www.trjfas.org
ISSN 1303-2712
Turkish Journal of Fisheries and Aquatic Sciences 14: 879-885 (2014) DOI: 10.4194/1303-2712-v14_4_05

Effect of Season and Treatment of Seed with Antibiotics on Grow out Culture of Tiger shrimp, *Penaeus monodon* (Fabricius, 1798) at Sunderban, India

R. Ananda Raja[1,*], N. Kalaimani[1], A. Panigrahi[1], A. G. Ponniah[1]

[1] Central Institute of Brackish water Aquaculture (ICAR), 75, Santhome High Road, R. A. Puram, Chennai, 600028, India.

* Corresponding Author: Tel.: +91.44 24616948; Fax: +91.44 24610311;
E-mail: anandarajars@gmail.com

Received 20 November 2013
Accepted 31 October 2014

Abstract

Experiments in hapa and pond were carried out with antibiotic treated tiger shrimp, *Penaeus monodon* seeds to assess the morphological and hematological parameters during two different seasons viz, monsoon (March to August) and winter (October to February). Survival rates of the antibiotic treated seeds during transport were found to be significantly more compared to untreated control seeds. Average body weight (ABW), feed conversion ratio (FCR) and survival rate after 134 days of culture (DOC) showed no significant difference between antibiotic treated and untreated control seeds during both the seasons. On comparison of seasonal performance, the ABW was 25.79±2.29 g in monsoon and 13.53±2.23 g in winter batch with corresponding FCR of 1.62±0.03 and 2.57±0.01 and survival rate of 55.62±4.94 and 24.62±4.78 per cent respectively with significant difference. Total hemocyte count (THC), granular hemocyte (GH) and nongranular hemocyte (NGH) counts were not statistically significant between antibiotic treated and untreated animals. Significant difference was observed in water and soil quality parameters between seasons but not between antibiotic and control group experimental ponds.

Keywords: Shrimp hematology, chloramphenicol, furazolidone, ciprofloxacin.

Introduction

The demands for food production remain challenging with the new innovative sustainable technologies in agriculture, animal husbandry and fishery sector as the world's population continues to grow exponentially. As the new technologies emerge in biological system, the parallel increase of problems mainly associated with the infectious diseases is inevitable. Despite many advances in vaccines, biosecurity and health management, we have neither eliminated infectious diseases nor will this be accomplished in the foreseeable future. Overstreet (1987) estimated that the loss of cultured shrimp due to parasites and diseases varies from 20 to 50 per cent at larval stages and 20 per cent in post-larval stages. The gross national loss in India due to shrimp diseases was estimated as 48,717 metric tonnes valued 164.21 million USD, and employment of 2.15 million man days during the period of 2006-08 (Kalaimani *et al*., 2013). So, there will be a steady demand for safe and effective antibiotics to treat bacterial infections in aquatic animals with judicious use principles. There is a global concern about the consumption of aquatic food containing low levels of antibiotics (Chafer-Pericas *et al*., 2010). In this sense, the European Union has established maximum residue limits (MRLs) in order to guarantee the safety of aquaculture produced marine fish (Official Journal of the European Communities, 1990). Coastal aquaculture authority in India has imposed ban on the list of 20 antibiotics and other pharmacologically active substances and permitted certain level of residue for four antibiotics and antimicrobials in fish and fishery products (Coastal Aquaculture Authority, 2006). But as on date, there is lack of sufficient scientific data on the application of the antibiotics in the shrimp aquaculture. There is a belief among the farmers that the usages of antibiotics in shrimp hatchery lead to better performance in grow out culture. To test this hypothesis, experiments were conducted in grow out culture and hapa with an objective to evaluate the effect of application of the antibiotics such as chloramphenicol, furazolidone and ciprofloxacin at hatchery level. We describe in this paper the effect of application of antibiotics at hatchery level along with shrimp morphological and hematological parameters with pond water and soil microbial and physico-chemical characteristics during two different culture operations.

Materials and Methods

Experimental Site and Ponds

Two experiments were carried out in the brackish water tide-fed ponds of the Kakdwip Research Centre of Central Institute of Brackishwater Aquaculture (KRC of CIBA), Kakdwip (Latitude - 21°51′15.01″–21°51′30.77″N, Longitude - 88°10′58.44″–88°11′12.09″ E), South 24 Parganas, West Bengal, India for a period of 134 days each, during March 2010 to February 2011. The monsoon batch was during March to August 2010 and the winter batch during October 2010 to February 2011. Earthen ponds of 0.06 ha each were selected for grow-out culture.

Pond Preparation

The ponds were filled with strained brackishwater taken through tide from nearby creek of Muriganga river to a depth of 150 cm. During water let in 120x120 wire mesh net was used at inlet sluice to avoid entry of unwanted materials or carriers in to the pond (Ananda Raja *et al*., 2012a). After 3 days of water intake, bleaching was done at the rate of 50 mg/kg water after sunset since sunlight fastens the oxidation of chlorine. After one day, residual chlorines were removed by running aerators (12 armed 2 aerators/ha) for one hour consecutively for two days preferably during mid-day. Dolomite was applied at the rate of 20 mg/kg water on third day of bleaching to increase the buffering capacity of the water. Crab and bird fencing were done for strict biosecurity measures. Zero water exchange farming was practiced throughout the culture.

Stocking and Management

The first trial was conducted with the antibiotics such as chloramphenicol and furazolidone treated and untreated control seeds during monsoon followed by chloramphenicol, furazolidone and ciprofloxacin treated and untreated control seeds in the second trial during winter season. The seeds were transported from Marakkanam near Chennai, Tamil Nadu to KRC of CIBA. Stocking was done at the rate of 5 pc/sq. m on 8th day of bleaching. In addition, 200 animals were stocked in hapa (2.5x1x1 m) in duplicate for each antibiotic and control group during both seasons. The hapas were cleaned and washed once in a week to avoid clogging. Seeds were acclimatized by floating the seed bags and adjusting the salinity. Dolomite was applied up to 30 days of culture (DOC) at weekly interval followed by biweekly application up to 60 DOC to increase bloom and replaced by lime stone powder (LSP) to control bloom towards the end of the culture at the rate of 10 mg/kg water to maintain the optimum pH in the pond water. No aerator was used during the culture.

Feeding and Management

Initially, blind feeding was done twice a day (morning and evening) up to 60 DOC followed by four times a day. The commercial feed was used throughout the culture. Out of the calculated ration, 40 per cent of the feed was given during day time and 60 per cent in the night and feeding ration was regulated through regular check tray monitoring.

Morphometry

The morphological parameters such as sex, carapace length, total length and weight were recorded. The carapace length was measured from the orbital groove to the posterior edge of the cephalothorax and the total length was measured from the tip of the rostrum to the tip of the telson (Owens and O' Neill, 1997; Ananda Raja *et al*., 2012b).

Total Haemocyte Count

Haemolymph (0.1 ml) was withdrawn from the ventral sinus of the first abdominal segment with equal volume of fixative (10 per cent formalin in 0.45M NaCl) in a syringe and transferred to micro centrifuge tube for total haemocyte count (THC), granular haemocyte (GH) and nongranular haemocyte (NGH) count as reported by Kallaya *et al.* (2005) and Ananda Raja *et al.* (2012b). After 10 min, 20 µl of the fixed haemocyte suspension was mixed with same volume of Rose Bengal solution (1.2 per cent Rose Bengal in 50 per cent ethanol) and incubated at ambient temperature (27-35°C) for 20 min. before being used to determine THC. Haemocytometer (improved Neubauer, Marienfeld, Germany) counts were made in 5/25 squares (vol. of one square = 0.2x0.2x0.1 mm^3). THC was calculated as:

THC ml^{-1} of haemolymph = 5 × count × 10^4 × dilution factor.

Granular Hemocyte Count

For GH and NGH counts, smears were prepared from the fixed and Rose Bengal stained hemocyte suspension. The smears were completely dried before counterstaining with haematoxylin solution (50g aluminium or potassium alum, 1 g haematoxylin crystals, 0.2 g sodium iodate, 1 g citric acid, 50g chloral hydrate and distilled water to 1L) for 7 to 10 min. The slides were then rinsed with tap water for 10 min. followed by dehydration with ascending grades of ethanol (10 dips each). After dehydration, the slides were cleared in xylene (3 times for 3 min. each) before being mounted with DPX mountant (Merck) and covered with a cover glass. The proportions of GH that included both large-granular and small-granular/semigranular hemocytes in 200 total hemocytes were recorded and those proportions were

used to calculate the total number of GH (i.e. GH count/200 × THC). NGH count was calculated in the same manner.

Sample Collection and Analysis

The shrimp samples were collected at fortnight interval for two-step polymerase chain reaction (PCR) amplification for white spot syndrome virus (WSSV) as reported by Kimura *et al.* (1996). Soil parameters such as pH, electrical conductivity and organic carbon, and water quality parameters such as temperature, total alkalinity, transparency, turbidity, pH, morning dissolved oxygen (DO), gross primary productivity, net primary productivity, nitrite-nitrogen (NO_2-N), nitrate-nitrogen (NO_3-N), phosphate-phosphorus (PO_4-P) and ammonia-nitrogen (NH_3-N) were recorded following standard methods (APHA, 1998) and salinity was assessed using a refractometer (ATAGO, Japan) at weekly interval.

Microbial Dynamics Study

Water samples were collected fortnightly from the culture ponds and the total plate (TPC) and *Vibrio* (TVC) count were done to monitor the microbial dynamics throughout the culture. For that purpose, one ml of water sample from respective ponds was serially diluted in autoclaved normal saline, plated aseptically under laminar airflow on sterilized Tryptone Soya Agar (TSA) and Thiosulfate Citrate Bile Sucrose Agar (TCBS) (Hi-Media, Mumbai) plates in duplicate. Those culture plates were incubated at 30°C for 24 h. They were then examined for TPC and TVC. The number of colonies formed on each plate was multiplied by the reciprocal value of dilution to determine the colony numbers per unit sample volume of water (Biswas *et al*., 2012).

Harvesting

The shrimps were harvested by continuous drag netting and hand picking at the end of the culture (134 DOC).

Statistical Analysis

Shrimp growth performance, hematological parameters, water and soil quality parameters, and microbial populations between untreated and treated seeds and between seasons were analyzed with independent samples t-test and one way ANOVA and Duncan's Multiple Range Test using SPSS for Windows v.17.0 programme (SPSS Inc. 2007). All data were expressed as mean ± standard error (SE).

Results

The survival rate of the chloramphenicol, furazolidone and ciprofloxacin treated seeds were 89.79, 88.57 and 85.06 per cent, respectively against the survival rate among the untreated control seeds were 69.08 per cent on transportation. The transportation was approximately covering duration of 12 h to reach KRC of CIBA. The survival rate among the antibiotics treated seeds were found to be significantly more ($P<0.05$) when compared to the untreated seeds. It showed the benefit of survivability during seed transport due to antibiotics application. But in grow out culture, the average body weight (ABW) after 134 DOC was 17.40±6.10 g in control and 21.92±6.17 g in antibiotic treated seeds. The feed conversion ratio (FCR) was found to be 2.11±0.46 and 2.08±0.49, and survival rate of 40.20±20.36 and 40.04±10.64 per cent in control and antibiotic group respectively with no statistically significant differences. Since there was no significant difference between control and treatment, the entire data was combined and analyzed for seasonal variation (Table 1). The analysis showed that the ABW after 134 DOC was 25.79±2.29g in monsoon and 13.53±2.23 g in winter batch with FCR of 1.62±0.03 and 2.57±0.01 and survival rate of 55.62±4.94 and 24.62±4.78 per cent (Table 1) respectively with significant difference in total production ($P<0.05$), survival rate ($P<0.05$) and FCR ($P<0.01$). Morphological parameters such as carapace length, total length and weight were not statistically significant between sexes, antibiotic treated and untreated grow out pond trials, and seasons. In hapa, there was significant difference observed within the seasons in terms of total length and weight but not in carapace length (Table 2). Significant difference was observed in carapace length, total length and weight between seasons ($P<0.05$). THC, GH and NGH counts were ranging from $13.96\pm1.55\times10^6$ to $16.38\pm1.05\times10^6$; $2.4\pm0.52\times10^6$ to $3.52\pm0.26\times10^6$; and $11.05\pm1.21\times10^6$ to $13.05\pm0.91\times10^6$, respectively. Fortnightly examination of animals for WSSV revealed negative by PCR. Significant difference ($P<0.01$) was observed

Table 1. Comparison of production parameters of *P. monodon* for monsoon and winter batch

Production parameters	Monsoon batch	Winter batch
Average Body Weight (g)	25.79±2.29	13.53±2.23
Male Weight (g)	24.49±1.58	14.11±2.66
Female Weight (g)	26.93±2.92	13.26±2.06
Total Production (kg)*	37.25 ± 0.25^a	8.90 ± 3.1^b
Survival Rate (%)*	55.62 ± 4.94^a	24.62 ± 4.78^b
FCR**	1.62 ± 0.03^a	2.57 ± 0.01^b

*P<0.05, **P<0.01; [a, b] - Values bearing different superscripts in a row differ significantly.

Table 2. Comparison of growth in hapa reared *P. monodon* during different seasons

Treatment	15th Day*	30th Day	45th Day	60th Day**	75th Day**	90th Day	105th Day	120th Day	134th Day
	Hapa samples total length (mm) in monsoon batch								
Control	36.70± 0.80[a]	45.55± 2.65	50.70± 1.80	59.65± 0.95[a]	63.70± 1.70[a]	80.10± 2.30	86.50± 0.40	98.62± 1.62	102.02± 3.75
Chloramphenicol	40.85± 0.05[b]	52.60± 0.80	60.02± 2.49	74.85± 1.95[c]	79.05± 1.25[b]	82.70± 3.60	91.85± 7.65	102.43± 0.90	103.10± 0.80
Furazolidone	41.32± 0.62[b]	47.20± 0.30	52.54± 0.44	66.95± 0.05[b]	68.33± 0.70[a]	84.04± 5.77	85.52± 4.59	93.22± 2.39	97.85± 3.85
	Hapa samples total weight (g) in monsoon batch								
Control	0.36± 0.06[a]	0.68± 0.12	1.12± 0.10	1.71± 0.13[a]	2.16± 0.18[a]	3.87± 0.31	5.23± 0.15	9.13± 0.49	10.34± 1.21
Chloramphenicol	0.57± 0.01[b]	1.01± 0.06	1.65± 0.27	3.60± 0.50[b]	3.99± 0.19[b]	4.48± 0.54	6.55± 1.69	9.59± 0.08	10.40± 0.05
Furazolidone	0.56± 0.00[b]	0.71± 0.01	1.14± 0.03	2.28± 0.11[ab]	2.56± 0.01[a]	4.79± 0.80	5.17± 0.82	7.84± 0.86	8.91± 1.39
	Hapa samples total length (mm) in winter batch								
Control	22.40± 0.90[a]	32.97± 2.17[a]	47.47± 1.63	55.12± 0.38	60.28± 1.55	62.32± 1.91	64.59± 1.84	69.10± 0.20	72.50± 1.25
Chloramphenicol	32.87± 0.07[b]	46.08± 1.35[b]	47.78± 1.18	54.78± 0.65	64.02± 0.08	65.02± 0.55	65.58± 0.58	69.80± 2.27	72.20± 3.13
Furazolidone	36.85± 1.65[b]	41.33± 1.40[b]	43.98± 1.18	51.08± 2.68	55.12± 0.92	61.07± 0.23	62.28± 0.95	63.65± 1.02	64.94± 1.41
Ciprofloxacin	25.90± 2.70[a]	46.00± 2.60[b]	49.85± 1.05	53.05± 1.72	58.08± 2.42	61.77± 0.81	63.97± 1.16	66.03± 0.97	68.16± 1.05
	Hapa samples total weight (g) in winter batch								
Control	0.05± 0.01[a]	0.26± 0.07[a]	0.82± 0.11	1.21± 0.04	1.77± 0.08	2.11± 0.09	2.21± 0.04	2.81± 0.23	3.15± 0.39
Chloramphenicol	0.24± 0.00[bc]	0.63± 0.05[b]	0.78± 0.07	1.25± 0.03	2.21± 0.07	2.31± 0.01	2.32± 0.01	2.59± 0.09	2.73± 0.14
Furazolidone	0.34± 0.06[c]	0.48± 0.05[ab]	0.63± 0.05	1.04± 0.17	1.47± 0.04	1.94± 0.12	2.08± 0.19	2.28± 0.13	2.44± 0.14
Ciprofloxacin	0.11± 0.04[ab]	0.78± 0.12 [b]	0.97± 0.06	1.14± 0.09	1.68± 0.22	2.19± 0.12	2.27± 0.11	2.32± 0.13	2.39± 0.13

*P<0.05, **P<0.01; [a, b] - Values bearing different superscripts in a row column significantly.

in water quality parameters such as temperature, salinity, turbidity, pH, DO, NO_2-N, NO_3-N, PO_4-P and NH_3-N, and organic carbon of soil between monsoon and winter batch (Table 3). TPC and TVC in pond water were lowest of 0.01±0.00 $x10^5$ and 0.00±0.00 $x10^2$ cfu/ml in initial phase of culture after bleaching and peak of 920±35$x10^5$ and 11.75±0.85$x10^2$ cfu/ml in monsoon batch, and 370±5$x10^5$ and 8.35±0.5$x10^2$ cfu/ml in winter batch respectively. Fortnightly TPC exhibited increasing trend and reached peak towards the end of the culture in monsoon batch while it was peak during 4th month then decreased towards the end of the culture in winter batch. TVC exhibited decreased during 2nd month and were found at the same level till the end in monsoon batch while it was peak during 2nd month and decreasing towards the end of the culture (Figures 1, 2).

Discussion

Owens and O' Neill (1997) reported that the female *P. monodon* were larger in total length than their male counterparts of same age group after adolescence. Moreover, they reported no differences in carapace length between the summer and winter populations. However, there was a difference between the weights of the two populations with shrimps that were sampled in the summer being twice as heavy as those sampled in winter is in close association with the present results. In *P. monodon*, the THC ranged from 2.10$x10^7$ (flow cytometry) to 2.33$x10^7$ (haemocytometer) (Owens and O' Neill, 1997). Chang *et al.* (1999) reported that *P. monodon* THC values ranged from 2.67±0.44$x10^7$ (ATP analysis) to 2.72±0.31$x10^7$ (haemocytometer) among the cultured populations. However, the present study showed lesser than the earlier reported values with no statistical significance between treated and untreated seeds and between seasons. The animals were found apparently healthy throughout the culture which ruled out the chances of drastic changes in the hematological parameters due to diseases. Owens and O' Neill (1997) reported that the NGH counts were significantly different between the sexes, with females having higher levels than males while THC and GH count within male and female prawns were not significantly different. In wild caught shrimp, THC and NGH ($x10^6$) count differs significantly among both the sexes between different months of the year. Moreover, significant difference is observed between two seasons (pre-monsoon and monsoon) in THC, GH and NGH count within sexes. No significant difference in hematological parameters is observed between sexes (Ananda Raja *et al.*, 2012b). In wild condition, the shrimp is exposed to different seasons and atmospheric conditions from birth but not so happens in grow out culture with hatchery produced

Table 3. Physico-chemical characteristics of experimental pond water and soil

Water quality parameters	Monsoon batch (n=18)	Winter batch (n=18)
Temperature (°C)**	32.37±0.37[a] (30-35)	25.13±1.31[b] (14-32.5)
Salinity (g/L)**	16.84±0.65[a] (13.3-21)	7.11±0.59[b] (3.5-13.5)
Total alkalinity (mg $CaCO_3$/L)	183.78±3.94 (140-216)	188.78±9.85 (116-250)
Transparency (cm)	27.67±0.50 (24-31)	25.78±1.14 (19-35)
Turbidity (NTU)**	43.67±4.93[a] (17-76)	22.67±1.20[b] (15-35)
Morning pH	8.13±0.058 (7.9-8.79)	8.32±0.076 (7.76-9)
Evening pH**	8.26±0.06[a] (7.97-8.95)	8.75±0.09[b] (8.2-9.89)
Morning dissolved oxygen**	4.66±0.32[a] (3-7.9)	6.25±0.08[b] (5.8-7.2)
Gross primary productivity (mg Carbon/m^3/hr)	309±24.03 (175.77-585.9)	280.81±21.04 (125-400)
Net primary productivity (mg Carbon/m^3/hr)	166.90±23.72 (48-445.28)	127.39±10.91 (37.5-200)
NO_2-N (mg/L)**	0.043±0.00[a] (0.025-0.069)	0.029±0.00[b] (0.012-0.044)
NO_3-N (mg/L)**	0.153±0.01[a] (0.09-0.34)	0.067±0.00[b] (0.035-0.096)
PO_4-P (mg/L)**	0.103±0.00[a] (0.081-0.148)	0.03±0.00[b] (0.009-0.043)
NH_3-N (mg/L)**	0.13±0.00[a] (0.083-0.198)	0.072±0.01[b] (0.028-0.109)
Soil quality parameters	Monsoon batch (n=18)	Winter batch (n=18)
Soil pH	7.28±0.047 (6.89-7.5)	7.26±0.07 (6.75-7.98)
EC (µ mhos)	8.14±0.22 (6.02-9.6)	7.90±0.08 (7.4-8.36)
Organic carbon (%)**	0.97±0.02[a] (0.78-1.11)	1.0±0.01[b] (0.9-1.08)

Figures in parentheses are range. *P<0.05, **P<0.01; [a, b] - Values bearing different superscripts in a row differ significantly.

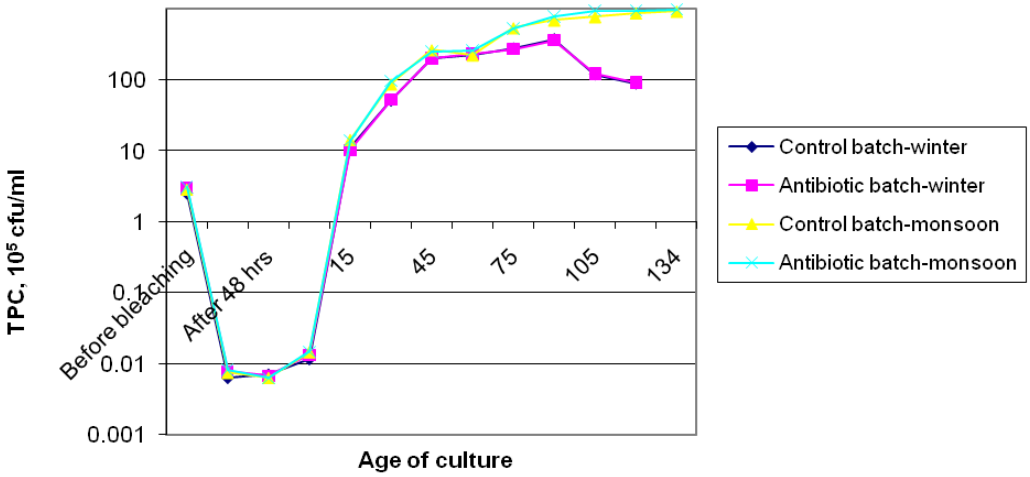

Figure 1. Seasonal influences on total plate count (TPC) of *P. monodon* grow out culture ponds.

seeds. Water temperature affects metabolism, oxygen consumption, growth, and survival and influences environmental parameters such as salinity and oxygenation of the water as reported by Moullac and Haffner (2000). Qing *et al.* (2007) reported that the greater the temperature change the greater the immune parameters affected. The THC decreased while phenol oxidase activity increased with the change of temperature in a short time. But after a period of adaptation, all immune parameters tended to be stable (Qing *et al.*, 2007). The recorded water quality parameters were within the optimum ranges for brackishwater shrimp and finfish culture (Ali *et al.*, 1999; Bhowmik *et al.*, 1992; Chakraborti *et al.*, 2002). The application of LSP and dolomite contributed optimum water quality. However, water temperature ranged from 30.0 to 35.0 and 14.0 to 32.5°C in monsoon and winter seasons, respectively during the experimental period. The low temperature during the winter season might be responsible for low survival and growth of shrimp (Wyban *et al.*, 1995; Yuan *et al.*, 2010) as compared to the survival rate (%), ABW (g) at harvest and FCR of 59, 28.64 and 1.36 during 2008 (CIBA, 2008) and 67.74, 26.9 and 1.37 during 2009 (CIBA, 2009) respectively in the conventional shrimp ponds. The sizes of the animals in hapa were considerably lesser than the pond reared animals which might be due to more stocking density and confinement.

TPC and TVC exhibited sharp fall after bleaching. TPC was found continuously increasing in population towards the end of the culture while TVC was found to rise during mid of the culture. Overall microbial population was found more in monsoon batch and less in winter batch at the end of the culture. The increase in bacterial load could be due to the uneaten feed, feces and plankton die-offs as the culture advances. But, decrease in the bacterial load towards the end of the culture during winter might be due to sharp reduction in pond water temperature.

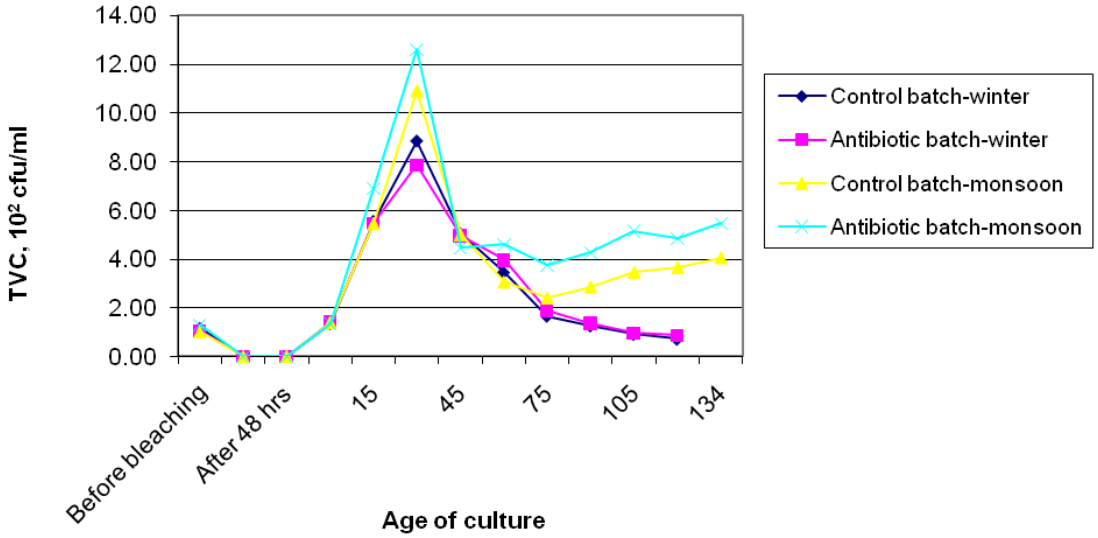

Figure 2. Seasonal influences on total *Vibrio* count (TVC) of *P. monodon* grow out culture ponds**.**

Similarly, total heterotrophic bacterial (THB) and TVC were reported decreasing significantly after disinfection and increasing towards the progress of the shrimp culture. Bleaching at 60 and 30 mg/kg water performed for disinfection in shrimp monoculture and polyculture system respectively was found to be effective to significantly decrease the THB and TVC (Ananda Raja *et al*., 2010; Biswas *et al*., 2012). Chlorine is widely used in hatcheries and ponds (Ananda Raja *et al*., 2012a), but its use stimulates the development of multiple antibiotic resistance genes in bacteria and become pathogenic. The resistant microbiota will then grow rapidly in the absence of their competitors and either predisposes the animals for disease or itself causes disease (Moriarty, 1999). But in the present experiments, the production was appreciable with no disease outbreak. It needs further validation to understand the level of chlorination and the development of resistance among the bacterial population.

The application of the antibiotics such as chloramphenicol, furazolidone and ciprofloxacin at hatchery increased the seed survival rate on transport but no statistically significant advantages were observed in grow out culture system. Mere advantage in seed survivability does not guarantee the use of these antibiotics in shrimp hatchery. Romero-González *et al.* (2007) reported that sulfonamides and tetracycline are two classes of antimicrobials broadly used in aquaculture to treat infections in fish. However, these compounds can come into contact with humans by means of the food chain (Kan and Meijer, 2007). It is important to pay attention to this contamination because of the potential hazards associated to the presence of these products in edible tissues. Chloramphenicol induced mutation was demonstrated in *Vibrio parahaemolyticus* strain isolated from freshwater fishes by Nithya Quintal *et al.* (2009). There are chances that the antibiotics may enter the environment by means of leaching from faecal materials and uneaten feeds when they are used in aquaculture. Recent report shows no uses of antibiotics in India by the farmers in shrimp grow out culture due to better export awareness (Ananda Raja *et al*., 2012a). However, more research is needed to better understand the processes and pathways of antibiotics and their metabolites in the sediments and other aquatic organisms before put them in to use. We have an obligation to have zero tolerance for banned antibiotics and use the allowed antibiotics prudently as per the interests of both public and aquatic animal health.

Acknowledgements

The authors express the deep sense of veneration and obligation to Director, CIBA, Chennai, Dr. K. P. Jithendran, SIC, AAHED, Dr. S. M. Pillai (Rtd.), Dr. T.C. Santiago (Rtd.) and Officer-in-Charge, KRC of CIBA for providing the required facilities and support to carry out the experiment. Field staffs of KRC of CIBA are duly acknowledged for their support.

References

Ali, M.S., Shofiquzzoha, A.F.M. and Ahmed, S.U. 1999. Effect of submerged aquatic vegetation on growth and survival of *Penaeus monodon* (Fab.). Bangladesh Journal of Fisheries Research, 3: 145–149.

Ananda Raja, R., Panigrahi, A. and Sujeet Kumar. 2012a. Epidemiological investigation of brackishwater culture systems in West Bengal, India, Journal of Applied Aquaculture, 24: 49-59. doi: 10.1080/10454438.2012.652029

Ananda Raja, R., Panigrahi, A., Shyne Anand, P.S., Biswas, G., De, D., Ghoshal, T.K. and Sujeet, K. 2010. Microbial dynamics of shrimp, *Penaeus monodon* culture and polyculture system in West Bengal. In: AMSCO 2010 International conference on Aquatic microbiology (Status, Challenges and Opportunities), 2-4 September 2010. Parangipettai, India: 30-31. (Abstracts).

Ananda Raja, R., Sujeet, K., Sundaray, J.K., De, D., Biswas, G. and Ghoshal, T.K. 2012b. Hematological parameters in relation to sex, morphometric characters and incidence of white spot syndrome virus in tiger shrimp *Penaeus monodon* Fabricius, 1798 from Sunderban, West Bengal. Indian Journal of Fisheries, 59: 169-174.

APHA/AWWA/WPCF. 1998. Standard Methods for the Examination of Water and Wastewater, 20th edition, American Public Health Association, Washington, DC, USA, 1325 pp.

Bhowmik, M.L., Chakraborti, R.K., Mandal, S.K. and Ghosh, P.K. 1992. Growth of *Penaeus monodon* (Fab.) under variable stocking densities. Environment and Ecology, 10: 825–828.

Biswas, G., Ananda Raja, R., De, D., Sundaray, J.K., Ghoshal, T.K., Shyne Anand, P. S., Sujeet Kumar., Panigrahi, A., Thirunavukkarasu, A.R. and Ponniah, A.G. 2012. Evaluation of productions and economic returns from two brackishwater polyculture systems in tide-fed ponds. Journal of Applied Ichthyology, 28: 116–122.
doi: 10.1111/j.1439-0426.2011.01909.x

Chakraborti, R.K., Sundaray, J.K. and Ghoshal, T.K. 2002. Production of *Penaeus monodon* in the tide fed ponds of Sunderbans. Indian Journal of Fisheries, 49: 419–426.

Chang, C.F., Su, M.S. and Chen, H.Y. 1999. A rapid method to quantify total haemocyte count of *Penaeus monodon* using ATP analysis. Fish Pathology, 34: 211-212.

CIBA 2008. Annual Report 2007-2008, Central Institute of Brackishwater Aquaculture, Chennai, India: 53-57.

CIBA. 2009. Annual Report 2008-2009. Central Institute of Brackishwater Aquaculture, Chennai, India: 45-51.

Coastal Aquaculture Authority - Compendium of Act, Rules, Guidelines and Notifications. 2006. Tamil Nadu, India: 116-124.

Chafer-Pericas, C., Maquieira, A. and Puchades, R. 2010. Multiresidue determination of antibiotics in fish samples by immunoassay. Safety control in cultivated fish. International conference on food innovation. 25-29 October 2010. Spain: 1-4.

Kalaimani, N., Ravisankar, T., Chakravarthy, N., Raja, S., Santiago, T.C. and Ponniah, A.G. 2013. Economic losses due to disease incidences in shrimp farms of India. Fishery Technology, 50: 80-86.

Kallaya., S., Gangnonngiw., W., Archakunakorn., S., Fegan, D. and Flegel, T.W. 2005. Bacterial clearance rate and a new differential hemocyte staining method to assess immunostimulant activity in shrimp. Diseases of Aquatic Organisms, 63: 89–94.

Kan, C.A. and Meijer, G.A.L. 2007. The risk of contamination of food with toxic substances present in animal feed. Animal Feed Science and Technology, 133: 84-108.
doi: 10.1016/j.anifeedsci.2006.08.005

Kimura, T., Yamano, K., Nakano, H., Monoyama, K., Hiraoka, M. and Frousp, K. 1996. Detection of penaeid rod shaped DNA (PRVD) by PCR (in Japanese). Fish Pathology, 31: 93-98.

Moriarty, D.J.W. 1999. Disease Control in Shrimp Aquaculture with Probiotic Bacteria. Microbial Biosystems: New Frontiers. In: C.R. Bell, M. Brylinsky and P. Johnson-Green (Eds.), Proceedings of the 8th International Symposium on Microbial Ecology, Atlantic Canada Society for Microbial Ecology, Halifax, Canada: 237-243.

Moullac, G.L. and Haffner, P. 2000. Environmental factors affecting immune responses in crustacea. Aquaculture, 191: 121-131.

Nithya Quintoil, M., Porteen, K., Wilfred Ruban, S., Abraham, T.J. and Pramanik, A.K. 2009. Chloramphenicol induced mutation frequency of *Vibrio parahaemolyticus* strains isolated from freshwater fishes. Indian Veterinary Journal, 86: 451-453.

Official Journal of the European Communities (1990, L224, 1-20) L224, of 18 August 1990, Council Regulation 2377/90/EC; consolidated version of the Annexes I to IV updated up to 01.02.2007 obtained from http://www.emea.eu.int/htms/vet/mrls/a-zmrl.htm# (accessed September 24, 2013).

Overstreet, R.M. 1987. Solving parasite-related problems in culture crustacean. International Journal of Parasitology, 17: 309-318.

Owens, L. and O' Neill, A. 1997. Use of a clinical cell flow cytometer for differential counts of prawn *Penaeus monodon* haemocytes. Diseases of Aquatic Organisms, 31: 147-153.

Qing., P.L., Bo., F., Ling-Xu, J. and Jing, L. 2007. The Effect of temperature on selected immune parameters of the white shrimp, *Litopenaeus vannamei*. Journal of World aquaculture Society, 38: 326-332. doi: 10.1111/j.1749-7345.2007.00105.x.

Romero-González, R., López-Martínez, J.C., Gómez-Milán, E., Garrido-Frenich, A. and Martínez-Vidal, J.L. 2007. Simultaneous determination of selected veterinary antibiotics in gilthead seabream (*Sparus Aurata*) by liquid chromatography-mass spectrometry. Journal Chromatography, 857(B): 142-148.

SPSS Inc. 2007. SPSS Base 17.0 for Windows User's Guide. SPSS Inc., Chicago IL. http://www.spss.com.

Wyban, J., Walsh, W.A. and Godin, D.M. 1995. Temperature effect on growth, feeding rate and feed conversion of the pacific white shrimp (*Penaeus vannamei*). Aquaculture, 138: 267–279. doi: 10.1016/0044-8486(95)00032-1.

Yuan, D., Yi, Y., Yakupitiyage, A., Fitzimmons, K. and Diana, J.S. 2010. Effects of addition of red tilapia (*Oreochromis* spp.) at different densities and sizes on production, water quality and nutrient recovery of intensive culture of white shrimp (*Litopenaeus vannamei*) in cement tanks. Aquaculture, 298: 226–238. doi:10.1016/j.aquaculture.2009.11.011.