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*Short Communication*

# Incidence of bacteria involved in nitrogen and sulphur cycles in tropical shrimp culture ponds

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Pond management is an integral part of shrimp culture activity. Deterioration of pond water and sediment quality would cause stress to shrimp making them susceptible to pathogens. Nutrient budgets of intensive shrimp culture ponds have been studied by a few investigators (Briggs and Funge-Smith, 1994; Lin and Muthuwan, 1995). Briggs and Funge-Smith (1994) reported that only 21% of the nitrogen and 13% of the phosphorus introduced through feed is incorporated into shrimp flesh and a major portion of nitrogen (31%) and phosphorus (84%) input is retained in the pond sediment causing nutrient loading. Shrimp are a poor converter of feed. Of the total feed given (dry weight), 15% is left unconsumed, 20% is lost as feces and 48% as metabolites with only 17% being incorporated into the flesh of shrimp (Primavera, 1994). Hence, more than 50% of the feed input into the pond goes to waste. This further leads to deterioration of the environment by reducing dissolved oxygen and increasing the ammonia and hydrogen sulphide levels (Wickins, 1976; Mevel and Chamroux, 1981).

Microorganisms are known to play an important role in nutrient cycling and decomposition (Anderson, 1987; Coleman and Edwards, 1987; Fry, 1987; Rheinheimer, 1992). Hence, water quality in aquaculture systems is to a great extent controlled by microbial degradation of organic matter (Avnimelech et al., 1995). Organic matter is degraded by a wide array of microorganisms. For instance, heterotrophic microorganisms oxidize organic

matter consuming oxygen and releasing carbon dioxide in the process, whilst autotrophic nitrifying and sulphur bacteria consume oxygen and carbon dioxide during the process of oxidizing ammonia, nitrite and sulphide. In shrimp culture systems, ammonia and sulphide are the forms of nitrogen and sulphur that are toxic to shrimp. Hence, nitrogen and sulphur cycle bacteria are most important in recycling lethal forms of nitrogen and sulphur.

Studies have been carried out on heterotrophic bacterial populations in shrimp culture ponds (Moriarty, 1986; Ruangpan et al., 1995; Anand et al., 1996); however, these studies did not include the bacterial flora involved in nutrient recycling. In this communication, we present data on the bacterial population involved in nitrogen and sulphur cycles in tropical shrimp culture ponds and their correlation with water quality parameters.

Quantification of bacterial populations involved in nitrogen and sulphur cycles were carried out for one culture cycle period from two shrimp ponds situated near Tallur estuary on the south west coast of India. Prior to stocking, the ponds were dried thoroughly, limed and fertilized with cow dung at 500 kg $ha^{-1}$. Shrimp ponds of 0.7 ha area were stocked with 22 postlarvae $m^{-2}$ 15 days after filling with water. High quality pelleted feed was given at recommended rates (2–8% based on the body weight) five times a day regularly until the end of culture. Each pond was provided with four aerators, one placed in each of the four corners. Except during feeding times, aerators were on during the rest of the period. Water exchange was at the rate of 70% per day. Plankton blooms in the ponds were maintained by the addition of inorganic fertilizers (urea, superphosphate) and liming intermittently. Chemicals such as iodophor, formalin, chlorine and tea seed cake were also used by the farm technicians to control the bacterial and other ectocommensal ciliates whenever necessary.

Fortnightly sampling of water and sediment was done aseptically at the inlet (inside) of the pond, which was also used as the outlet during neap tide. For bacteriological analysis, water samples were collected from a depth of 0.6 m below the surface and sediment samples from the bottom of the pond in pre-sterilized bottles (250 ml) and plastic bags respectively. Samples for water quality analysis were collected in 500 ml capacity polyethylene bottles. They were stored in an icebox and brought to the laboratory within 2 h of sampling. Physical and chemical parameters of water were analyzed following standard procedures. Water temperature, pH and salinity were recorded in the field using thermometer, pH meter and salinometer respectively. Dissolved oxygen was analysed following azide modification of Winkler's method (American Public Health Association, 1998). Total ammonia-N (phenate method), nitrite-N (Budschneider and Robinson method) and nitrate-N (Armstrong and Richard method using

*Table 1.* Water quality parameters of the shrimp culture ponds

| Water quality parameters | Mean (SE) | Range |
|---|---|---|
| Water temperature (°C) | 30.42 (0.35) | 27.0–32.5 |
| pH | 7.38 (0.07) | 6.88–7.80 |
| Dissolved oxygen (mg/l) | 7.37 (0.13) | 6.5–8.2 |
| Salinity (‰) | 26.9 (0.5) | 22.9–30.2 |
| Total ammonia-N (mg/l) | 0.694 (0.080) | 0.283–1.714 |
| Nitrite-N (mg/l) | 0.006 (0.002) | 0.001–0.0031 |
| Nitrate-N (mg/l) | 0.036 (0.021) | 0.002–0.213 |
| Phosphate-P (mg/l) | 0.151 (0.050) | 0.038–0.921 |
| Silicate-Si (mg/l) | 0.019 (0.009) | 0.005–0.033 |

cadmium column) were analysed as described by Strickland and Parsons (1972). Analysis of phosphate-P (ascorbic acid method) was as described by American Public Health Association (1998). Qualitative estimation of sulphide in water and sediment was done using lead acetate strips.

For bacteriological analysis of water and sediment samples, serial ten fold dilutions were made using 0.85% sterile physiological saline. Sediment samples after dilution were placed in an ultrasonic bath (Bransonic, Mexico) for 5 min to separate the bacterial cells from the sediment. Total plate count (TPC) was determined by plating water and sediment samples on tryptone soy agar (HiMedia, Bombay) having 1% sodium chloride. The inoculated plates were incubated at 30(2)°C for 48 h, enumeration done and counts represented as colony forming units (cfu).

Most probable number (MPN) technique (five-tube) was followed to enumerate the bacteria involved in nitrogen and sulphur cycles (American Public Health Association, 1992). Ammonia oxidizing and nitrite oxidizing bacteria were enumerated using Winogradsky's modified medium for ammonia oxidizers and nitrite oxidizers (Rodina, 1972). Sulphur oxidizing and sulphate reducing bacteria were also enumerated using sulphur oxidizing bacterial medium (Schneider and Rheinheimer, 1988) and sulphate reducing bacterial medium (tryptone 1%, sodium sulphite 0.1% and ferric ammonium citrate 0.001%) respectively. Water and sediment samples were suitably diluted before inoculating into respective culture media. All tubes of SRB medium were overlaid with sterile liquid paraffin after inoculation of sample. Nitrogen and sulphur cycle bacteria were estimated by incubating the inoculated tubes in the dark at 30 (2) °C for 6–15 days. Presence of SRB

was indicated by development of black colour due to formation of ferrous sulphide. Statistical analysis was carried out using "Statistica" package.

Water quality parameters in both the ponds showed similar trends (Table 1). Statistically, there were no significant differences ($P > 0.05$) between the ponds. Water temperature, ranged from 27–32 °C. Dissolved oxygen levels were high (>6 mg $l^{-1}$) throughout the culture period. Fluctuation in pH values was minimal (6.88–7.80). Similarly, salinity values ranged from 22.0–30.2 ‰ (Table 1). Nutrient levels such as ammonia-N and phosphate-P of both the ponds varied greatly whereas nitrite-N and nitrate-N levels were low. In general, ammonia-N levels in both the ponds increased towards the later period of culture (0.283–1.714 mg $l^{-1}$). The ammonia-N levels were very high on day 63 and 77 of culture (Figure 1a). The safe levels of ammonia-N for shrimp culture has been reported to be <1 mg $l^{-1}$ (MPEDA, 1992) and the values recorded here were high. Briggs and Funge-Smith (1994) and Tookwinas et al. (1994) reported levels ranging from 0.19 to 0.66 mg $l^{-1}$ in intensive shrimp culture farms of Thailand. High concentrations of ammonia recorded towards the end of the culture could be due to the increased feed input as the shrimp culture progressed. It is also possible that the ammonia build up was due to the non-oxidization of ammonia by resident ammonia oxidizing bacteria.

Nitrate-N levels were low during the entire culture period in the ponds, ranging from 0.002 to 0.213 mg $l^{-1}$ with a mean of 0.036 mg $l^{-1}$ (Table 1). Nitrite-N levels were below the safe level i.e. <0.25 mg $l^{-1}$ (MPEDA, 1992) throughout the culture period in both the ponds. Both nitrite and nitrate values were low compared to the levels reported by Briggs and Funge-Smith (1994) from the Thai shrimp ponds which had a mean value of 0.04 to 0.10 for nitrite-N and 0.08 to 0.16 mg $l^{-1}$ for nitrate-N. The phosphate-P levels increased during the last few days of culture period, peak being 0.92 mg $l^{-1}$ on day 91 of culture (Figure 1b). Phosphate-P values for both the ponds were high compared to the values reported for Thailand ponds (Briggs and Funge-Smith, 1994) which could be due to higher amounts of fertilizer input. Hydrogen sulphide was present throughout the culture period except for the first sampling.

In general, the numbers of bacteria present in sediment were higher than in the water sample. The total aerobic count in water ranged from $10^3$–$10^4$ cfu $ml^{-1}$ while it was $10^3$–$10^5$ cfu $g^{-1}$ in sediment (Table 2, Figure 2a). The high bacterial load in the sediment could be because of the high content of organic matter (Fletcher, 1979). Further, application of sanitizers such as chlorine, iodophor or any other chemicals into shrimp ponds would primarily act on the water column and thus reduce the bacterial load there. The results presented here are similar to those reported by Nayyarahamed et al. (1995)

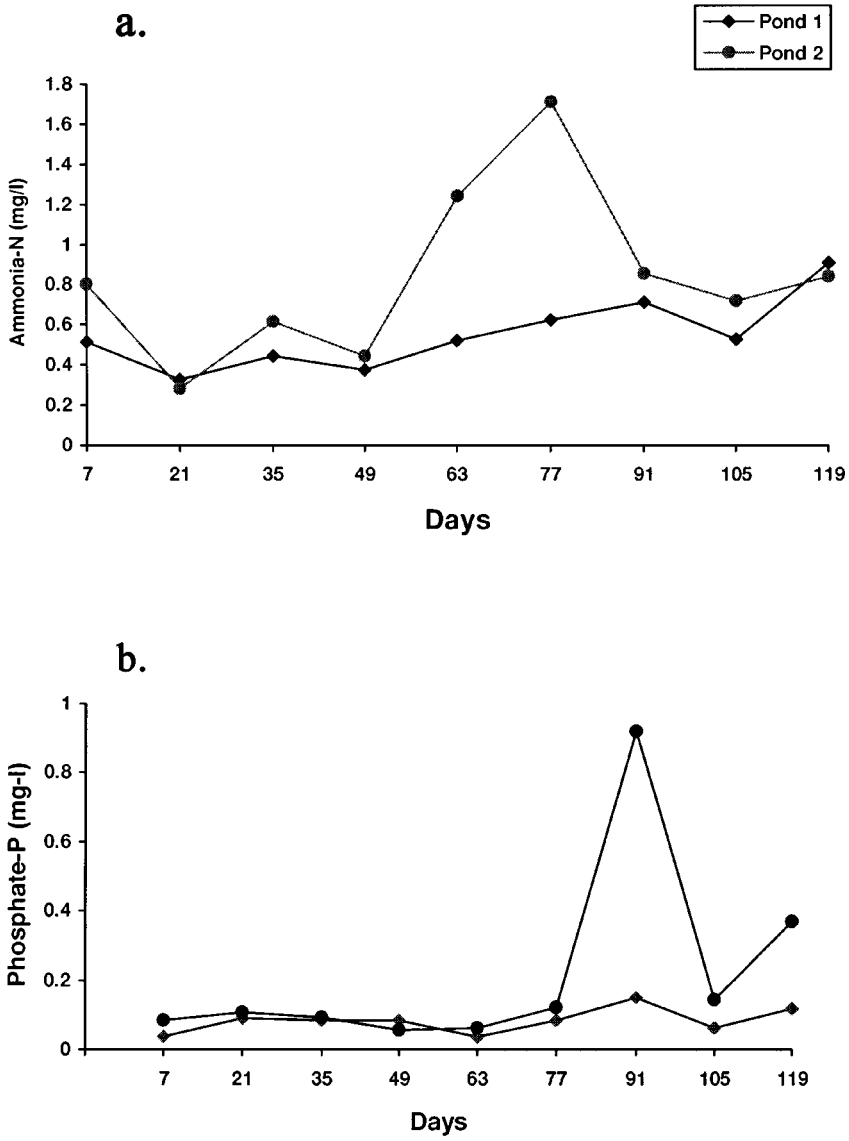


*Figure 1.* (a) Different concentrations of ammonia-N in two shrimp ponds during the culture period. (b) Change in Phosphate-P levels in both the shrimp ponds during the culture period.

*Table 2.* Different groups of bacteria in shrimp culture ponds

| Bacteria | Mean (SE) (Range) | |
|---|---|---|
| | Water | Sediment |
| Total aerobic count (cfu/ml; cfu/g) | 11,983 (3,573) (1,400–34,000) | 231,267 (3,573) (2600–610,000) |
| Ammonia oxidizing bacteria (*Nitrosomonas* sp.)(no./l;no./kg) | 65 (30) (<2–230) | 382 (247) (<2–2,000) |
| Nitrite oxidizing bacteria (*Nitrobacter* sp.) (no./l; no./kg) | 84 (22) (18–200) | 1,420 (462) (400–4,700) |
| Sulphur oxidizing bacteria (no./l; no./kg) | 7,828 (3,470) (230–>24,000) | 80,922 (1,250) (2,300–>240,000) |
| Sulphate reducing bacteria (no./l; no./kg) | 11,644 (2,396) (540–>24,000) | 194,000 (24,680) (54,000–>240,000) |

for shrimp farms practising extensive culture in India. Ruangpan et al. (1995) reported total bacterial counts ranging from $10^3$–$10^5$ cfu $ml^{-1}$ in water of shrimp culture ponds in Thailand. However, Anand et al. (1996) reported higher bacterial counts in water and sediment of shrimp culture ponds viz. $10^6$–$10^7$ cfu $ml^{-1}$ and $10^6$–$10^8$ cfu $g^{-1}$ respectively in India. In the present study, lowest levels were seen on day 21 of culture, which was probably due to the application of bactericidal chemicals during that period. However, peak values of TPC were recorded on day 77 of culture, which coincided with high ammonia-N values. Significant positive correlation was also found between total ammonia-N and total aerobic bacteria in the sediment ($r = 0.84$, $p < 0.05$) (Figure 2b). Ruangpan et al. (1995) also reported significant positive correlation between nitrite-N and total aerobic bacteria in intensive shrimp culture ponds of Thailand.

The number of ammonia oxidizers varied from $<2 - 230$ $l^{-1}$ of water and $<2 - 2{,}000$ $kg^{-1}$ of sediment and that of nitrite oxidizers ranged from 18–200 $l^{-1}$ of water and 400–4,700 $kg^{-1}$ of sediment. Ammonia oxidizers could not be detected in the water on day 49 and 63 of sampling. Though a low level ($0.018 \times 10^1$ $l^{-1}$) was detected on day 77, the levels were below detectable limits between day 91 and 119. In the sediment, ammonia oxidizers were detectable only on day 7 and 77 and were undetectable during the rest of the period. There is not much information on the incidence of ammonia oxidisers in shrimp ponds.

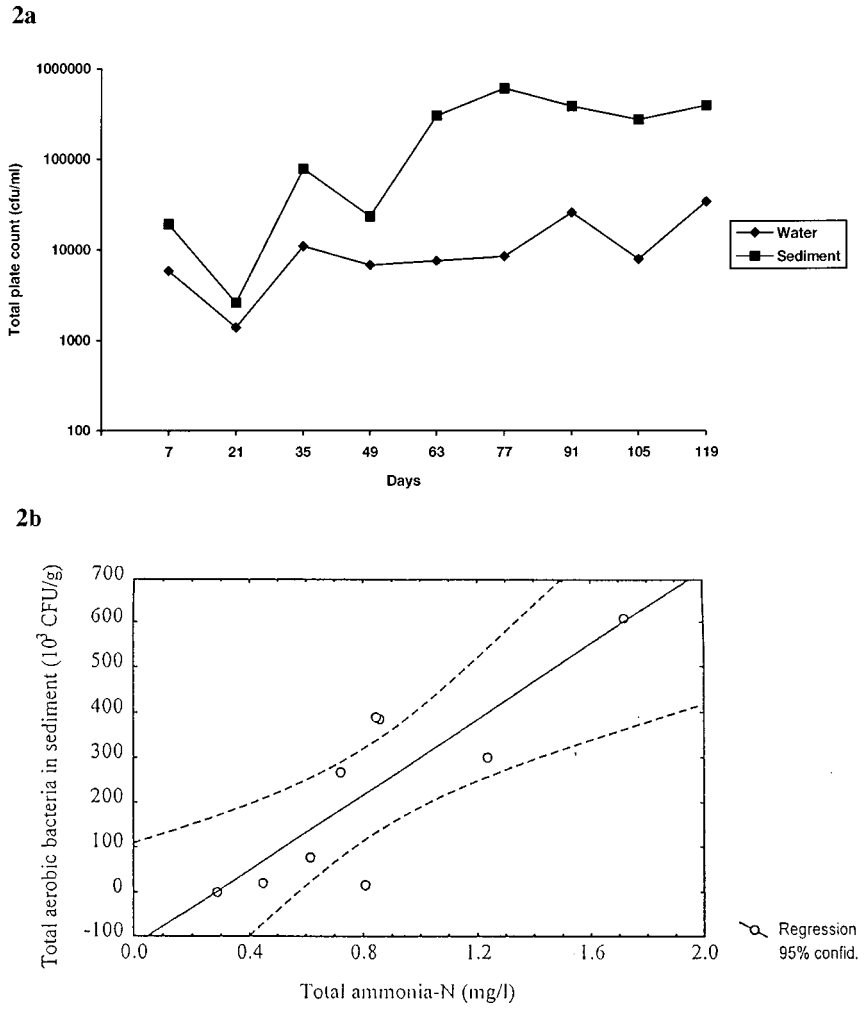


*Figure 2.* (a) Levels of aerobic bacteria in water and sediment from shrimp ponds during the culture period. (b) Graph representing positive correlation between total aerobic count in sediment and total ammonia-N.

Bianchi and Bianchi (1982) reported $1.0 \times 10^7$ nitrifying bacteria $l^{-1}$ of water from a shrimp farm. Joye and Hollibaugh (1995) observed that nitrification rates either get reduced or inhibited when hydrogen sulphide concentration increases in the marine sediment. In the present study, the detection of low number or absence of nitrifying bacteria in shrimp pond could be due to the presence of hydrogen sulphide in water and sediment or due to the effect of water sanitizers and chemicals used (Brown

and Turner, 1982). A significant positive correlation ($p < 0.05$) was found between ammonia-N concentration and the number of ammonia oxidizers in the sediment ($r = 0.67$) and also between nitrite-N and nitrite oxidizers in the sediment ($r = 0.86$). This suggests that the load of nitrifying bacteria in the environment is substrate dependent.

Bacteria involved in the sulphur cycle were present in high numbers throughout the culture period compared to nitrogen cycle bacteria. Levels of sulphur oxidizing bacteria (SOB) ranged from $230 - > 24{,}000\ l^{-1}$ in water and $2{,}300 - > 240{,}000\ kg^{-1}$ of sediment (Table 2). The levels of sulphate reducing bacteria (SRB) ranged from $5{,}400 - > 24{,}000\ l^{-1}$ in water and $54{,}000 - > 240{,}000\ kg^{-1}$ sediment (Table 2). A significant positive correlation was found between the levels of SOB and ammonia-N levels in water (at $p < 0.05$, $r = 0.70$), between SOB counts and ammonia-N in sediment ($p < 0.10$, $r = 0.61$), between SOB in water and SOB in sediment ($p < 0.05$, $r = 0.963$), between SOB in both sediment and water with ammonia oxidizers in sediment ($p < 0.05$, $r = 0.856$ and 0.935 respectively) and between SOB in sediment and SRB in water ($p < 0.05$, and $r = 0.885$).

Information on the levels of SRB in shrimp culture ponds is scanty. The number of SRB recorded in the present study is considerably low compared to SRB count of 20,000 to 130,000 $cm^{-3}$ in mud of Limfjord (Jorgensen, 1977a). Suplee and Cotner (1996) reported SRB counts of 9,300–42,000 $cm^{-3}$ of the sediment in the experimental ponds stocked with penaeid shrimp and fish. Even though SRB are anaerobic in nature, they are recorded in both sediment and water of shrimp farms and this is probably due to the occurrence of reduced microniche in sediment particles suspended in water. In this environment, aerobic heterotrophs consume oxygen, creating anaerobic conditions towards the centre of the particle thus allowing SRB to flourish (Jorgensen, 1977b). The counts of SRB were very high on day 77 of culture. This could be due to the higher availability of organic matter which might be related to excessive feed input leading to wastage during that period of culture. Suplee and Cotner (1996) also observed that the SRB count increased as the culture progressed and they correlated the sulphate reduction with availability of organic matter.

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