



Comparative Analysis of Growth and Microbial Dynamics of Monoculture and Polyculture Shrimp Farming Systems in Sundarbans, West Bengal

R. ANANDA RAJA^{1*}, A. PANIGRAHI¹, SUJEET KUMAR¹, P.S. SHYNE ANAND¹, D. DE²,
G. BISWAS³, and T.K. GHOSHAL³

¹ICAR-Central Institute of Brackishwater Aquaculture, Chennai - 600 028, Tamil Nadu, India

²ICAR - Central Institute of Brackishwater Aquaculture, Kakdwip Research Centre,
Kakdwip - 743 347, West Bengal, India

³ICAR - Central Institute of Fisheries Education, Kolkata Centre, Salt Lake, Kolkata,
West Bengal - 700 091, India

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Different sets of on-farm trials were conducted to standardize shrimp, *Penaeus monodon* monoculture at two stocking densities (6 and 12 post larvae [PLs] m⁻²) with conventional, organic principles and Bio-secured Zero Water Exchange (BZEST), and two shrimp-fish polyculture systems with different species combinations (TI - *Mugil cephalus*, *Liza tade*, *L. Parsia* and *P. monodon* and TII - *Chanos chanos* and *P. monodon*). The pond water was treated with 60 and 30 ppm of calcium hypochlorite before stocking in shrimp monoculture and polyculture ponds, respectively. Real-time microbial dynamics were studied throughout the culture and compared. BZEST average production was 3109 kg ha⁻¹ with 7.95%, 6.96% and 10.24% gain in terms of production, survival rate and FCR in comparison to conventional ponds, respectively. The total production in polyculture was 687.44±27.64 and 717.08±43.75 kg ha⁻¹ with FCR of 1.74±0.02 and 1.99±0.25 in T-I and T-II, respectively. Total heterotrophic bacterial (THBC) and *Vibrio* (TVC) counts in shrimp monoculture and polyculture ponds were significantly decreased immediately after bleaching. It revealed that the THBC and TVC in organic, BZEST and TI ponds were lesser than the conventional and T-II ponds, but not statistically significant throughout the culture. The influence of age of culture over the THBC in both monoculture and polyculture and TVC in monoculture ponds was highly significant. Further, it was observed that the treatment and age of culture interaction had a significant role in TVC in both the system of culture and THBC in polyculture ponds. Multiple comparisons of microbial dynamics in monoculture and polyculture ponds with reference to the age of culture revealed that there was a significant difference in THBC at the mid-culture and in TVC at the start and the end of the culture. Results obtained in this study would serve as a baseline real-time data on microbial dynamics in brackishwater aquaculture systems.

(**Key words:** Microbial dynamics, Shrimp aquaculture, *Penaeus monodon*, Monoculture, Polyculture)

In recent years, cultured penaeid shrimp have wreaked havoc worldwide due, inter alia, to the spread of disease (Ananda Raja *et al.*, 2012, 2017a). As a result, the boom and bust of this risky but highly profitable venture has shifted a large number of farmers to practice polyculture. In intensive aquaculture systems, there is usually an accumulation of high organic load in the pond bottom due to uneaten feed, faeces and plankton die-offs. Thus, water quality is to a large extent controlled by the microbial biodegradation of organic residues (Avnimelech *et al.*, 1995).

Microbial processes affect water quality mainly due to utilization of oxygen, regeneration of inorganic nutrients and production of toxic metabolites like ammonia, nitrite and sulphide (Moriarty, 1996). Microbes

are very important and have a critical role in aquaculture systems because water quality and disease control are directly related and closely affected by microbial activity (Jory, 1998). Few studies have been conducted to assess the microbial dynamics of brackishwater aquaculture systems (Lalramchhani *et al.*, 2019). The present study was undertaken to standardize and compare the different brackishwater aquaculture systems. Different sets of on-farm trials were conducted to standardize the shrimp monoculture with two stocking densities (6 and 12 PLs m⁻²) and polyculture with different species combinations to standardize the suitable species combination for Sundarbans agro-climatic condition for sustainability in the brackishwater aquaculture industry. As a part of those studies, we also studied the *in situ* microbial

*Corresponding author: E-mail: anandarajars@gmail.com

dynamics of shrimp, *Penaeus monodon* and polyculture ponds throughout the culture, which is presented in this manuscript.

MATERIALS AND METHODS

Giant tiger shrimp, *P. monodon* monoculture and polyculture were carried out at Kakdwip Research Centre (KRC) of the Indian Council for Agricultural Research-Central Institute of Brackishwater Aquaculture (ICAR-CIBA). The shrimp monoculture and polyculture pond water were disinfected with calcium hypochlorite (bleaching powder with 35% available chlorine) @ 60 and 30 parts per million (ppm), respectively before stocking. Shrimp monoculture was done following the organic and conventional farming systems in replicate ponds with a stocking density of 6 no. m⁻². Low fish meal feed with a crude protein content of 34% was applied in the organic ponds along with organic juice (25 kg rice bran, 10 kg jaggery and 250 g bakery yeast fermented for 48 hours and applied per hectare at fortnight intervals) and vermicompost (500 kg ha⁻¹) as other organic inputs while conventional ponds were grown without organic inputs. In another set of on-farm trial, Bio-secured zero water exchange shrimp culture (BZEST) and conventional farming were carried out in replicates with a stocking density of 12 no. m⁻². The ponds were maintained with strict biosecurity measures and zero-tolerance for antibiotics and other chemicals. Beneficial microbes such as *Lactobacillus* sp., *Bacillus* sp., *Pseudomonas* sp. and *Saccharomyces* sp. were

applied at fortnight intervals (10⁹ CFU mL⁻¹ of 100 L ha⁻¹) only to BZEST ponds along with feed for controlling the pathogenic microbiota.

In addition, polyculture with two different species combinations in duplicate ponds was also carried out. In treatment I (T - I), mullet species (*Mugil cephalus* - 0.45 no. m⁻², *Liza tade* - 0.15 no. m⁻², *L. parsia* - 0.2 no. m⁻²) and *P. monodon* (2 no. m⁻²) and in treatment II (T - II), *Chanos chanos* (0.8 no. m⁻²) and *P. monodon* (2 no. m⁻²) were stocked. Low-cost pellet feed (Table 1) prepared from locally available feed ingredients was given as supplementary feed @ 2-5% body weight depending on the age of fish/shrimp.

Water samples were collected at fortnightly intervals from shrimp monoculture ponds and every month from the polyculture ponds throughout the culture period. The total viable heterotrophic bacterial (THBC) and *Vibrio* count (TVC) were estimated to monitor the microbial dynamics throughout the culture. For that purpose, one milliliter of water sample from respective ponds was serially diluted in autoclaved normal saline, plated aseptically under laminar air flow on sterilized Tryptone Soya Agar (TSA) and Thiosulfate Citrate Bile Sucrose Agar (TCBS) (Himedia Laboratories, Mumbai) plates in duplicate. Those culture plates were incubated at 30° C for 24 h. THBC and TVC were assessed. The number of colonies counted on each plate was multiplied by the reciprocal value of dilution to determine the colony

Table 1. Nutritional composition of low cost polyculture feed. Values are means and standard errors.

Ingredients	Levels of inclusion
DM%	96.02±0.11
CP%	29.77±0.06
EE%	5.00±0.03
CF%	8.72±0.04
OM%	88.70±0.03
AIASH%	3.72±0.05
NFE%	45.21±0.01
Calcium (%)	2.23
Phosphorous (%)	2.04
Vitamin A (µg/100 g)	300.03
Vitamin C (mg/100 g)	4.28

DM - Dry Matter; CP - Crude Protein; EE - Ether Extract; CF - Crude Fibre; OM - Organic Matter; AIASH - Acid Insoluble Ash; NFE: Nitrogen Free Extract

numbers per unit volume of water. From polyculture ponds, mucus was collected from all the finfishes (*M. cephalus*, *L. parsia* and *L. tadar*) and well diffusion test was performed against *Vibrio mimicus* isolated from the WSD infected farmer's pond (Ananda Raja *et al.*, 2017b). The shrimp samples were collected at fortnight intervals and screened by polymerase chain reaction (PCR) for viral infections such as white spot syndrome virus (WSSV), infectious hypodermal and hematopoietic necrosis virus (IHHNV), yellow head virus (YHV) and gill associated virus (GAV). Data were analyzed with analysis of variance (ANOVA) and Duncan's Multiple Range Test (DMRT) at $p < 0.05$ using the SPSS version 17.

RESULTS AND DISCUSSION

The total production following organic principles was 1289 ± 5.5 kg ha⁻¹ with 1.28 feed conversion ratio (FCR) against the conventional ponds of 1121 ± 3.5 kg ha⁻¹ with 1.37 FCR while BZEST gave average production of 3109 ± 4.5 kg ha⁻¹ with 7.95%, 6.96% and 10.24% gain in terms of production, survival rate and FCR in comparison to the conventional ponds respectively. After 170 days of culture in polyculture ponds, total biomass production was 687.44 ± 27.64 and 717.08 ± 43.75 kg ha⁻¹ with FCR of 1.74 ± 0.02 and 1.99 ± 0.25 in T-I and T-II, respectively.

Both THBC and TVC in shrimp monoculture and polyculture ponds were reported in Tables 2 and 3. It was observed that both THBC and TVC in shrimp monoculture and polyculture ponds were found significantly decreased after bleaching at the concentration of 60 and 30 ppm respectively during the starting of the culture. Chlorine is widely used in hatcheries and ponds for disinfection purposes, but its use may lead to the development of multiple antibiotic resistance (MAR) genes in bacteria and the bacteria may become pathogenic (Moriarty 1999; Ananda Raja *et al.*, 2023). The resistant microbiota may then grow rapidly in the absence of their competitors and either predisposes the animals for disease or itself cause disease (Moriarty 1999). But in the present experiments, the production was appreciable with no disease outbreak. Oseko *et al.* (2006) reported effective inactivation of WSSV even at

a low free chlorine concentration of 0.5 ppm. The free chlorine concentrations used by most of the farmers were between 3.1 and 23.4 ppm (Ananda Raja *et al.*, 2012). Since there are wide range of decapod crustaceans acting as carriers to WSSV (Escobedo-Bonilla *et al.* 2008), further study is needed to estimate the minimum free chlorine concentration required to inactivate the pathogen in carriers without the development of resistance among the bacterial population (Ananda Raja *et al.*, 2012).

As a whole, in both the types of culture, the bacterial load was increasing towards the progress of the culture with turn down in the population at the end of the culture. Moreover, it revealed that the THBC and TVC in organic, BZEST and T - II ponds were lesser than the conventional and T - I ponds but not statistically significant throughout the culture. There was a significant difference in the microbial load of the ponds between the treatment and the age of culture with the application of biotherapeutic agents like yeast based organic preparations or probiotics in organic culture ponds (Tables 2 & 3). The production was comparatively more in organic (1289 ± 5.5 kg ha⁻¹), BZEST (3109 ± 4.5 kg ha⁻¹) and T - II (717.08 ± 43.75 kg ha⁻¹) treatment ponds than in conventional shrimp monoculture (1121 ± 3.5 kg ha⁻¹) and T - I (687.44 ± 27.64 kg ha⁻¹) ponds. It is evident from this study that the use of organic inputs and probiotics in shrimp monoculture, and *C. chanos* with *P. monodon* species combination not only reduced the bacterial load but also helped in enhancement of the production. Competitive exclusion is one of the ecological processes that can be manipulated to modify the microbial species composition of a soil or water body. The complete species composition in natural environments is largely unknown, but it is possible to change species composition by making use of competitive exclusion principles. Thus, bacteria can compete by secreting antimicrobial compounds that do not necessarily kill all their competitors, but increase mortality rates just enough to tip the balance in resource utilization. According to Boyd (1995), some of the benefits of using bacterial products include the reduction

Table 2. Comparison of total viable heterotrophic bacterial (THBC) and Vibrio count (TVC) in polyculture ponds. Values are means and standard errors.

Treatments	Bacterial load	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6	
Organic	THBC cfu	0.38±	0.01±	1.74±	12.65±	29.78±	1035.00±	
Con-trol**	x 10 ⁵ ml ⁻¹	0.09	0.01	1.22	3.50	7.53	40.00	
Organic	THBC cfu	0.26±	0.01±	1.04±	15.13±	35.26±	430.00±	
Treat-ment**	x 10 ⁵ ml ⁻¹	0.20	0.02	0.45	2.03	6.24	22.50	
BZEST	THBC cfu	0.27±	0.01±	0.38±	14.86±	36.50±	1395.00±	
Con-trol**	x 10 ⁵ ml ⁻¹	0.17	0.01	0.08	0.38	15.00	20.00	
BZEST	THBC cfu	0.32±	0.01±	1.00±	7.70±	26.64±	1672.50±	
Treat-ment**	x 10 ⁵ ml ⁻¹	0.03	0.01	0.64	1.75	1.13	1.32	
Level of significance		P=0.01*	P=0.24	P=0.66	P=0.05	P=0.80	P=0.53	P=0.65
Treatment & age of culture interaction (P=0.13)								
Organic	cfu x 10 ⁵	0.31±	0.01±	1.04±	4.80±	31.59±	722.50±	
Con-trol**	ml ⁻¹	0.52	0.01	0.91	3.50	7.03	100.76	
Organic	TVC cfu x	1.55±	2.68±	0.25±	8.00±	4.95±	0.45±	
Treat-ment**	10 ² ml ⁻¹	0.00	0.08	0.05	0.55	1.3	0.10	
Organic	TVC cfu x	0.93±	1.70±	0.38±	10.13±	3.98±	1.10±	
Treat-ment**	10 ² ml ⁻¹	0.08	0.50	0.13	4.58	0.38	0.75	
BZEST	TVC cfu x	1.45±	4.68±	0.30±	6.28±	4.18±	1.80±	
Con-trol**	10 ² ml ⁻¹	0.10	3.28	0.05	5.58	0.08	1.05	
BZEST	TVC cfu x	1.13±	7.18±	0.45±	5.30±	4.05±	1.10±	
Treat-ment**	10 ² ml ⁻¹	0.28	2.78	0.10	4.2	1.95	0.20	
Level of significance		P=0.12	P=0.40	P=0.48	P=0.82	P=0.93	P=0.68	P=0.35
Treatment & age of culture interaction (P=0.00**)								
Organic	TVC cfu x	1.26±	4.06±	10.36±	7.54±	4.29±	3.81±	
Con-trol**	10 ² ml ⁻¹	0.31	0.08	0.12	4.96	1.34	2.43	
Organic	TVC cfu x	0.38±	4.06±	6.37±	7.03±	4.29±	3.81±	
Treat-ment**	10 ² ml ⁻¹	0.08	3.22	0.12	4.96	1.34	2.43	
Organic	TVC cfu x	0.38±	4.06±	6.37±	7.03±	4.29±	3.81±	
Treat-ment**	10 ² ml ⁻¹	0.08	3.22	0.12	4.96	1.34	2.43	
Level of significance		P=0.05	P=0.05	P=0.05	P=0.05	P=0.05	P=0.05	P=0.05

*P < 0.05; **P < 0.01. † Values in the respective row and column differ significantly

Table 3. Comparison of total viable heterotrophic bacterial (THBC) and *Vibrio* count (TVC) in polyculture ponds. Values are means and standard errors.

Treatments	Bacterial load	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Polyculture Treatment I*	THBC cfu x 10 ⁵ ml ⁻¹	0.06±0.01	0.87±0.73	2.50±2.35	6.42±5.25	7.31±6.11	6.84±5.24
Polyculture Treatment II*	THBC cfu x 10 ⁵ ml ⁻¹	0.09±0.03	0.68±0.44	0.93±0.33	2.20±0.26	8.38±0.58	7.12±0.48
Level of significance		P=0.49	P=0.85	P=0.58	P=0.51	P=0.88	P=0.96
Treatment & age of culture interaction (P=0.04*)							
Polyculture Treatment I	THBC cfu x 10 ⁵ ml ⁻¹	0.76±0.03	0.78±0.70	1.71±2.14	4.31±4.94	7.84±5.05	6.98±4.30
Polyculture Treatment II	TVC cfu x 10 ² ml ⁻¹	3.48±0.98	8.63±6.53	9.15±3.05	12.79±10.09	12.21±0.14	12.70±2.15
Level of significance		P=0.90	P=0.89	P=0.90	P=0.83	P=0.55	P=0.59
Treatment & age of culture interaction (P=0.00**)							
	TVC cfu x 10 ² ml ⁻¹	3.55±0.84	9.16±5.52	9.37±2.51	11.48±9.01	12.05±0.42	11.70±2.80

*P < 0.05; **P < 0.01. † Values in the respective row and column differ significantly

Table 4. Multiple comparisons of total viable heterotrophic bacterial (THBC) and Vibrio count (TVC) in shrimp monoculture and polyculture ponds with reference to age of culture. Values are means and standard errors.

Treatments	Bacterial load	Month 1	Month 2	Month 3	Month 4	Month 5	Month 6
Polyculture Treatment I	THBC cfu x 10 ⁵ ml ⁻¹	0.06±0.01	0.87±0.73	2.50±2.36	6.42±5.25	7.31±6.11	6.84±5.24
Polyculture Treatment II	THBC cfu x 10 ⁵ ml ⁻¹	0.09±0.01	0.68±0.44	0.93±0.33	2.20±0.26	8.38±0.58	7.12±0.48
Organic Control	THBC cfu x 10 ⁵ ml ⁻¹	0.01±0.00	0.01±0.01	1.52±5.14	21.05±5.50	216.49±6.26	530.00±19.75
Organic Treatment	THBC cfu x 10 ⁵ ml ⁻¹	0.01±0.02	0.01±0.01	1.27±2.26	29.83±9.00	432.76±29.43	33.75±16.00
BZEST Control	THBC cfu x 10 ⁵ ml ⁻¹	0.01±0.00	0.02±0.01	4.99±41.80	28.54±6.66	307.00±15.50	95.75±25.75
BZEST Treatment	THBC cfu x 10 ⁵ ml ⁻¹	0.01±0.01	0.01±0.01	3.89±11.80	26.85±6.88	96.06±61.63	853.50±65.88
Level of significance		P=0.62	P=0.51	P=0.39	P=0.03*	P=0.21	P=0.26
Polyculture Treatment I	TVC cfu x 10 ² ml ⁻¹	3.48±1.00	8.63±6.53	9.15±3.05	12.79±10.09	12.21±0.14	12.70±2.15
Polyculture Treatment II	TVC cfu x 10 ² ml ⁻¹	3.63±0.33	9.70±1.60	9.59±0.29	10.18±4.08	11.89±0.44	10.70±2.25
Organic Control	TVC cfu x 10 ² ml ⁻¹	0.38±0.03	6.33±2.93	2.96±0.19	6.26±1.16	4.49±1.16	0.44±0.09
Organic Treatment	TVC cfu x 10 ² ml ⁻¹	0.40±0.10	1.62±0.34	5.25±0.90	12.78±0.95	4.55±1.03	1.03±0.53
BZEST Control	TVC cfu x 10 ² ml ⁻¹	0.35±0.14	9.33±7.53	2.75±2.28	4.99±2.86	2.99±0.56	1.05±0.05
BZEST Treatment	TVC cfu x 10 ² ml ⁻¹	0.38±0.04	11.56±2.91	2.46±1.74	5.11±3.24	4.18±2.25	0.89±0.26
Level of significance		P=0.00**	P=0.69	P=0.08	P=0.71	P=0.00**	P=0.00**

*P < 0.05; **P < 0.01. † Values in the respective column differ significantly

of blue green algal populations thus preventing off-flavor, nitrate, nitrite, ammonia and phosphate levels, increased dissolved oxygen (DO) concentrations and promotion of organic matter decomposition. However, a reliable documentation on the positive benefits of applying bacterial products in aquaculture systems needs further scaling up (Queiroz and Boyd, 1998). The applications of probiotics lessen pathogenic vibrios and enhance beneficial bacilli in the culture leading to improved water quality, promoted growth and survival rates and increased the health status of the shrimp without stress and disease outbreaks (Kumar *et al.*, 2017; Matias *et al.*, 2002). Thus, the application of probiotics could lead to disease-free and profitable shrimp culture operations which will be helpful for shrimp farmers. Further, the well diffusion test performed against *V. mimicus* showed prominent inhibitory zone (8 mm) with *C. chanos* mucus but not with mucus collected from the mullets. The result corroborates the finding of Tendencia *et al.* (2006) that the milk fish mucus has inhibitory effect on the growth of luminous bacteria.

It was found that the age of culture influences the THBC in both shrimp monoculture and polyculture ponds with high significant role of TVC in shrimp monoculture ponds. Moreover, it was observed that the treatment and age of culture interaction had a significant role in the TVC in both systems of culture. This could be due to the accumulation of uneaten feed, feces and plankton die-offs as the culture advances. Multiple comparisons of microbial dynamics in shrimp monoculture and polyculture ponds with reference to age of culture revealed that there was significant difference in THBC at the mid-culture and in TVC at the starting and the end of the culture (Table 4).

In present study, the shrimp produced were healthy and found to be free from WSSV, IHHNV, YHV and GAV diseases by PCR screening. Moriarty (1996) strongly advocates that the successful use of probiotic to promote sustainable aquaculture greatly depends on an understanding of the nature of competition between

species and strains of bacteria. Suhendra *et al.* (1997) supported this view as they found that routine use of commercial probiotics in shrimp farm resulted in reduced incidence of Vibriosis and other viral outbreaks, enhanced environmental conditions, reduced organic matter accumulation, improved water quality and increased shrimp size and total production. Moriarty (1998) found that the use of probiotics could prevent luminescent *Vibrio* by either lowering or completely eliminating luminous *Vibrio* in pond water and sediment. In another probiotic experiment, the populations of total heterotrophic bacteria (THB) and *Bacillus* spp. in the experimental pond increased and the vibrios decreased after each application of probiotics. But the result of the control pond showed an increasing trend of the populations of THB, *Bacillus* spp. and vibrios towards days of culture. The control pond had lower levels of THB and *Bacillus* spp. and higher levels of vibrios than the probiotic applied (experimental) pond (Dalmin *et al.*, 2001). Furthermore, works of Rengpipat *et al.* (1998) showed similar results although no obvious effect was found with regard to water quality in shrimp ponds. The effect of probiotics was reflected in the significantly higher survival of shrimp which could be corroborated by the higher survival rate in the organic and BZEST system of culture in the present study.

CONCLUSION

The health of animals was improved by improving water quality through the more rapid degradation of waste organic matter by the application of organic inputs and probiotics. It needs further research to understand the stochastic phenomena, that is, deterministic and predictable factors that allow one species to grow and divide more rapidly than others to dominate numerically. With regard to the microbial load, the kind of microbes and their characteristics and interaction are more important to influence productivity. Though there are not much variations in the THBC and TVC among the treatment ponds, the ponds with microbial interventions demonstrated better performance. Bioaugmentation and the use of probiotics are significant management tools for

aquaculture, but their efficacy depends on understanding the nature of competition between particular species or strains of bacteria.

CONFLICTS OF INTEREST

There is no conflict of interest.

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