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Shrimp Production under Zero Water Exchange Mode Coupled with Bioremediation and Application of Probiotics

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Abstract: The study evaluates field level performance of a zero water exchange shrimp farming protocol in terms of production of shrimp biomass and maintenance of water and sediment qualities in 10 earthen modified semi-intensive farms in different parts of Kerala, India which were managed through bioremediation and application of probiotics. Water and sediment qualities during the culture were maintained by the addition of Detrodigest™, an indigenous bioaugmentor. The ponds were stocked with healthy *Penaeus monodon* seed (PL 20), tested nested PCR negative for White Spot Syndrome Virus (WSSV), at an average stocking density of 5 m^{-2}. The gut probiotic Enterotrophotic™ and an antagonistic probiotic PS-1 were used through out the culture period. Eh and pH of the sediment did not vary significantly during different phases of the culture and water quality parameters, except salinity and total hardness, also followed the same trend, maintaining stable environment quality throughout the culture period compared to the control ponds. Routine animal health assessment showed no outbreak of diseases in any of the ponds under study whereas in the control pond there was mortality and culture failure. Cost benefit ratio indicated profitability and the average feed conversion ratio was close to the optimum.

Key words: Zero water exchange, shrimp farms, bioremediation, probiotics, water quality, sediement quality, shrimp health management, shrimp diseases

INTRODUCTION

Zero water exchange shrimp farming technology has grown over the years in shrimp producing countries, as a sustainable practice to combat outbreak of diseases and management of culture environment quality. In conventional shrimp culture, water used to be exchanged in order to maintain optimum environment quality required for growth (Hopkins *et al*., 1993). This practice besides causing eutrophication in the adjacent coastal waters (Shang *et al*., 1998), also opens up avenue for horizontal transmission of pathogens (Chamberlain, 1997). With the intensification of farming practice the risk of disease outbreaks has also been increasing simultaneously (Kautsky *et al*., 2000; Guan *et al*., 2003; Carbajal-Sanchez *et al*., 2008.) The stage has come now that without proper aquaculture environment management, it will not be possible to get a successful crop (Flegel, 1996) and accordingly the shrimp culture has begun to get transformed from the traditional open system with frequent water exchange to closed systems with little or no water exchange at all (Aquacop, 1985; Hopkins *et al*., 1993; Sandifer and Hopkins 1996; Thakur and Lin, 2003). Meanwhile, as a result of growing requirements for increased biosecurity and effluent quality control (Pruder and Bullis, 2001), the shrimp culture industry in many countries has increasingly been developing biosecured closed production systems (Menasveta, 2002; Schuur, 2003), including zero water exchange or recirculation aquaculture systems by employing in-situ and ex-situ biofiltration (Avnimelech, 2000; Cohen *et al*., 2001; McAbee *et al*., 2003; Sowers *et al*., 2005; Azim and Little, 2008; Balasubramanian *et al*., 2005; Lezama-Cervantes *et al*., 2010). However during their operation, several essential nutrients progressively get depleted from water column and digestive/excretory metabolites or feed contaminants get progressively accumulated to toxic levels (McNeil, 2000). This suggested the requirement of the implementation of an appropriate waste management strategy, as poor water and sediment qualities lead to disease outbreaks (Mohan, 1996). To tide over the situation, the practice of applying viable bacteria and their products has been introduced which would regulate water

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and sediment qualities presuming that the added bacteria produce greater quantities of a range of exoenzymes breaking down organic compounds (Moriarty, 1997). Moreover, the added organisms might stabilize or enhance a microbial community in the gastrointestinal tract and within the culture system favorable to the animal and improve the growth, survival and disease resistance of the cultured animals (Douillet, 2000; Horowitz and Horowitz, 2001; Karunasagar *et al*., 2000; Sonnenholzner and Boyd, 2000; Vine *et al*., 2006; Castex *et al*., 2008). Adequate scientific evidence could be cited concerning the beneficial effects of probiotics in clear water hatchery conditions, however this had not always been the case under real life pond grow-out conditions (Fegan, 2000). A zero water exchange shrimp culture system was developed in India employing indigenous bioaugmentors and probiotics which in due course got widely extended to different shrimp farms of different state, such as Kerala, Tamil Nadu and Andhra Pradesh. This study presents preliminary evaluation of the technology at field level in terms of water and sediment qualities and its economic viability in a few selected shrimp farms of Kerala, India.

MATERIALS AND METHODS

Study area: The 10 earthern shrimp grow out ponds maintained in a zero water exchange mode, located in Ernakulam (Lat. 10°00' N, Long. 76°15 E), Thrissur (Lat. 10°30' N, Long. 76°15 E), Alappuzha (Lat. 9°30' N, Long. 76°23 E) and Kozhikode (Lat. 11°15' N, Long. 75°49 E) and 2 control ponds at Ernakulum (Lat. 10°00' N, Long. 76°15 E), Kerala, India were subjected for the study. General characteristics of the ponds are given in Table 1.

Protocol for closed system shrimp culture

Pond preparation: Surface soil samples (100 g) collected in polythene bags from different locations of the ponds using a mini grab were maintained airtight and transported in thermo cool boxes at 4°C to laboratory and analyzed for pH, Eh and total organic carbon followed by Walkley and Black Method (Allison, 1965). The ponds were eradicated of the weed fishes by applying lime (1275 kg ha^{-1}) and ammonium sulphate at a ratio of 5:1 (255 kg ha^{-1}) and tea seed powder at the rate 100 kg ha^{-1} (Jhingran, 1988; Pillai, 1995). On completing the eradication, water was pumped in to the ponds through 100 μ bag net to have maximum 5-10 cm at the top most portion of the pond. After recording pH and Eh, Detrodigest™ was applied to the experimental ponds for the speedy degradation of the dead weed animals. The ponds were subsequently filled with water up to 70 cm through 100 μ bag net fitted to the sluice gate. The sluice gate was then closed by mud packing.

Table 1: General characteristics of the closed system shrimp ponds of Kerala, India

Regions	Farm code	Area (ha)	Depth (cm)	Initial TOC (%)
Ernakulam	E-1	1.6	100	4.36
	E-2	0.4	150	2.87
	E-3	2.4	90	1.80
Thrissur	T-1	0.9	65	ND
	T-2	0.4	80	4.530
Alappuzha	A-1	2.8	80	0.525
	A-2	1.2	90	1.96
Kozhikode	K-1	1.0	100	ND
	K-2	2.8	100	ND
	K-3	2.0	100	0.96
Control	C-1	0.8	90	1.90
	C-2	1.0	90	1.90

ND = No Data

Detrodigest™ is an indigenous probiotic preparation containing the bacterium Bacillus cereus sensu lato MCCB101 (GenBank Accession No. EF062509) having 10^9-10^{12} cells mL^{-1}. This was applied to the ponds by brewing (an aliquot of 300 mL preparation/1 ha) for 24-48 h in 100 L medium composed of filtered (through muslin cloth of 100 μ size), chlorinated (7% chlorine as sodium hypochlorite) and dechlorinated (after 6 h by adding 20 g sodium thiosulphate) pond water from the respective pond, supplemented with cooked 100 g each shrimp feed and rice bran. Generation of fermented smell and froth were the signs of brewing. The preparation was subsequently diluted with pond water and sprayed over the ponds during morning hours. The treatment continued till the optimum soil conditions were attained.

Fertilization: A combination of nutrients, such as Nutrimix 650 g ha^{-1} (NCAAH, CUSAT), triple super phosphate 15 kg ha^{-1} (FACT, Kerala) and 100 L aqueous extract generated from 150 kg cow dung were applied to all the experimental ponds during morning hours after regulating pH to 7-7.5 by applying dolomite. Nutrimix per kg contains sodium nitrate (588 g), sodium phosphate (39 g), sodium silicate (270 g), copper sulphate (25 g) and ferric chloride (78 g). In addition, another nutrient preparation Micromix containing ferric chloride and magnesium chloride at a ratio 1:1 was also applied at a rate of 300 mL ha^{-1}, under the situations when there was delay in the development of phytoplankton bloom. In cases where the phytoplankton failed to appear within 3 days of application, an additional dosage of 40 L extract from 40 kg cow dung, 200 g Nutrimix, 620 g single super phosphate and 200 mL Micromix were applied per hectare.

Management of the culture: When the water and sediment pH remained between 7-8.5, sediment Eh between -100 to -125 mV, alkalinity between 70-120 mg L^{-1} all the ponds were stocked with healthy *Penaeus*

monodon seed (PL 20), tested for nested PCR negative for WSSV, at an average stocking density of 5 m^{-2}. To document survival of post larvae subsequent to stocking, 100 of them were maintained in a nylon hapa and counted after 48 h presuming it to reflect the overall survival in the pond.

The ponds were maintained without mechanical aeration throughout the culture. The bioaugmentor Detrodigest™ application continued till harvest at an interval of 10 days in all the ponds except the control ones. Feeding the post larvae started 15 days after stocking and the consumption was monitored through check trays and the quantity regulated. Seepage of water and evaporation loss was compensated by topping up water from the adjacent creek by pumping through a bag net of 100 µ mesh size during high tide.

Application of the gut probiotic Enterotrophotic™ (NCAAH, CUSAT) coated on to the feed at a rate of 50 mL per 10,000 animals was commenced from the 30th day of culture except in the control ponds. Enterotrophotic is a gut probiotic preparation composed of Bacillus MCCB 101 (GenBank Accession No. EF062509) and Arthobacter MCCB 103 (Jayaprakash *et al*., 2005), blended in equal proportion to attain 10^8-10^9 CFU mL^{-1}. An antagonistic probiotic PS-1™, containing *Pseudomonas aeruginosa* MCCB 102 (GenBank Accession No. EF062514; Vijayan *et al*., 2006) was brewed and applied during times of higher Vibrio population (10^2 mL^{-1}) or during the presence of luminescent bacteria. Brewing protocol of the probiotic was the same followed for Detrodigest™, at a rate of 300 mL of inoculum to 100 L medium per hectare. The animal samples were subjected for health assessment from the 30th day of culture onwards once in 7-10 days. They were observed for colour, length of antennae, fullness of pleopod and uropod setae, black spot on shells, gill colouration, nature of spots on the inner side of the carapace, fullness of gut, colour of hepatopancreas and epibionts such as Zoothamnium.

Analyses of water and sediment quality: From the day of stocking to harvest, analyses of sediment and water were performed weekly. The water samples were collected from a depth of 10-15 cm using a glass water sampler and sediment samples using sediment grab sampler and transported to the laboratory in glass bottles and polythene bags, respectively. Sampling was made at an interval of 10 days. Sediment pH was measured using a digital pH meter (Euteck, Singapore) and Eh using an ORP meter (Euteck, Singapore). The total organic carbon was estimated following the Walkley and Black Method (Allison, 1965). Water samples were analyzed for pH using digital pH meter (Euteck-Singapore), salinity using refractometer (Erma-Japan), alkalinity, total hardness and phosphate following standard methods (APHA, 1998), total ammonia nitrogen (NH_3-N) by phenol hypochlorite method (Solorzano, 1969), nitrite-nitrogen (NO_2-N) following Bendschneider and Robinson (1952) and phytoplankton count using a haemocyctometer.

Statistical analyses: The mean and standard deviation of the sediment and water quality parameters for each pond were calculated for different phases of the culture, such as initial (0-30 days), mid (31-60 days) and final (61-90 days). The sediment pH and Eh data from farms 2 and 3 of Kozhikode region could not be analyzed statistically due to lack of sufficient data. The 2 way Analysis of Variance (ANOVA) was done to assess the variation in environmental quality between different phases of the culture and between different ponds in the control and experimental ponds. Productivity of each pond in terms of yield per hectare was worked out. The average Feed Conversion Ratio (FCR) and survival rates were also calculated. The operational cost for implementing the protocol was worked out in terms of seed, feed, probiotics, nutrients and labour, based on which cost-benefit ratio was calculated.

RESULTS AND DISCUSSION

Pond characteristics: General characteristics of the ponds selected are shown in Table 1. The initial total organic carbon ranged from 0.53-4.36% in the experimental ponds and 1.9% in the control ponds. In farm 1 and 2 of Ernakulam region and in farm 2 of Thrissur region, the organic carbon contents were >2% and could be brought down to <2% before stocking by regular application of Detrodigest™. Depth of the ponds ranged from 65-100 cm and area from 0.4-2.8 ha.

Water and sediment quality: In the experimental ponds water pH, total alkalinity, ammonia, nitrite and phosphate between different phases of the culture did not show significant difference whereas salinity and total hardness differed significantly. pH of the water ranged from 6.30-8.58, salinity from 3.4-30 g L^{-1} and total alkalinity from 33-195 mg L^{-1} in experimental ponds and 6.8-8.08, 13-21 g L^{-1} and 22.5-60 mg L^{-1}, respectively in the control ponds (Fig. 1-3). Total hardness ranged from 820-6244 and 2650-4225 mg L^{-1} in the experiment and control ponds, respectively (Fig. 4). Total ammonia recorded lowest (0.049 mg L^{-1}) in farm 1 and highest (0.79 mg L^{-1}) in farm 2 of Ernakulam region and nitrite

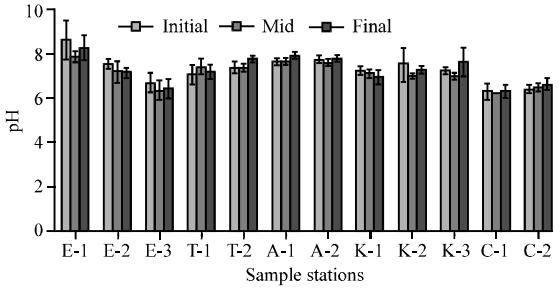

Fig. 1: pH in the zero water exchange shrimp ponds during different phases of culture; E-1 to E-3 = Ernakulam; T-1 to T-3 = Thrissur; A-1, A-2 = Alappuzha; K-1 to K-3 = Kozhikode; C-1, C-2 = Control

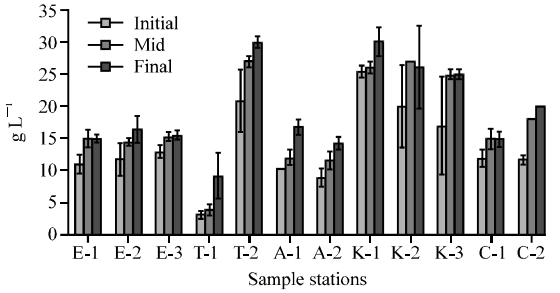

Fig. 2: Salinity (g L^{-1}) in the zero water exchange shrimp ponds during different phases of culture; E-1 to E-3 = Ernakulam; T-1 to T-3 = Thrissur, A-1, A-2 = Alappuzha; K-1 to K-3 = Kozhikode; C-1, C-2 = Control

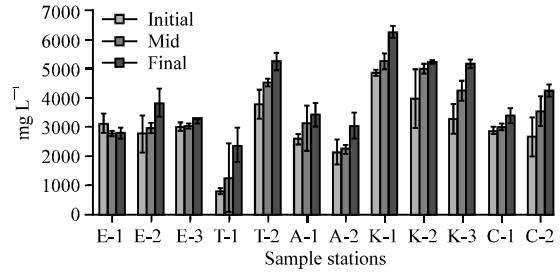

Fig. 3: Total hardness (mg L^{-1}) in the zero water exchange shrimp ponds during different phases of culture; E-1 to E-3 = Ernakulam; T-1 to T-3 = Thrissur; A-1, A-2 = Alappuzha; K-1 to K-3 = Kozhikode; C-1, C-2 = Control

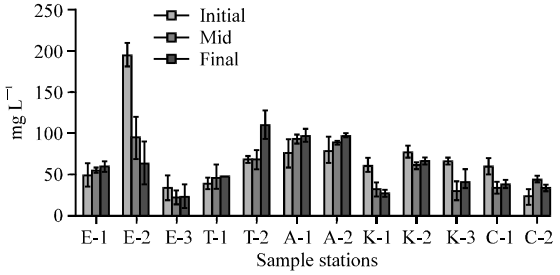

Fig. 4: Alkalinity (mg L^{-1}) in the zero water exchange shrimp ponds during different phases of culture; E-1 to E-3 = Ernakulam; T-1 to T-3 = Thrissur; A-1, A-2 = Alappuzha; K-1 to K-3 = Kozhikode; C-1, C-2 = Control

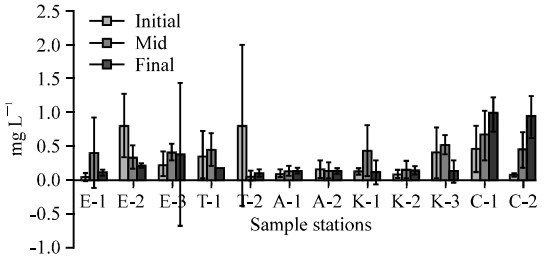

Fig. 5: Total ammonia nitrogen (mg L^{-1}) in the zero water exchange shrimp ponds during different phases of culture; E-1 to E-3 = Ernakulam; T-1 to T-3 = Thrissur; A-1, A-2 = Alappuzha; K-1 to K-3 = Kozhikode; C-1, C-2 = Control

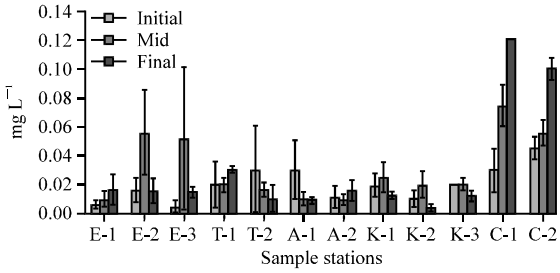

Fig. 6: Nitrite (mg L^{-1}) in the zero water exchange shrimp ponds during different phases of culture; E-1 to E-3 = Ernakulam; T-1 to T-3 = Thrissur; A-1, A-2 = Alappuzha; K-1 to K-3 = Kozhikode; C-1, C-2 = Control

ranged from 0.004-0.06 mg L^{-1} in experimental ponds. In control ponds, total ammonia ranged from 0.09-1.108 mg L^{-1} and nitrite 0.02-0.124 mg L^{-1}, respectively with significantly higher value compared to experimental ponds (Fig. 5 and 6). The phosphate concentration was, also significantly different between the experiment and control ponds, as it ranged from 0.008-0.037 and 0.008-0.078 mg L^{-1}, respectively (Fig. 7). In the control ponds, there was significant difference in the values of ammonia ($p<0.01$), nitrite ($p<0.05$) and phosphate ($p<0.05$) between different phases of the culture. The phytoplankton density in the experimental farms ranged from 1×10^4-71×10^4 cells mL^{-1} (Table 2).

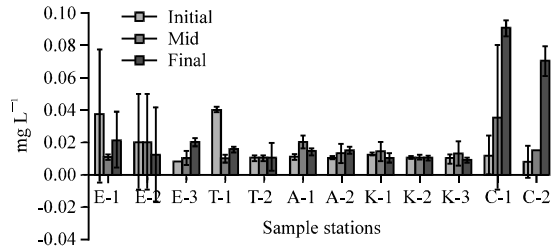

Fig. 7: Phosphate (mg L^{-1}) in the zero water exchange shrimp ponds during different phases of culture; E-1 to E-3 = Ernakulam; T-1 to T-3 = Thrissur; A-1, A-2 = Alappuzha; K-1 to K-3 = Kozhikode, C-1, C-2 = Control

Table 2: Mean cell count of phytoplankton during different phases of the culture period in zero water exchange shrimp

	Phytoplankton cell count $\times 10^4$ cells mL^{-1}		
Farms	Initial phase	Mid phase	Final phase
E-1	8×10^4	5.6×10^4	6.8×10^4
E-2	4.2×10^4	4×10^4	12×10^4
E-3	16.35×10^4	1×10^4	1×10^4
T-1	ND	5.45×10^4	ND
T-2	8×10^4	3.6×10^4	11×10^4
A-1	7.60×10^4	4.77×10^4	57.3×10^4
A-2	4.58×10^4	4.45×10^4	47.35×10^4
K-1	8×10^4	4.8×10^4	2×10^4
K-2	2×10^4	4×10^4	2×10^4
K-3	2.2×10^4		71×10^4

E-1 to E-3 = Ernakulam; T-1 to T-3 = Thrissur; A-1, A-2 = Alappuzha; K-1 to K-3 = Kozhikode; ND = No Data

Sediment pH ranged from 6.62-8.01, Eh from -20 to -225 mV in the experimental ponds and 6-8.1 and -80 to -268 mV in the control ponds (Table 3). There was no data available for Eh in the final phase of the culture from farms 1 and 3 of Kozhikode region. Results of ANOVA showed that pH and Eh of the sediment samples in the farms remained without significant changes from one phase of the culture to another in the same pond, even though it varied significantly between the farms.

The multiple regression analysis of yield and sediment quality parameters in the experimental ponds did not give any significant results. The fitted multiple regression analysis of yield against the water quality parameters gave the following equation ($p<0.001$; r^2 0.643):

$$Y = 2138-251X_1-16.6X_2-2.35X_3+ 0.48X_4+210X_5+461X_6-688X_7$$

Where:

X_1 = pH
X_2 = Salinity
X_3 = Alkalinity
X_4 = Hardnes
X_5 = Ammonia
X_6 = Nitrite
X_7 = Phosphate

However, multiple regression analysis could not be done in the case of control ponds, as mortality occurred after 80 days of culture.

Cost-benefit analysis: Stocking density, survival, yield, FCR and cost-benefit ratios for the zero water exchange shrimp farms validated are given in Table 4. The average survival rate and yield were 51% and 429 kg ha^{-1}, respectively in experimental ponds. The average body weight of the animals during the final phase of the culture in all the farms was 25 g with an FCR of 1.9. The cost-benefit ratio ranged from 0.076-0.77 with an average of 0.46. In control ponds, mortality occurred after 80 days of culture.

The study was focused on validation of a zero water exchange shrimp culture system developed employing indigenous bioaugmentors and probiotics in India. The striking observation was the lack of wide fluctuations in sediment and water quality parameters within experimental ponds during different phases of the culture. However, there were variations in several of the parameters between the farms. In shrimp ponds, in general deterioration of pond bottom, increase in the amount of waste accumulation and development of anaerobic conditions are principally due to incomplete utilization of feed. This generally leads to accumulation of toxic metabolites, a situation which might lead to retarded growth as well (Avnimelech and Ritvo, 2003). In an experimental study, Hopkins *et al.* (1994) reported that in plastic lined ponds with no water exchange, 100% mortality had occurred when the accumulated sludge was left untreated. Meanwhile, Lemonnier and Faninoz (2006) reported that sediment quality did decrease with reduced water exchange.

However in the study, experimental ponds sediment quality could be maintained without significant variations in the ponds well within the limited range with enhanced redox potential through out the culture period. This may be attributed to the exceptionally high hydrolytic activity of Bacillus MCCB 101 contained in Detrogiest™ applied regularly in these ponds. In control ponds, sediment redox potential was up to -268 mV during the end period of the culture due to the accumulation of organic matter in the bottom of the ponds and there was significant variation ($p<0.01$) between different phases of the culture. Noticeably, pH, alkalinity, ammonia, nitrite and phosphate in experimental ponds were found not varying significantly during the culture in a pond. During validation of the technology, pH stood within a range of

Table 3: Sediment quality in zero water exchange shrimp culture farms of Kerala, India during different phases of the culture

	Culture Phase[NS]					
	pH			Eh (mV)*		
Farms	Initial	Mid	Final	Initial	Mid	Final
E-1	7.0±0.063	7.120±0.08	7.52±0.17	-177±-128	-225±-59	-225±-35
E-2	7.21±0.28	7.43±0.44	7.77±0.07	-138±-51	-150±-31	-167±-16
E-3	6.89±0.23	6.77±0.23	6.85±0.09	-131±-43	-157±-44	-174±57
T-1	8.01±0.3	7.54±0.31	7.63±0.40	-139±-59	-150±-29	-138±-1.41
T-2	6.95±0.32	7.40±0.19	7.60±0.02	-152±-10	-134±42	-107±20
A-1	7.35±0.24	7.09±0.04	7.41±0.19	-125±-10	-126±-16	-156±3.0
A-2	6.91±0.21	7.09±0.08	7.41±0.19	-108±0.21	-82±34	-146±-3
K-1	6.85	7.12±0.18	ND	-73	-120±-10	ND
K-2	6.95±0.05	7.10±0.12	7.07±0.12	-131±-19	-116±28	-119±-38
K-3	6.63	6.93±0.06	ND	-68	-20±14	ND
C-1	7.68	8.1	8.08	-155	-230	-268
C-2	6.5	6.85	6.0	-84	-170	-201

*$p<0.01$ for Eh between farms; [NS]No significant difference between different phases of the culture; ND = No Data; E-1 to E-3 = Ernakulam; T-1 to T-3 = Thrissur; A-1, A-2 = Alappuzha; K-1 to K-3 = Kozhikode; C-1, C-2 = Control

Table 4: Survival rates, yield, FCR and cost-benefit ratios for the closed system shrimp farms studied

Farms	Survival (%)	Yield (kg ha^{-1})	FCR	Cost-benefit ratio
E-1	46	206.25	2.72	0.75
E-2	46	188	2.30	0.77
E-3	36	208	3.00	0.076
T-1	60	333	1.5	0.47
T-2	40	1000	1.38	0.34
A-1	55	321	2.22	0.57
A-2	57	500	1.96	0.46
K-1	50	400	1.00	0.34
K-2	80	533	1.30	0.36
K-3	44	600	1.66	0.43
Average	51.4±12.55	429±247	1.90±0.65	0.46±0.21

E-1 to E-3 = Ernakulam; T-1 to T-3 = Thrissur; A-1, A-2 = Alappuzha; K-1 to K-3 = Kozhikode; C-1, C-2 = Control

6.30-8.58 in experimental ponds without any visible negative impact on the animals' health, even though according to Ramakrishna (2000) and Soundarapandian and Gunalan (2008), 7.5-8.5 was the most ideal pH in shrimp farms. Meanwhile, significant variation in salinity and hardness could be experienced between the phases of the culture, the situation attributed to rain fall during the initial phase and evaporation loss subsequently. This was in agreement with the study reported by Guerrero-Galvan *et al.* (1998), Mmochi *et al.* (2002) and Everett *et al.* (2007). This can not be considered as a negative impact of the closed culture technology, as in Taiwan the shrimp farmers routinely used to vary salinity between 15-20 g L^{-1} to stimulate moulting and to enhance weight gain (Chien, 1989). Even though, the ideal salinity suggested ranged between 15-25 g L^{-1} (Boyd, 1989), in the study salinity variations ranging from 3.4-30 g L^{-1} were found not to affect the overall shrimp yield.

During the validation, Total Ammonia Nitrogen (TAN) stood within a range of 0.049-0.79 mg L^{-1}, well within the safe level of 1 mg L^{-1} in the pH regime up to 8.0 (Chien, 1992). Prabhu *et al.* (1999) and Rao *et al.* (2000) reported NH_3-N above 1.0 mg L^{-1} in Indian shrimp culture ponds and Abraham *et al.* (2004) observed that the mean TAN did increase with days of culture which were extending to 120 days in semi-intensive systems. However in the study, the ammonia concentration was not significantly varying between different phases of the culture, alike the nitrite concentration which also was within the safe levels of 1 mg L^{-1} (Chien, 1992). In an experimental study by Thakur and Lin (2003), the total nitrogen and total phosphorus concentrations in water were found going up with the progress of culture and within the closed system they supported growth of natural food organisms thereby contributed positively to shrimp growth. The mean values of ammonia and nitrite in control ponds during the initial period of culture was 0.075 and 0.004 mg L^{-1}, respectively. When culture progressed, it reached up to 0.96 and 0.233 mg L^{-1}, respectively.

In support of the study, Wang *et al.* (2005) observed higher yield and improved water quality in shrimp ponds treated with commercial probiotics than those of the controls. One of the significant observations in the study was the consistence in the availability of nutrients in the water column during the culture period, a situation attributed to the continuous mineralization of organic matter due to the periodic addition of Detrodigest™.

Pond management studies are usually limited due to the non availability of culture systems for experimentation to maintain adequate replicates (Lemonnier and Faninoz, 2006). In such situations, regression analysis of data from trials with single or duplicate ponds for each treatment over a wide range of parameters is a more appropriate means of obtaining information on pond management (Wudtisin and Boyd, 2005). The multiple regression analysis revealed that variability in the yield could not be significantly related to the sediment quality parameters in the present study, however could be related by 64.3% to the water quality parameters studied. This implies that

35.7% of the variability in the yield is dependent on the other parameters not included in the present study, such as dissolved oxygen, nitrate nitrogen, total nitrogen, total phosphorus, silicates, primary productivity, secondary productivity and heterotrophic bacterial production.

The cost benefit ratio showed profitability in all the farms studied with an average value of 0.46. The average FCR arrived at was 1.9, well within the country estimate for India (2.0) and comparable to the average shrimp FCR in the other leading Asian shrimp producing countries like Thailand (1.8), Indonesia (1.9), lower compared to that of China (2.3) and Vietnam (2.5) (Tacon, 2002). Supporting the present study, Castex *et al.* (2008) demonstrated that probiotic treatment improved the final biomass. The feed costs could be reduced considerably in the present case, as it was the natural biotic communities in these systems contributed substantially to the overall nutritional requirements of the cultured shrimp. McIntosh (2000) reported that the dietary crude protein level could be decreased from 31-24% (with FCR decreasing from 2.2-2.0) and body N retention efficiency increased from 23-37% in shrimp (*P. vannamei*) reared under commercial zero water-exchange culture conditions. The average survival rate in the farms under the present management strategy was 51%, substantially higher compared to 26.5% survival in Chilka farms (Balasubramanian *et al*., 2005). As the input costs were comparatively low, the survival rates did not affect much the economic feasibility of the shrimp farming system (Balasubramanian *et al*., 2005). The average production for the reported 100,000 shrimp farms in India with an area of 130,000 ha in production is at the rate of 538 kg ha^{-1} (Rosenberry, 1999). However, the average pond production for zero water exchange systems in the Chilka region was 145 kg ha^{-1} (Balasubramnaian *et al*., 2005). Meanwhile in the study, it was 429 kg ha^{-1}, comparable to the national average and very high compared to the production attained so far in zero-water exchange farms in the Chilka region. The zero water exchange culture systems offer the advantages, such as increased biosecurity (Pruder and Bullis, 2001), reduced feed utilization and minimal water use (Chamberlain and Hopkins, 1994; Boyd, 2000). Shrimp in closed systems grow by consuming both externally supplied compounded aqua feeds and endogenously produced biotic community comprising bacteria, algae, fungi, protozoans, metazoans, rotifers, nematodes and gastrotrichs (Tacon *et al*., 2002). Notable features of the zero water exchange technology validated here is the maintenance of fairly stable environmental conditions with reduced stress to the animals and the subsequent lesser chances of disease outbreak due to opportunistic pathogens. This is apart from the biosecurity offered by the system. Experimenting with *Litopenaeus vannamei*, Cohen *et al.* (2005) demonstrated limited water exchange improved water quality, growth and survival. Even though, more studies are required to understand the dynamics and interrelationships between yield and the environmental parameters, the closed system protocol validated here has been observed specially suited for the tropics in general and Indian conditions in particular, primarily because the systems are biosecured, environmentally sustainable and economically viable.

CONCLUSION

The results indicated that zero water exchange shrimp culture coupled with bioremediation and application of probiotics could maintain environmental quality in the culture systems.

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