain water into the brackish water farms.

Table 3. Physicochemical parameters of Kannamaly farm during pre-monsoon, monsoon and post-monsoon

Parameters	Pre-monsoon	Monsoon	Post-monsoon
Temperature, °C	31	29	32
DO, mg.l^{-1}	3.4	8	9.2
pH	7.06	6.4	8.3
Light penetration,cm	39	32	28
Salinity, ppt	10.64	5.2	7.3

Microbial flora also changed with the season as shown in Table 4. During monsoon period water, sediment and shrimp had high TPC, *E.coli*, and total coliforms count. This could be due to the low salinity and inflow of rain water during rainy season.

Table 4. Microbial parameters of Kannamaly farm during pre-monsoon, monsoon and post-monsoon

	Water	Sediment	Shrimp
Pre-monsoon period			
TPC ($cfu.ml^{-1}/cfu.g^{-1}$)	$2.8x10^4$	$2.5x10^5$	$5.8x10^5$
E. coli ($cfu.ml^{-1}/cfu.g^{-1}$)	2	0	5
Total coliforms ($cfu.ml^{-1}/cfu.g^{-1}$)	25	38	76
Vibrios ($cfu.ml^{-1}/cfu.g^{-1}$)	$1.2x10^1$	$8.4x10^3$	$3.1x10^3$
Sulphite reducing clostrida (MPN)	450 $.100ml^{-1}$	>140 $.g^{-1}$	>140 $.g^{-1}$
Monsoon period			
TPC ($cfu.ml^{-1}/cfu.g^{-1}$)	$9.5x10^6$	$2.6x10^7$	$1.1x10^7$
E. coli ($cfu.ml^{-1}/cfu.g^{-1}$)	2	$2x10^2$	$2x10^4$
Total coliforms ($cfu.ml^{-1}/cfu.g^{-1}$)	54	$5.2x10^3$	$1.02x10^5$
Vibrios ($cfu.ml^{-1}/cfu.g^{-1}$)	$1.2x10^1$	$8.4x10^3$	$3.1x10^3$
Sulphite reducing clostrida (MPN)	450 $.100ml^{-1}$	>140 $.g^{-1}$	>140 $.g^{-1}$
Post-monsoon period			
TPC ($cfu.ml^{-1}/cfu.g^{-1}$)	$1.7x10^5$	$4.5x10^6$	$6.5x10^6$
E. coli ($cfu.ml^{-1}/cfu.g^{-1}$)	0	0	0
Total coliforms ($cfu.ml^{-1}/cfu.g^{-1}$)	$1.2x10^1$	$4.5x10^1$	$3.8x10^3$
Vibrios ($cfu.ml^{-1}/cfu.g^{-1}$)	$3.8x10^2$	$4.2x10^3$	$1.68x10^4$
Sulphite reducing clostrida (MPN)	45 $.100ml^{-1}$	2.5 $.g^{-1}$	25 $.g^{-1}$

The study showed that majority of the brackish water farms were polluted with indicator organisms as well as other pathogens. Physicochemical parameters were well within the acceptable limits, but a sudden change in pH is a matter of concern.There was no significant variation in the vibrio count during different seasons, whereas total microbial load and indicator organisms were less in number during pre-monsoon and post-monsoon period, the ideal time for harvesting of shrimps.

References

Budisusilowati, S. and Haryani, E.S. (1995) *FAO Fish. Rep.* **514**, Suppl., FAO, Rome

Horsley, R.W. (1971) *J. Appl. Bact.* **36**, 3177

Lobrerra, T.A., Bulalacao, L.M. and Tan, A. (1990) *FAO Fish. Rep.* **401** Suppl., FAO, Rome

Nayyarahamed, I., Karunasagar, I. and Karunasagar, I. (1995) *FAO Fish. Rep.* No. **514**, Suppl., FAO, Rome

Nedoluha, P.C. and Westhoff, D. (1997) *Food Microbiol.* **14**, 255

Putro, S., Angyawati, A.M., Fawzya, Y.N. and Ariyani, F. (1990) *FAO Fish. Rep.* No. **401**. Suppl., FAO, Rome

Rattagool, P., Wongcherida, N. and Sanghtong, N. (1990) *FAO Fish. Rep.* **401**, Suppl. FAO, Rome

Shewan, J.M. (1962) in *Recent Advances in Food Sciences*, (Howthorn, J. and Leitch, J.M., Eds.), p. 167, Butterworths, London

Surendran, P.K., Thampuran, N. and Nambiar, V.N. (2000) *Fish. Technol.* **37**, 25

Surendran, P.K., Thampuran, N., Gopakumar, K. (1994) *FAO Fish. Rep.* **514**, Suppl., FAO, Rome

Toor, K.S., Kour, K. and Dhawan, A. (1991) *Aquaculture Production*, Oxford and IBH Pub. Co. Pvt. Ltd., New Delhi

Trivedy, R.K. and Goel, P.K. (1984) *Chemical and Biological Method for Water Pollution Studies,* Enviromental Pub., Karad, India

Twiddy, D.R. and Reilly, P.J.A. (1995) *FAO Fish. Rep.* **514**, Suppl., FAO, Rome

USFDA (2001) *Bacteriological Analytical Manual*, 8th edn. (revised), US Food and Drug Administration, Association of Official Analytical Chemists, Washington DC