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Attenuation of Negative Impacts by Micro Algae and Enriched *Artemia salina* on *Penaeus monodon* and *Litopenaeus vannamei* Larval Culture

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ABSTRACT

Shrimp farming is one of the most important aquaculture and economically lucrative practices of maritime countries. Providing a Specific Pathogen Free (SPF) shrimps to the farmers is a big challenge that must be addressed to meet the demand. In general, microalgae are utilized in aquaculture as a live feed for the shrimps. However, their importance in the attenuation of negative impacts of pathogenic microbial load, eutrophication and promotion of shrimps growth has to be delineated by experimental investigations to justify the above specific pathogen free shrimps. The present study was carried out to investigate the feeding of five different microalgae and algae enriched *Artemia salina*. Nauplii on digestive enzyme activity, growth, survival rate, microbial load on *Penaeus monodon* and *Litopenaeus vannamei* from zoea to post larvae (20 stages) and other water quality. Microalgae such as *Isochrysis galbana*, *Cheatoceros calcitrans*, *Tetraselmis* sp., *Chlorella* sp. and *Nannochloropsis* sp. were obtained from AMET Microbial Culture Collection Centre, Department of Marine Biotechnology, AMET University. *Penaeus monodon* and *Litopenaeus vannamei* at PL 20 stage showed maximum protease and amylase (digestive enzyme) activity, maximum length and survival rate when fed with *Artemia salina* nauplii enriched with *C. calcitrans* followed by *Chlorella* sp. On studying the water quality parameters such as, pH, temperature, salinity, dissolved oxygen and ammonia it was found better in tank II where the shrimps were fed with *Artemia salina* enriched with *C. calcitrans*. Regarding the *vibrio* load at different stages of *P. monodon* and *L. vannamei* larvae and cultured water it was comparatively lower in tank II, where the shrimps were fed with *Cheatoceros calcitrans* and enriched *Artemia salina* than other groups. It is concluded that, the use of *Cheatoceros calcitrans* and enriched *Artemia salina* will reduce the potential negative impacts in the environment and promote production of shrimp larvae in hatcheries and fetch benefits to local economies.

Key words: Shrimp seeds, micro algae, *Artemia salina*, digestive enzyme, water quality

INTRODUCTION

Shrimp farming is one of the most important aquaculture practices worldwide especially in Asia due to their high economic value. It is estimated that approximately more than 5 million Mt of shrimp are annually produced but the current global demand for both the wild and farmed shrimps

is approximately more than 6.5 million Mt per annum. Artificial culture of shrimps in grow out ponds has been intensified to keep shrimp production on par to the demand. As a sequel semi intensive and extensive methods of culture brought various ecological, economical and social issues (FAO., 2012). In general, during the shrimp growth after metamorphosis it is considerably affected by the gradual change from planktonic to benthic existence coinciding with changes in the alimentation. During early post larval development, high mortality was noticed due to the changes in the gut associated digestive enzyme production levels (Madhumathi and Rengasamy, 2011a, b). The first feeding during the growth of any cultivable organism is the most 'critical phase' of their life cycle for their survival. Hence, developing a new technology and new live feed may offer great hope for the future with a promise for blue revolution in the century to match the green revolution. Protein is the major component in the natural food of penaeids shrimps. Thus, feeding the penaeids with protein rich live diets such as, phytoplankton such as microalgae (2-20 μm) and zooplankton such as rotifers (50-200 μm) and brine shrimp, *Artemia salina* (200-300 μm) can increase the gut associated digestive enzymes (Anonymous, 2000).

Micro algae and *Artemia salina* like zooplankton help to stabilize and improve the water quality improve the oxygen production, promote pH stabilization (the action of some excreted biochemical compounds along with the induction of behavioral processes like initial prey catching) and regulate disease causing bacterial population and above all the probiotics and stimulate immunity in the host animal. The microalgae most frequently used in aquaculture include *Chlorella*, *Tetraselmis*, *Isochrysis*, *Pavlova*, *Phaeodactylum*, *Chaetoceros*, *Nannochloropsis*, *Skeletonema* and *Thalassiosira* (Spolaore *et al*., 2006). Moreover, *Artemia salina* are biologically uncontaminated readily available and acceptable larval feed and established as a standard live feed for over 85% of marine species. The present study was aimed to examine the digestive enzymes (amylase and protease) activity, survival rate (%), average growth (mm), water quality parameters and bacterial (*Vibrio*) load in *Penaeus monodon* and *Litopenaeus vannamei* shrimp culture from zoea to postlarvae 20 stages by feeding with five different microalgae such as, *Isochrysis galbana*, *Cheatoceros calcitrans*, *Tetraselmis* sp., *Chlorella* sp., *Nannochloropsis* sp. and algae enriched *Artemia salina* nauplii.

MATERIALS AND METHODS

Microalgae: The five different microalgae such as *Isochrysis galbana*, *Cheatoceros calcitrans*, *Tetraselmis* sp., *Chlorella* sp. and *Nannochloropsis* sp. were obtained from AMET Microbial Culture Collection Centre, Department of Marine Biotechnology, AMET University.

Experimental animal: The shrimp (nauplii 24 h) of *Penaeus monodon* and *Litopenaeus vannamei* were obtained from a commercial shrimp hatchery located in Marakanam, Kanchipuram, Tamil Nadu, India. They were kept in seawater with aeration for a period of 6 h in order to avoid any stress to the animals and then used for the experiments.

Experimental design: The experiments were conducted at the Department of Marine Biotechnology, AMET University, Chennai. A total of twelve, 100 L glass tanks (Six tanks for *Penaeus monodon* and another six tanks for *Litopenaeus vannamei*) were added with 70 L of filtered seawater at 32% salinity and kept at ambient temperature (28±1°C) and aerated continuously. The seeds were transferred in the tank with a stocking density of 75 nauplii L^{-1}.

Feeding schedule from zoea to post larvae: The zoea of *Penaeus monodon* and *Litopenaeus vannamei* kept in the experimental tanks were fed with five different microalgae such

as *Isochrysis galbana*, *Cheatoceros calcitrans*, *Tetraselmis* sp., *Chlorella* sp. and *Nannochloropsis* sp. On 1st day of the Zoea (Z) of I- III stages were fed thrice with 30×10^4 cells mL^{-1} of algal cells. On the 2nd day of the Mysis (M) (I- III/Postlarvae 1) of *Penaeus monodon* and *Litopenaeus vannamei* were fed thrice with 40×10^4 cells mL^{-1} of algal cells. From 3rd day up to 20th day they were fed thrice with 3-8 No/mL of *Artemia salina* nauplii enriched with different microalgae. The 24 h old *Artemia salina* nauplii enriched with *Isochrysis galbana*, *Cheatoceros calcitrans*, *Tetraselmis* sp, *Chlorella* sp and *Nannochloropsis* sp at 20, 30, 40, 50 and 60×10^4 cells mL^{-1} at 16 and 9 h, respectively for a period of 24 h as fed to *Penaeus monodon* and *Litopenaeus vannamei*. Filtered seawater was exchanged daily and the debris settled at the bottom was siphoned out without disturbing the animals. This experiment was conducted up to the stages of PL20. At the end of the experiments, the animals from all the experimental tanks were randomly selected and the survival rate and the average length were recorded.

Digestive enzyme activities of shrimps fed with different microalgae: The shrimps were collected on 1st day (Zoea III stages) after naupliar stage, 2nd day (Mysis 111), 5th day (PL5), 10th day (PL10), 15th day (PL15) and 20th day (PL20) from all the experimental tanks and they are subjected for different enzyme analysis (Madhumathi and Rengasamy, 2011a, b). Protease assay was performed using the method followed by Walter (1984). Amylase assay was performed using the method followed by Rick and Stegbauer (1974).

Microbiological analysis: Water samples were taken from the *Penaeus monodon* and *Litopenaeus vannamei* larval rearing tanks (all the experimental tanks) during various larval stages of shrimps (i.e. nauplii, zoea, mysis and post larvae) and the *Vibrio* sp., load was enumerated by growth on TCBS agar (Karthik *et al*., 2014).

RESULTS AND DISCUSSION

In general, traditional and non-scientific shrimp farms depend upon the shrimp seeds caught from the wild or those entered with the tides for stocking. Such seeds collected naturally from tides may have fortuitous infection with microbes and bound to affect shrimp culture. There is a demand for healthy and quality seeds throughout the year. Thus, the successful shrimp culture is dependent upon stocking disease free, healthy seeds in the hatcheries (Soundarapandian and Babu, 2010). The applications of microalgae for aquaculture are associated with nutrition and other biological activities (Hemaiswarya *et al*., 2011). The study of digestive enzymes constitutes a denominator role to consider the nutritional requirements of specific stages of development in shrimps (Le Moullac *et al*., 1997). In the present study, the zoea to postlarvae 20 of *Penaeus monodon* and *Litopenaeus vannamei* were fed with five different microalgae such as, *Isochrysis galbana*, *Cheatoceros calcitrans*, *Tetraselmis* sp., *Chlorella* sp. and *Nannochloropsis* sp. and algae enriched *Artemia salina*. The protease activity at all the different stages of *P. monodon* and *L. vannamei,* the animals fed with *Cheatoceros calcitrans* and enriched *Artemia salina* exhibited a maximum protease activity of (*P. monodon* 17.02±0.01 U mL^{-1} and *L. 1vannamei* 16.55±0.08 U mL^{-1}) at pH 8 and a minimum total activity of (*P. monodon* 16.19±0.01 U mL^{-1} and *L. vannamei* 16.13±0.13 U mL^{-1}) at pH 6 followed by animals fed with *Chlorella* sp. and enriched *Artemia salina*, respectively (Table 1-2). A decrease of protease activity was found from PL6 to PL20 stages of animals fed with other microalgae in all the groups. Same results were obtained when the animals fed with *Artemia salina* enriched with five different microalgae (Table 3-7).

Table 1: Water quality parameters of both control and experimental larval rearing tanks of *Penaeus monodon*

	Penaeus monodon				
Water quality parameters	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5
Salinity (ppt)	30.00±0.78	30.00±0.13	30.00±0.22	30.00±0.81	30.0±0.23
pH	8.00±0.17	8.10±0.12	8.10±0.12	8.10±0.17	8.10±0.15
Water temperature (°C)	31.00±0.50	31.00±0.50	31.00±0.50	31.00±0.50	3.00±0.50
Dissolved oxygen (mL L^{-1})	5.58±0.12	6.19±0.10	5.73±0.19	6.10±0.17	5.70±0.32
Ammonia (mg L^{-1})	0.16±0.19	0.14±0.11	0.17±0.18	0.15±0.13	0.17±0.11

Table 2: Water quality parameters of both control and experimental larval rearing tanks of *Litopenaeus vannamei*

	Litopenaeus vannamei				
Water quality parameters	Tank 1	Tank 2	Tank 3	Tank 4	Tank 5
Salinity (ppt)	30.00±0.32	30.00±0.43	30.00±0.51	30.00±0.19	30.0±0.37
pH	8.00±0.11	8.10±0.14	8.10±0.02	8.10±0.11	8.10±0.03
Water temperature (°C)	31.00±0.50	31.00±0.50	31.00±0.50	31.00±0.50	31.0±0.50
Dissolved oxygen (mL L^{-1})	5.22±0.13	6.10±0.10	5.22±0.22	6.08±0.18	5.38±0.35
Ammonia (mg L^{-1})	0.17±0.19	0.14±0.34	0.16±0.32	0.16±0.44	0.17±0.23

Table 3: Protease activity of Z3-M3 stages of *Penaeus monodon* and *Litopenaeus vannamei*

	Penaeus monodon		*Litopenaeus vannamei*	
	Total activity (U mL^{-1})		Total activity (U mL^{-1})	
Algal sources	pH 6	pH 8	pH 6	pH 8
Isochrysis sp.	15.10±0.20	15.44±0.12	15.17±0.09	15.02±0.21
Cheatoceros sp.	16.19±0.01	17.02±0.31	16.13±0.13	16.55±0.08
Tetraselmis sp.	15.02±0.40	15.44±0.17	15.17±0.18	15.43±0.11
Chlorella sp.	15.17±0.20	15.55±0.07	16.01±0.09	16.35±0.09
Nannochloropsis sp.	13.10±0.10	13.44±0.22	14.07±0.06	14.61±0.03

Table 4: Protease activity of PL 1-PL5 stages of *Penaeus monodon* and *Litopenaeus vannamei*

	Penaeus monodon		*Litopenaeus vannamei*	
	Total activity (U mL^{-1})		Total activity (U mL^{-1})	
Algal sources	pH 6	pH 8	pH 6	pH 8
Isochrysis sp.	0.42±0.04	20.09±0.30	0.35±0.20	18.11±0.13
Cheatoceros sp.	0.49±0.03	20.55±0.21	0.44±0.11	20.60±0.11
Tetraselmis sp.	0.08±0.12	19.48±0.18	0.11±0.16	19.12±0.21
Chlorella sp.	0.47±0.19	20.44±0.12	0.40±0.14	19.55±0.19
Nannochloropsis sp.	0.44±0.08	20.22±0.11	0.38±0.12	19.46±0.07

Table 5: Protease activity of PL 6-PL10 stages of *Penaeus monodon* and *Litopenaeus vannamei*

	Penaeus monodon		*Litopenaeus vannamei*	
	Total activity (U mL^{-1})		Total activity (U mL^{-1})	
Algal sources	pH 6	pH 8	pH 6	pH 8
Isochrysis sp.	0.39±0.21	18.19±0.09	0.28±0.13	17.00±0.15
Cheatoceros sp.	0.45±0.18	19.42±0.13	0.40±0.22	20.32±0.19
Tetraselmis sp.	0.15±0.13	19.00±0.18	0.17±0.18	18.12±0.18
Chlorella sp.	0.40±0.03	19.20±0.09	0.36±0.15	19.30±0.16
Nannochloropsis sp.	0.38±0.09	18.20±0.06	0.34±0.19	17.22±0.12

Madhumathi and Rengasamy (2011a, b) also observed similar results, while studying the ontogenetic changes in the digestive enzyme of *Penaeus monodon* at Z3 to PL20 stages, when fed with *C. calcitrans* and other four different microalgae and enriched *Artemia salina*.

Regarding, the enzyme activity all the different stages of *P. monodon* and *Litopenaeus vannamei* at different time intervals the *Artemia* nauplii fed with *Cheatoceros calcitrans* and enriched exhibited a maximum amylase and protease total activity at 15 min time interval and it

Table 6: Protease activity of PL 11-PL15 stages of *Penaeus monodon* and *Litopenaeus vannamei*

	Penaeus monodon		*Litopenaeus vannamei*	
	Total activity (U mL^{-1})		Total activity (U mL^{-1})	
Algal sources	pH 6	pH 8	pH 6	pH 8
Isochrysis sp.	0.11±0.12	0.42±0.16	0.09±0.08	0.44±0.09
Cheatoceros sp.	0.48±0.18	1.12±0.11	0.38±0.17	1.14±0.16
Tetraselmis sp.	0.20±0.19	0.55±0.18	0.17±0.14	0.51±0.12
Chlorella sp.	0.44±0.14	0.65±0.13	0.31±0.11	0.59±0.09
Nannochloropsis sp.	0.32±0.11	0.44±0.18	0.28±0.08	0.41±0.07

Table 7: Protease activity of PL 16-PL20 stages of *Penaeus monodon* and *Litopenaeus vannamei*

	Penaeus monodon		*Litopenaeus vannamei*	
	Total activity (U mL^{-1})		Total activity (U mL^{-1})	
Algal sources	pH 6	pH 8	pH 6	pH 8
Isochrysis sp.	0.42±0.17	0.20±0.13	0.33±0.05	0.18±0.19
Cheatoceros sp.	0.55±0.11	0.78±0.15	0.42±0.13	0.62±0.12
Tetraselmis sp.	0.11±0.13	0.56±0.12	0.10±0.21	0.32±0.14
Chlorella sp.	0.45±0.14	0.58±0.17	0.36±0.19	0.40±0.08
Nannochloropsis sp.	0.33±0.18	0.41±0.19	0.30±0.09	0.37±0.11

Table 8: Amylase activity of Z3-M3 stages of *Penaeus monodon* and *Litopenaeus vannamei*

	Penaeus monodon			*Litopenaeus vannamei*		
	Total activity (U mL^{-1}) at different time intervals (min)			Total activity (U mL^{-1}) at different time intervals (min)		
Algal sources	15	30	45	15	30	45
Isochrysis sp.	0.36±0.02	0.32±0.11	0.29±0.30	0.34±0.18	0.31±0.06	0.28±0.12
Cheatoceros sp.	1.10±0.04	0.92±0.09	0.80±0.07	1.02±0.15	0.78±0.08	0.68±0.11
Tetraselmis sp.	0.66±0.04	0.58±0.12	0.55±0.16	0.64±0.09	0.61±0.12	0.56±0.18
Chlorella sp.	0.78±0.01	0.69±0.07	0.66±0.09	0.76±0.08	0.71±0.09	0.65±0.14
Nannochloropsis sp.	0.38±0.06	0.35±0.02	0.31±0.03	0.36±0.02	0.30±0.11	0.27±0.19

Table 9: Amylase activity of PL 1-PL5 stages of *Penaeus monodon* and *Litopenaeus vannamei*

	Penaeus monodon			*Litopenaeus vannamei*		
	Total activity (U mL^{-1}) at different time intervals (min)			Total activity (U mL^{-1}) at different time intervals (min)		
Algal sources	15	30	45	15	30	45
Isochrysis sp.	0.55±0.12	0.52±0.08	0.58±0.11	0.57±0.08	0.48±0.02	0.62±0.16
Cheatoceros sp.	0.92±0.17	0.88±0.12	0.94±0.15	0.91±0.12	0.86±0.06	0.93±0.11
Tetraselmis sp.	0.47±0.15	0.39±0.15	0.49±0.18	0.46±0.11	0.38±0.11	0.52±0.13
Chlorella sp.	0.66±0.11	0.59±0.19	0.69±0.12	0.68±0.13	0.61±0.03	0.72±0.08
Nannochloropsis sp.	0.52±0.09	0.47±0.18	0.56±0.09	0.51±0.18	0.47±0.19	0.53±0.19

Table 10: Amylase activity of PL 6-PL10 stages of *Penaeus monodon* and *Litopenaeus vannamei*

	Penaeus monodon			*Litopenaeus vannamei*		
	Total activity (U mL^{-1}) at different time intervals (min)			Total activity (U mL^{-1}) at different time intervals (min)		
Algal sources	15	30	45	15	30	45
Isochrysis sp.	0.66±0.02	0.58±0.14	0.55±0.07	0.64±0.18	0.61±0.50	0.56±0.08
Cheatoceros sp.	1.13±0.08	0.98±0.11	0.90±0.16	1.10±0.09	0.93±0.15	0.88±0.12
Tetraselmis sp.	0.38±0.12	0.35±0.05	0.31±0.02	0.36±0.04	0.30±0.07	0.27±0.16
Chlorella sp.	0.78±0.03	0.69±0.15	0.66±0.19	0.76±0.12	0.71±0.17	0.65±0.17
Nannochloropsis sp.	0.36±0.14	0.32±0.13	0.29±0.09	0.34±0.13	0.31±0.04	0.28±0.07

Table 11: Amylase activity of PL 11-PL15 stages of *Penaeus monodon* and *Litopenaeus vannamei*

	Penaeus monodon			*Litopenaeus vannamei*		
	Total activity (U mL^{-1}) at different time intervals (min)			Total activity (U mL^{-1}) at different time intervals (min)		
Algal sources	15	30	45	15	30	45
Isochrysis sp.	0.56±0.09	0.52±0.09	0.48±0.70	0.57±0.08	0.52±0.10	0.45±0.13
Cheatoceros sp.	1.09±0.03	1.15±0.10	0.99±0.16	1.12±0.12	0.96±0.09	0.96±0.18
Tetraselmis sp.	0.66±0.12	0.62±0.12	0.58±0.18	0.64±0.11	0.62±0.18	0.58±0.17
Chlorella sp.	0.88±0.16	0.76±0.13	0.69±0.12	0.85±0.19	0.71±0.06	0.62±0.04
Nannochloropsis sp.	0.58±0.07	0.55±0.05	0.49±0.14	0.51±0.05	0.49±0.02	0.40±0.08

Table 12: Amylase activity of PL 16-PL20 stages of *Penaeus monodon* and *Litopenaeus vannamei*

	Penaeus monodon			*Litopenaeus vannamei*		
	Total activity (U mL^{-1}) at different time intervals (min)			Total activity (U mL^{-1}) at different time intervals (min)		
Algal sources	15	30	45	15	30	45
Isochrysis sp.	0.66±0.19	0.58±0.05	0.55±0.07	0.64±0.13	0.61±0.10	0.56±0.05
Cheatoceros sp.	1.07±0.02	1.18±0.11	0.75±0.15	1.02±0.18	0.98±0.04	0.72±0.19
Tetraselmis sp.	0.58±0.14	0.55±0.07	0.49±0.12	0.51±0.03	0.49±0.16	0.40±0.11
Chlorella sp.	0.67±0.17	0.59±0.02	0.69±0.02	0.68±0.07	0.63±0.05	0.65±0.14
Nannochloropsis sp.	0.36±0.06	0.32±0.06	0.29±0.17	0.34±0.08	0.31±0.09	0.28±0.06

slightly decreased from 30 and 45 min (Table 3-12). However, there was a gradual increase in the enzyme activity up to PL5, then it decreased in PL10. Again it increased during the stages of PL15, the enzyme activity decreased during the stages of PL20.

Understanding the connection between the water quality and aquatic productivity is absolutely essential for optimum growth and production. The quality of water during the culture period will go down mainly due to the accumulation of organic wastes produced by the animals and unutilized feed (Soundarapandian and Babu, 2010). Thus, in this study, during the entire culture period some important water quality parameters such as, salinity, pH, temperature, dissolved oxygen and ammonia were monitored. Salinity is the most important factor influencing many functional responses of the organisms as metabolism, growth, migration, osmotic behavior, reproduction etc. The marine organisms maintain their internal salt concentration (salt concentration of blood and body fluids) by osmoregulation. In shrimp hatchery the recommended salinity range is 28-35 ppt (Kannupandi *et al*., 2002). In the present study the salinity was maintained at 30 ppt in all the experimental tanks. Similarly, Krishnaprakash (2007) also maintained the salinity for the larval rearing of *P. monodon* at 31 ppt. During the culture period the pH was found to be 8.1-8.2 in the entire experimental tanks and the temperature was found to be in the range of 31±1.1°C in both the *P. monodon* and *L. vannamei* reared experimental tanks. Similarly, Kannupandi *et al*. (2002) also reported that the required range of pH for shrimp larval culture is 8.2-8.5.

Dissolved Oxygen (DO) in the larvae/adult animals rearing water is an important factor for the respiration of aquatic organisms and also to maintain favorable chemical and hygienic environment of the water body. Moreover, it also controls many oxidation reactions and maintains aerobic conditions in water. Because, when oxygen level is very low and anaerobic conditions exist, nitrate is reduced into ammonia, which will be toxic to the larvae or adult in the culture water and it also increases the pH. Furthermore, low-level of oxygen tension hampers metabolic performances in shrimp larvae and it will reduce the growth and finally cause huge mortality (Le Moullac, 2001). So, in this study the oxygen level in the culture medium was maintained in the desired range by

aeration continuously during the whole culture period. In *P. monodon* culture while checking the dissolved oxygen it was found to be higher in experimental tank 2 (6.19±0.17 mL L^{-1}) followed by tank 4 (6.10±0.17 mL L^{-1}). Moreover, the ammonia was found to be low in experimental tank 2 (0.14±0.11 mg L^{-1}) followed by tank 4 (0.15±0.13 mL L^{-1}). In *L. vannamei* culture whereas checking the dissolved oxygen it was found to be higher in experimental tank 2 (6.10±0.10 mL L^{-1}) followed by tank 4 (6.08±0.18 mL LL^{-1}). Moreover, the ammonia was found to be low in experimental tank 2 (0.14±0.34 mg L^{-1}) followed by tank 4 (0.16±0.44 mL L^{-1}).

Kurmaly *et al*. (1989) has stated that poorest algal diet in terms of survival and development of the larvae of shrimp fed with *Dunaliella* sp., could be due to larger cell size of the alga when compared to the diatom. Kuban *et al*. (1985) reported that the survival rates, growth is superior in larvae fed with *Artemia* nauplii than microalgae alone. In the present study, whereas, monitoring the survival rate of the nauplii of *P. monodon* and *L. vannamei*, it was found to be higher in all the experimental tanks (Fig. 1a-b). At the end of the culture period (PL20), the average length of all the post larvae was found to be maximum higher in the experimental tank 2 where the animals fed with *Cheatoceros calcitrans* and enriched *Artemia salina* (Fig. 2a-b). Madhumathi and Rengasamy, (2011a, b), also observed the similar results, like high survival rate, length and weight of zoea-PL I of *P. monodon* fed with *C. calcitrans* and PL I- PL20 stages fed with *C. calcitrans* enriched *Artemia* nauplii diets. They also stated that, the zoea to PLI of shrimps had high protein content of 51% followed by carbohydrate 6% and lipid 50%, when it was fed with *C. calcitrans*.

In normal, diseases in aquaculture practices are mostly caused by luminous bacteria *Vibrio harveyi* and it has been referred to as the largest economic loss in the shrimp aquaculture due to mass mortalities (Sivakumar *et al*., 2014; Karthik *et al*., 2014). Furthermore, in general pathogenic microbes always remain in the water as opportunistic pathogens and they attack the larvae only when the larvae are weak due to environmental stress or nutritional deficiency (Soundarapandian and Babu, 2010). During larval rearing stages different microbes may enter the hatchery system through the eggs and live feeds such as algae and *Artemia* sp. In the present study while checking the *Vibrio* load at different stages of *P. monodon* and *L. vannamei* larvae and cultured water it was found to be comparatively lower in tank 2, where the animals fed with *Cheatoceros calcitrans* and enriched *Artemia salina* than other groups (Fig. 3a-d).

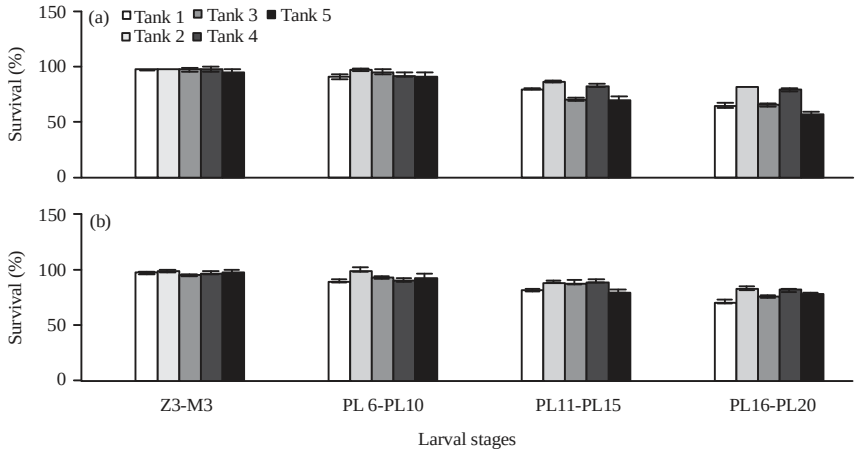

Fig. 1(a-b): Survival rate of different larval stages of (a) *Penaeus monodon* and (b) *Litopenaeus vannamei* reared in both control and experimental tanks

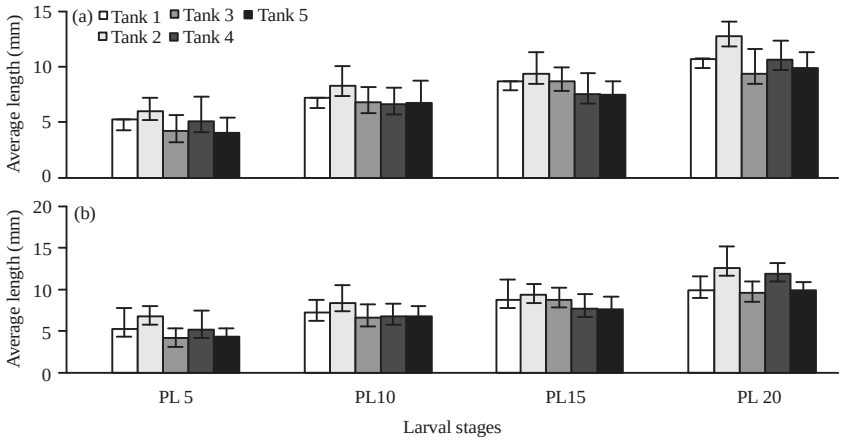

Fig. 2(a-b): Average length of (a) *Penaeus monodon* and (b) *Litopenaeus vannamei* post larvae reared in both control and experimental tanks

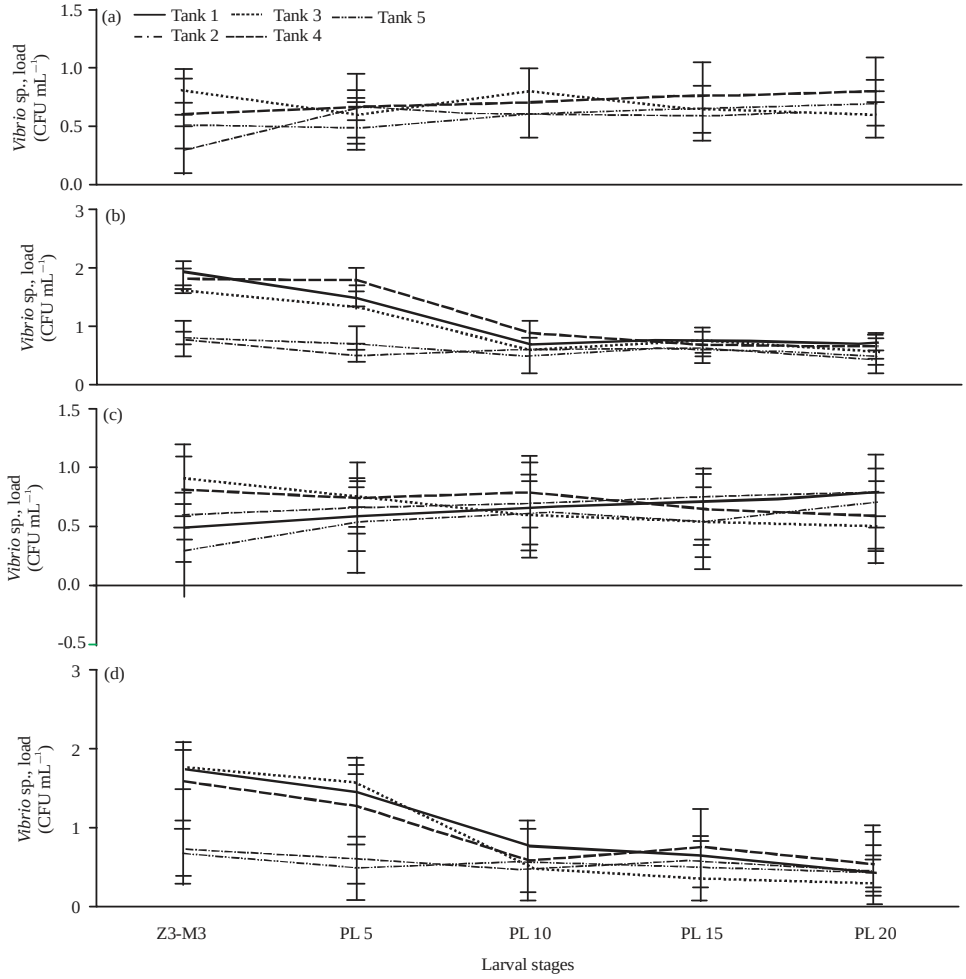

Fig. 3(a-d): Bacterial loads (*Vibriosis*) in the (a) Rearing water of *Penaeus monodon*, (b) Larvae of *Penaeus monodon*, (c) Rearing water of *Litopenaeus vannamei* and (d) Larvae of *Litopenaeus vannamei* (different stages) of both control and experimental tanks

CONCLUSION

Since, the demand for shrimp products in world markets continues to increase, the continuous supply of healthy, inexpensive and robust shrimp seed stocks to the farmers is important to maintain production of adult shrimps. Microalgae stay an important part of the aquaculture production chain particularly for hatchery shrimp feed, in spite of expensive culture installation and high production cost. From the results, it is concluded that, the use of *Cheatoceros calcitrans* and enriched *Artemia salina* will promote the successful production of shrimp larvae in hatcheries and reduce the potential negative impact of shrimp farming on the environment like, organic matter accumulation, ammonification, eutrophication and water toxicity and increase the productivity of the farms to the benefit of local economies.

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