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Trends in Beneficial and Pathogenic Bacterial Populations and their Relation with Environmental Parameters in Tiger Shrimp, *Penaeus monodon* Culture Ponds

P. K. Patil[1*], M. Muralidhar[1], H. G. Solanki[2], Krishna Patel[2], C. Gopal[3] and S. M. Pillai[3]

[1]Aquatic Animal Health and Environment Division, Central Institute of Brackishwater Aquaculture, R.A.Puram, Chennai, India
[2]Soil and Water Management Research Unit, Navsari Agricultural University, Navsari, Gujarat, India
[3]Crustacean Culture Division, Central Institute of Brackishwater Aquaculture, R.A.Puram, Chennai, India

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Correspondence to

**E-mail*: pkpatil@ciba.res.in

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Abstract

In aquaculture pond environment, application of several inputs leads to deterioration of pond bottom resulting in build up of toxicants like, ammonia and hydrogen sulphide. Hence it is essential to understand the beneficial effects of bacterial populations involved in nitrogen and sulphur recycling and the shifts in the opportunistic bacterial pathogens. The present investigation reports the influence of farm level interventions (fermented juice, soil and water probiotics) on nitrogen [Ammonia oxidizing bacteria (AOB) and Nitrite oxidizing bacteria (NOB)] and sulphur [Sulphur oxidizing and reducing bacteria (SOB and SRB)] recycling bacteria, opportunistic pathogens (*Vibrio* spp.) and total cultivable bacterial load and their relation with physico-chemical parameters of soil and water in three tiger shrimp *Penaeus monodon* culture ponds in Gujarat, India. During the culture period variation in different bacterial populations (x10^3 ml^{-1}) was observed; AOB: 4 to 28 (13±6) and 4 to 20 (12± 5), NOB: 4 to 28 (12±7) and 4 to 28 (14±6), SOB: 15 to 39 (23±8) and 15 to 120 (84±74), SRB: 15 to 39 (96.5±89) and 20 to 240 (166±75) in water and sediment samples respectively. Similar fluctuation was also noticed in water and sediment with respect to populations of presumptive *Vibrio* count (WPVC, SPVC) and total (heterotrophic) plate bacteria (WTPC and STPC). The hardness and alkalinity in pond waters was dominated by calcium and bicarbonate: ionic concentrations relate to organic carbon in soil and total ammonia N (TAN) (r=0.51) in water, AOB and nitrate N (r=0.59), TAN and WTPC (r=0.71)/WPVC (r=0.47), SOB *vs* NOB were positively correlated. The study indicated the importance of both groups of recycling bacteria in maintaining the pond water parameters at optimum level and to control the opportunistic pathogens in the culture environment.

1. Introduction

Recent intensification of shrimp culture systems with a host of inputs has lead to deteriorating water and soil quality causing poor nutrient utilization and increased susceptibility to diseases. Nutrient budgeting of shrimp culture systems revealed that 21% nitrogen and 13% phosphorus provided in the feed is accumulated into shrimp flesh, Lin and Muthuwan 1995) and rest of the nutrients are remained in pond sediment leading to pond environment deterioration by reducing dissolved oxygen and increasing the levels of ammonia and hydrogen sulphide which are known toxicants (Mevel and Chamroux, 1981).

Pond environment microbes play key role in decomposition of organic matter and nutrients cycling through the process of mineralization. Ammonia and nitrite oxidizing bacteria along with other bacterial communities carryout nitrogen fixation, ammonification, nitrification and denitrification in aquaculture pond (Avnimelech et al., 1995). Similarly sulphur reducing and oxidizing bacteria are predominately involved in reduction and oxidation of sulphates and hydrogen sulphide in pond bottom. Understanding the pond microbial dynamics is crucial for the effective management of shrimp culture environment. However, reports on importance of nutrient recycling bacteria are scanty (Devaraja et al., 2002, Abraham et al., 2004).

Farm level interventions like application of fermented rice juice and commercial probiotics is practiced in shrimp aquaculture to maintain the optimum water and soil quality. Beneficial effects of probiotic application as a method of biological control has been well documented (Gatesoupe, 1999). Hence, it is important to study the effect of such interventions on the microbial populations of the pond and in turn the overall pond health. In the present study, the influence of farm level interventions like addition of fermented rice juice and probiotics on the levels of pond water and soil physico-chemical parameters, environmental bacteria involved in nutrient recycling along with the total heterotrophic bacterial and pathogenic Vibrio bacteria and their association was assessed during the culture of *P. monodon* in three ponds.

2. Materials and Methods

2.1. Shrimp culture and management practice

Three ponds (0.15 ha each) located at Danti Experimental Station of Navsari Agricultural University (NAU), Navsari, Gujarat were stocked @ 16 nos. m^{-2} with postlarvae (PL15) of tiger shrimp *P.monodon*. The reservoir pond was tide-fed from an adjoining creek located about 1 km from the sea. The tiger shrimp (*P.monodon*) culture was carried out for 132 days during August to December 2011 and fed four times a day @ 5-10% of the shrimp body weight in equally divided quantities. Standard procedures of pond preparation [drying and scraping of subsurface soil, liming (Agril.lime @1 ton ha^{-1}) and filling filtered sea water, bleaching (calcium hypochlorite @300 kg ha^{-1}) followed by application of lime (Agril.lime @ 100 kg ha^{-1}), fertilization of water with fermented juice (100 kg rice bran+10 kg jaggery+ 100 g yeast per ha) in three doses each on every third day and addition of soil and water probiotics] were followed before stocking the seed. Management interventions pertaining to application of chemicals/products during the entire culture period is presented in Table 1.

2.2. Sample collection

Water and sediment samples were collected from five sites (four corners and centre of each pond) at regular intervals in sterile bottles and plastic bags respectively and transported on ice to laboratory. Samples were processed within four hours of collection and stored for further analysis.

2.3. Physico-chemical analysis

Water samples were analysed for pH, salinity, calcium, magnesium, total hardness, total alkalinity, nitrite nitrogen, nitrate nitrogen and total ammonia nitrogen (TAN) where as sediment samples were analyzed for pH, electrical conductivity (EC), organic carbon, available nitrogen and available phosphorus using standard procedures (APHA, 1989).

2.4. Bacteriological analysis

Both water and sediment samples were analysed for total plate count (TPC) (Gilliland et al., 1976), presumptive vibrio count (PVC) (Austin, 1988), ammonia oxidizing bacteria (AOB), nitrite oxidizing bacteria (NOB), sulphur oxidizing bacteria (SOB) and sulphur reducing bacteria (SRB) (Rodina, 1972).

2.5. Statistical analysis

The data was analysed for Pearson Correlation Coefficient to determine the significant association at $p<0.05$, between the levels of bacterial populations and physico-chemical parameters.

3. Results and Discussion

Nitrogen and sulphur plays a key role in nutrient recycling in the pond and becomes toxicants when accumulated at higher levels in the system (Burford and Lorenzen, 2004). Nitrogen and sulphur recycling beneficial heterotrophic bacteria through the process of mineralization mitigate the deterioration of pond water quality due to build up of toxicants. A wide range of microbes are involved in this process of microbial degradation. Accumulated organic matter is oxidized by heterotrophic bacteria by consuming oxygen and releases carbon dioxide. Where as autotrophic nitrifying and sulphur bacteria during the process of oxidation of ammonia, nitrite and sulphide consume oxygen and carbon dioxide. These microbial activities influence the water quality in aquaculture systems to a greater extent (Avnimelech et al., 1995).

3.1. Physico-chemical parameters of pond water and sediment

Water pH (7.94±0.38), salinity (17.89±1.19 ppt), calcium (512.66±35.69 ppm), magnesium (332.60±16.22 ppm), total

Table 1: Pond level interventions (pertaining to the application of chemicals/ products) during the culture period.

DOC	Treatment	DOC	Treatment
8	Fermented rice juice water probiotic	68	Fermented rice juice
12	Fermented rice juice	71	Drag chaining
17	Soil probiotics	77	Soil probiotics
20	Dolomite @ 20 kg ha^{-1}	78	Liming @25 kg ha^{-1}
25	water probiotics	81	Water probiotics
44	soil probiotics , zeolite @25 kg ha^{-1}	83	Fermented rice juice ½ dose
49	water probiotics, lime 20 kg ha^{-1}	93	Fermented rice juice
51	Fermented rice juice	94	Soil probiotics, Zeolite @ 25 kg ha^{-1}
58	Drag chaining	110	Fermented rice juice
59	soil probiotics	111	Dolomite 25 kg ha^{-1}
64	Water probiotic	120	Zeolite 20 kg ha^{-1} , Soil probiotic

Dose of water probiotic @ 350 g ha^{-1}; soil probiotic @ 2 L ha^{-1}; Fermented rice juice @100 lit ha^{-1}

hardness (2635±94.96 ppm), carbonate (8.68±4.93 ppm), bicarbonate (155.60±43.08 ppm), total alkalinity (150.13±31.49 ppm), nitrite nitrogen (0.16±0.19 ppm), nitrate nitrogen (0.21±0.08 ppm) and TAN (0.23±0.14 ppm) were within the optimal levels for shrimp farming (Table 2). The pond bottom sediment parameters like EC (3.85±1.06 ds m^{-1}), pH (8.58 ±0.31), organic carbon (0.22±0.10%), available nitrogen (123.52±44.78 mg kg^{-1} soil) and available phosphorus (38.29±18.22 mg kg^{-1} soil) were within the normal range (Table 3).

Variations in the trends of physico-chemical parameters of sediment were observed throughout the culture period. pH was stable throughout the culture period with average of 8.45 except a rise to 9.13 at 114 DOC. Though the salinity was higher during the initial period of culture (33.19±0.49 ppt) due to rains it reduced at 39 DOC (13.78±0.66 ppt) and there after stabilized latter at 18.39±1.3 ppt till harvest. Concentration of calcium, magnesium and the total hardness was maintained at steady levels and on the contrary higher fluctuations were observed in carbonate and bicarbonate and total alkalinity. The nitrite nitrogen steaily increased to reach 0.52±0.15 mg l^{-1} at harvest and however nitrate nitrogen was fluctuating throughout the culture. The concentration of TAN was 0.20±0.026 mg l^{-1}at the beginning, reached highest at 114 DOC (0.37±0.28 mg l^{-1}) and reduced to 0.18±0.02 mg l^{-1}at the time of harvest. The levels of nitrite nitrogen were within the optimum range though increase was registered at the end of the culture.

3.2. Bacterial population

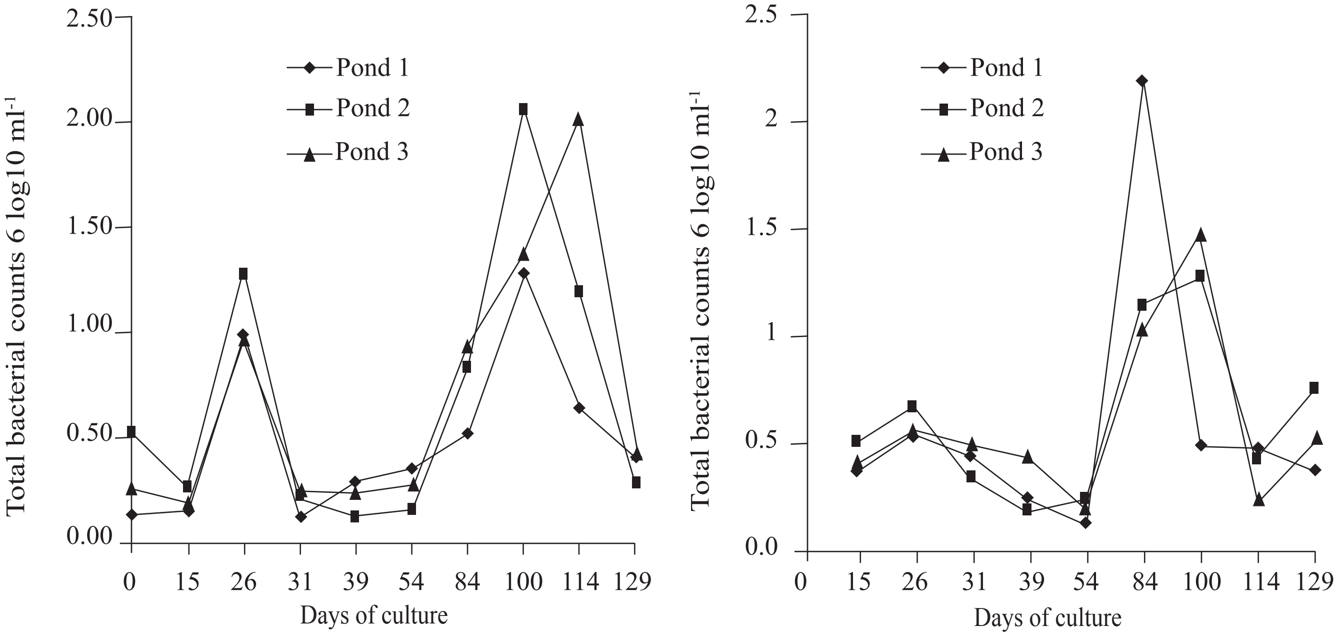
Bacterial populations showed similar trend among the three ponds throughout the culture period. The variation in different bacterial populations (x10^3 ml^{-1}) observed during the culture period were, AOB: 4 to 28 (13±6) and 4 to 20 (12± 5), NOB: 4 to 28 (12±7) and 4 to 28 (14±6), SOB: 15 to 39 (23±8) and 15 to 210 (84±74), SRB: 11 to 210 (96.5±89) and 20 to 240 (166±75) in water and sediment samples, respectively (Table 4). Similarly, average TPC (x10^6 ml^{-1}) 0.84±0.61 and 0.74±0.58 and PVC (nos. ml^{-1}) were 188±87.10 and 136.66±96.11 in water and sediments respectively.

Among the different bacterial populations evaluated in the study, TPC outnumbered AOB, NOB, SOB and SRB further indicating the role of abundant heterotrophic bacteria over the autotrophic ones. The average counts of heterotrophic bacteria as indicated by TPC were higher in sediment compare to water except at 84 and 100 DOC (Figure 1). Though similar fluctuations were observed between water and sediments, increasing trend was observed as culture progressed except at the last sampling. A general increasing trend of PVC in water was observed as the culture progressed despite some fluctuations, however similar observations were not recorded in sediment samples (Figure 1). The values of TPC observed in the present study were in agreement with previous reports for India (Rao et al., 2000) and Thailand (Ruangpan et al., 1995).

Throughout the culture period the populations of beneficial

Table 2: Physico-chemical parameters of water samples during the culture period (Mean ±SD), n=3 ponds.

DOC	Salinity (ppt)	Ca (ppm)	Mg (ppm)	Total Hardness (ppm as $CaCO_3$)	CO_3^{-2} (ppm)	HCO_3- (ppm)	Total Alkalinity (ppm as $CaCO_3$)	NO_2-N (ppm)	NO_3-N (ppm)	Total Ammonia-N (ppm)
15	33.19 (±0.49)	514.67 (±40.26)	334.79 (±26.33)	2662.67 (±9.24)	12.77 (±3.66)	152.03 (±17.30)	135.33 (±15.01)	0.01 (±0.00)	0.18 (±0.044)	0.20 (±0.026)
26	18.50 (±0.33)	554.67 (±16.65)	337.71 (±12.15)	2774.67 (±46.36)	15.17 (±1.38)	105.69 (±2.81)	99.33 (±3.05)	0.008449 (±0.001)	0.15 (±0.01)	0.20 (±0.03)
39	13.78 (±0.66)	501.33 (±16,65)	322.14 (±8.91)	2577.33 (±69.55)	6.39 (±1.38)	107.31 (±2.43)	93.33 (±3.05)	0.02 (±0.008)	0.21 (±0.006)	0.11 (±0.035)
54	17.10 (±0.23)	520.00 (±21.17)	325.06 (±10.25)	2636.00 (±94.32)	4.79 (±4.79)	121.13 (±3.72)	103.33 (±4.16)	0.03 (±0.02)	0.24 (±0.02)	0.13 (±0.01)
84	17.03 (±1.39)	485.33 (±20.13)	342.58 (±17.11)	2621.33 (±84.13)	11.18 (±6.91)	156.91 (±15.40)	138.00 (±6.93)	0.071 (±0.40)	0.19 (0.16)	0.22 (±0.06)
100	19.19 (±0.46)	538.67 (±28.09)	338.68 (±7.72)	2738.67 (±97.68)	3.99 (±3.66)	208.13 (±5.08)	174.00 (±4.00)	0.050 (±0.03)	0.25 (±0.08)	0.23 (±0.05)
114	17.74 (±1.02)	461.33 (±36.07)	334.79 (±23.60)	2529.33 (±20.53)	7.98 (±2.76)	193.49 (±49.28)	165.33 (±39.31)	0.156 (±0.03)	0.17 (±0.03)	0.37 (±0.28)
129	18.39 (±1.30)	525.33 (±16.65)	325.06 (±22.30)	2649.33 (±61.10)	7.188 (±2.36)	200.00 (±6.45)	170.00 (±4.00)	0.52 (±0.15)	0.21 (±0.06)	0.18 (±0.02)


Total bacterial counts in water and sediment samples of *P. monodon* culture ponds (Danti)

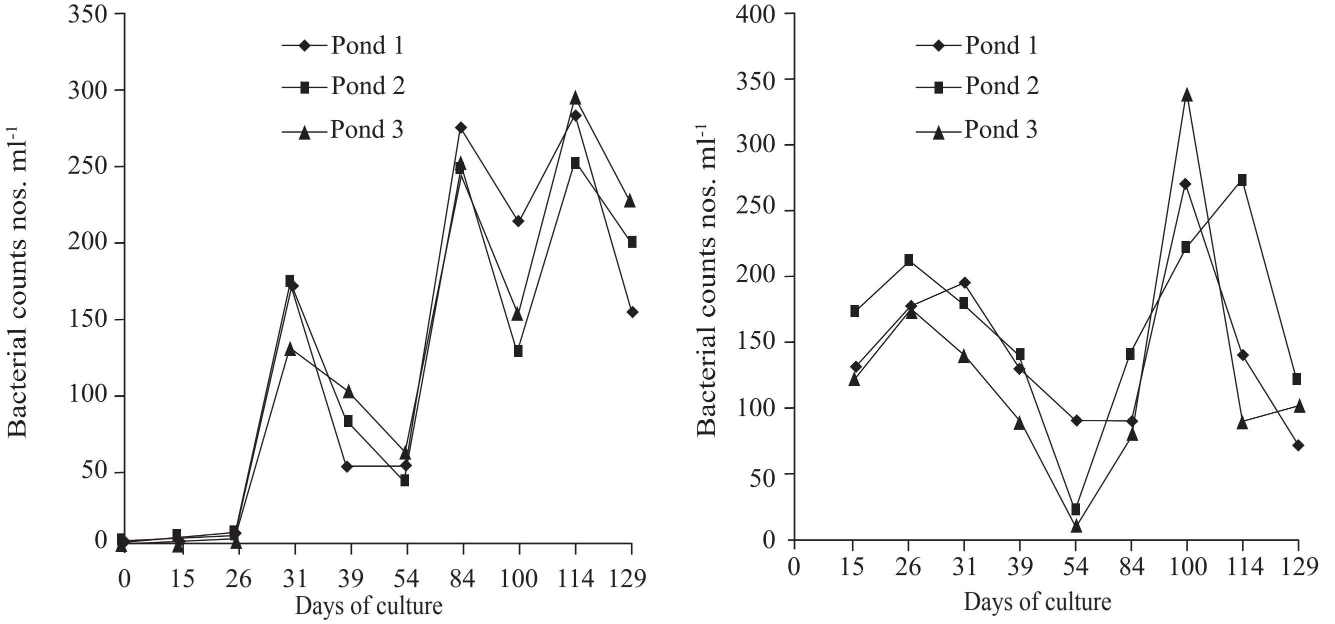

Total presumptive vibrio counts in water and sediment samples of *P. monodon* culture (Danti)

Figure 1: Trends in TBC and PVC in the water and sediment samples of the shrimp culture ponds. (TBC:Total bacterial counts, PVC: Presumptive vibrio counts)

Table 3: Physico-chemical parameters of pond sediment during the culture period (Mean ±SD), n=3 ponds.

DOC	EC (dS m^{-1})	pH	Organic carbon (%)	Available N (kg ha^{-1})	Available P (kg ha^{-1})
15	12.47 (±1.26)	8.47 (±0.04)	0.21 (±0.07)	108.74 (±15.44)	76.39 (±13.64)
54	3.89 (±0.60)	8.55 (±0.21)	0.23 (±0.05)	116.24 (±12.99)	41.36 (±7.94)
84	3.32 (±1.47)	8.37 (±0.08)	0.14 (±0.02)	108.20 (±11.40)	26.38 (±7.69)
100	4.20 (±0.84)	8.37 (±0.03)	0.14 (±0.05)	81.42 (±22.74)	49.82 (±33.86)
114	3.02 (±0.03)	9.13 (±0.11)	0.34 (±0.11)	172.48 (±78.48)	44.94 (±18.69)
129	4.81 (±1.26)	8.49 (±0.15	0.25 (±0.11)	139.27 (±1.85)	28.95 (±9.16)

Table 4: Density of different bacterial populations (103 ml^{-1}) in water and sediment during the culture period (Mean ±SD), n=3 ponds

	Water samples				Sediment samples			
DOC	AOB	NOB	SOB	SRB	AOB	NOB	SOB	SRB
39	6.00 (±1.73)	22.00 (±6.56)	19.00 (±3.46)	15.33 (±11.15)	7.67 (±6.35)	14.00 (±6.56)	90.00 (±104.40)	42.67 (±43.66)
54	12.67 (±6.66)	8.67 (±5.67)	25.67 (±4.04)	88.00 (±105.70)	13.00 (±8.18)	13.00 (±8.18)	176.67 (±57.73)	131.67 (±88.93)
84	14.00 (±6.56)	15.67 (±10.21)	23.33 (±4.04)	190.00 (±34.64)	9.67 (±4.62)	14.67 (±5.51)	68.67 (±70.44)	200.00 (±45.82)
100	16.67 (±10.60)	16.67 (±2.89)	27.33 (±12.01)	151.67 (±101.04)	12.33 (±4.62)	11.33 (±6.35)	96.00 (±62.19)	190.00 (±34.64)
114	8.67 (±5.69)	9.67 (±4.62)	16.67 (±2.89)	18.00 (±8.89)	11.33 (±6.35)	11.00 (±4.00)	19.33 (±7.50)	220.00 (±17.32)
129	15.67 (±1.14)	12.00 (±13.86)	23.00 (±13.86)	35.00 (±12.12)	14.00 (±6.56)	22.67 (±4.62)	61.67 (±76.54)	90.67 (±103.92)

DOC: Days of culture; AOB: Ammonia oxidizing bacteria; NOB: Nitrate oxidizing bacteria; SOB: Sulphur oxidizing bacteria; SRB: Sulphur reducing bacteria; TPC: Total plate count; PVC: Presumptive vibrio count

bacteria were significantly higher in pond sediment compared to water. As the culture progressed increase in the numbers of AOB was observed both in water and sediment samples except a dip on 114th day in water and on 84th day in the sediment, whereas, a decreasing trend was observed in NOB both in water and sediments except for the steep hike in the sediment samples at the end of the culture. The Fgrowth of AOBs is reported to be highly sensitive to change in temperature and nutrients and dissolved oxygen concentrations (Joye and Hollibaugh 1995). In the present study, water quality parameters at optimal range created favourable conditions for the observed higher growth of nitrifying bacterial populations. The recorded higher AOB and NOB levels were similar to the earlier reports of Cheng and Liu (2001). It could be speculated that high numbers of nitrifiers observed in turn through dynamic cycle, control the accumulation of toxicants as indicated by the concentration of ammonia and nitrite within the permissible limits during the culture. Role of bacteria in controlling the build up of toxicants like ammonia and nitrite has also been reported by Shan and Obbard (2001) and Fernandes et al. (2010).

Both SOB and SRB loads were also higher in sediment compared to water samples though no specific trend was observed as the culture progressed. Though SRBs are considered anaerobic bacteria, they were present both in pond bottom sediment and water column and are corroborated with the observations of Rao and Karunasagar (2000). The reason for higher SRB in water column might be attributed to possible creation of anaerobic conditions at the centre of micro-niche due to higher activity of heterotrophic bacteria (Schramm et al., 1999). Though sulphide concentrations could not be ascertained in the present study, levels of SRB is suggestive of efficient removal of sulphide from the culture system. However, levels recorded in the present study were much lower than the previous reports (Suplee and Cotner 1996; Rao et al., 2000). A significant increase of both SOB and SRB at 132 DOC coincides with the application of soil probitoic product containing the bacteria involved in sulphur recycling.

Levels of sulphate reducers and sulphide oxidizers reportedly suggest efficient sulfur cycling in an environment (Madrid et al., 2001). Throughout the culture period populations of bacteria involved in sulphur cycle were higher than those in nitrogen cycle contradicting the results of Fernandes et al. (2010), might be due to the anaerobic conditions and was in confirmity with the studies of Rao et al. (2000) and Devaraj et al. (2002). The observed higher SOB and SRB levels in sediment might be attributed to the availability of organic matter. Further, density of SRBs were recorded higher than that of SOB both in water and sediment samples and the reason is attributed to the possible creation of anaerobic conditions and release of reduced compounds like, acids, alcohols, carbon dioxide and hydrogen by fermenting bacteria as the culture progresses and utilization of these compounds by SRBs (Moriarty, 1997).

3.3. Relationship between physico-chemical parameters and bacterial populations in water and sediment

The physico-chemical parameters and bacterial populations revealed a negative correlation between TPC and available phosphorus in the present study, similar to the results of Fernades et al. (2010). Heterotrophic bacteria in culture pond were reported to compete for phosphate in addition to breaking down the organic matter (Drakare, 2002) and growth of bacteria is limited by the concentrations of inorganic phosphorus (Cotner et al., 2000). Enhanced phosphate uptake by heterotrophic bacteria could be the reason for the observed inverse correlation between the levels of TPC and available phosphorus. It is interesting to note that the peak TPC observed at end of the culture period coincide with that of ammonia levels. Similar significant correlation between the TPC and ammonia levels was observed by Ruangpan et al., 1995 and Rao et al., 2000.

The total hardness recorded in the study was positively correlated with the salinity of the pond water. Organic carbon in soil versus total ammonia N (TAN) (r=0.51) in water and AOB versus nitrate N (r=0.59) were positively correlated, indicating the oxidation of ammonia compounds into nitrite and then to nitrate. Positive correlation between TAN and WTPC (r=0.71)/WPVC (r=0.47) in addition to the positive correlation between oxidizing bacterial populations (SOB *vs* NOB) was also observed.

3.4. Influence of farm level intervention on water quality and microbial populations

Role of farm level interventions like application of fermented juice on the physico-chemical parameters and levels of microbes was evaluated by comparing the levels of water parameters and different bacterial populations. It was observed consistently that application of water probiotics on DOC 35, 49, 81 resulted in reduction of PVC both in water and sediment. Application of soil probiotics on DOC 59 and 77 registered an increase in NOB populations. Similarly, the observed increase in TPCs on DOC 100 could be attributed to the application of fermented rice juice on DOC 83 and 93.

It was observed that due to application of dolomite, the pH increased (on DOC 20) inspite of monsoon rains. The decrease in pH on 54th and 114th day (7.83) might be attributed to the application of fermented juice of 20 l per pond on 51st and 110th day though dolomite is applied. This indicates that dose of fermented juice @ 10 l/pond might be sufficient to maintain the pH. The application of water probiotics on 35th day correlates with the decrease in the nitrite nitrogen and TAN on 26th and 39th day. The repeated application of probiotics on 49th, 64th and 81st day helped in maintaining these parameters in optimum range. The increase in these parameters nearer the harvest time might be due to increase in shrimp biomass and the leftover uneaten feed. These observations suggest the need for repeated dose of probiotic applications to avoid the increase in these parameters.

Chain dragging on the pond bottom on 58th and 71st day might have resulted in the observed increase in nitrogen metabolites concentrations. Though dragging the chain is a good practice, it has to be carried out at regular interval followed by application of probiotics. Generally, pH decreases and organic carbon increases with the progress of culture. But in the present study the values did not change much compared to the initial values due to the maintenance of better quality pond environment.

4. Conclusion

In Indian shrimp culture system, major portion of expenditure on health management is through application of products like gut, water and soil probiotics. It is important to understand their mechanism of action and influence on the pond microbial dynamics to be able to effectively use these products for profitable shrimp production. In the present study the observed bacterial counts and physico-chemical parameters suggest the role of microbial processes in maintaining healthy pond environment. Further, research is required to identify the suitable strain and number of bacteria in the product, dose and schedule of application in different culture systems.

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