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# Crop Rotation as a Better Sanitary Practice for the Sustainable Management of *Litopenaeus vannamei* Culture

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## ABSTRACT

In the present study, finfishes (*Mugil cephalus* and *Chanos chanos*) were cultured between crops of *Litopenaeus vannamei* shrimps for crop rotation and the reduction in the incidence of shrimp diseases was observed. The fish seeds of 5-7 g individual weight were collected from the wild and required numbers were sorted out and stocked in the ponds at the rate of 7000 and 8000 numbers of *Mugil cephalus* and *Chanos chanos*, respectively in 1.3 ha. For shrimp culture, the seeds were purchased from a reputed hatchery and were stocked at a density of 7.7 shrimps $m^{-2}$. In the control pond, the shrimps were stocked at the same density. The first crop was a fish culture experiment for 6 months followed by a shrimp culture experiment for 4½ months in one pond and the other pond was used as control and two shrimp culture experiments were carried out. During the culture period, the water quality parameters such as, temperature, salinity, pH, transparency, dissolved oxygen, ammonia, nitrite, nitrate, phosphate, silicate and the microbial load were analyzed. While comparing the performance of shrimps in the experiment pond and control pond, experimental shrimp pond yield was interesting with 695.3 kg of more production, similarly survival rate was 25% higher, Average Body Weight (ABW) was 2.9 g higher and Average Daily Growth (ADG) was 0.16 g higher for 90 days of culture. The results of the study have clearly established the advantages of crop rotation with fish than continuous culture of shrimps in the same system.

**Key words:** Shrimp culture, vibriosis, finfish culture, crop rotation, profitability

## INTRODUCTION

Aquaculture production has grown enormously in recent years and among that penaeid shrimps are one of the most important cultured species worldwide especially in Asia due to their high economic value and export (Sekar *et al*., 2014). Approximately more than 5 million Mt of shrimps are annually produced but the current global demand for both the wild and farmed shrimp is approximately more than 6.5 million Mt per annum (Karthik *et al*., 2014). To overcome this, many shrimp farms are being created throughout the world to solve this increasing food demands (FAO., 2012). However, in recent years the shrimp farming ponds in many of these countries have declined due to persistent disease problem. Rather than *White spot syndrome virus* (WSSV), the luminous bacterium *Vibrio harveyi* has been associated with many of the disease out breaks and it has been spread worldwide and caused large scale mortalities and economic loss in shrimp culture particularly in Asia (Danya and Jagadish, 2014). Due to the continuous outbreak of this

WSSV disease in *Penaeus monodon* culture leading to loss of shrimp culture in India the farmers are seriously looking for alternative shrimp species for culture. In 2008, the Coastal Aquaculture Authority of India (CAA) introduced a new shrimp species *Litopenaeus vannamei* as an alternative penaeid species in India to culture and export. The penaeid shrimp *Litopenaeus vannamei* exhibits fast growth rate and its culture period is significantly reduces compared to *Penaeus monodon*, thus the *Litopenaeus vannamei* has been established as alternative to *Penaeus monodon* to shrimp farming in several countries such as, East, Southeast and South Asia (Karuppasamy *et al*., 2013). Fisheries in the East coast of India comprising of the coastal areas of Tamil Nadu, Andhra Pradesh, Orissa and West Bengal are dominated by shrimp culture. The rapid expansion of shrimp aquaculture has resulted in serious consequences mostly related to environmental impacts and over-dependence on the use of fish meal as the main protein ingredient in shrimp feeds (Porchas Cornejo *et al*., 2011). Continuous culture of shrimp over the past few years might have caused the increase of shrimp-pathogenic *Vibrio harveyi* in the culture systems and related environments. Although, many of the farms might have employed thorough pond preparation techniques, these bacteria would have passed over into succeeding cultures as they are protected by the biofilms. Bacterial biofilms are notably resistant to drying and disinfection (Paclibare *et al*., 1998). Karunasagar *et al*. (1996) found that *Vibrio harveyi* can survive in sediments that are treated with high doses of disinfectants. Because of the difficulty in reducing the concentration of pathogenic bacteria in shrimp ponds by conventional chemical disinfection, other effective means such as biological control should be explored.

Several management strategies have been developed to minimize the impacts of shrimp aquaculture, including the use of low or zero water exchange, recirculating systems, adoption of alternative feed ingredients and feeding strategies, polyculture rotation techniques and the use of natural feed (Thakur and Lin, 2003; Casillas-Hernandez *et al*., 2007; Muangkeow *et al*., 2007; Chi *et al*., 2009; Krummenauer *et al*., 2010; Markey *et al*., 2010; Porchas Cornejo *et al*., 2011). Assuming probiotics can reduce pathogenic bacteria in shrimp ponds, the method may still not be cost-effective to small farms because high amounts of the costly probiotic products must be added to the ponds frequently. The second approach, i.e., crop rotation for disease control in shrimp cultures is not yet widely recognized although it is already an established practice in agriculture (Sieczka, 1989; Reeves *et al*., 1984; Kommendahl and Todd, 1991) and suggested for adoption in shrimp farming.

Single crop production over the year in the same field may reduce the fertility of the soil and accumulate harmful nutrients in the pond bottom. Cultivable land reduction, seasonal inundation and disease problems can be minimized by adopting fish paddy crop rotation (Roy *et al*., 2013). Crop rotation in shrimp aquaculture is worth exploring and may prove feasible in view of the recent findings on the host specificity/preference of certain strains of *Vibrio harveyi*. Liuxy *et al*. (1996) found differences in the pathogenicity of *Vibrio harveyi* isolated from penaeid and non-penaeid sources. Keeping this in mind, in the present study finfishes (*Mugil cephalus* and *Chanos chanos*) were cultured between crops of *Litopenaeus vannamei* shrimps for crop rotation and the reduction in the incidence of shrimp diseases was observed.

## MATERIALS AND METHODS

The study was carried out in a shrimp farm situated on the Southern banks of Vellar estuary (Latitude 11°29' N, Longitude 79°46" E) near Agaram, Parangipettai. This shrimp farm has two

ponds, each with Water Spread Area (WSA) of 1.3 ha. The first crop was a fish culture experiment for 6 months followed by a shrimp culture experiment for 4½ months in one pond and the other pond was used as control and two shrimp culture experiments were carried out.

**Pond preparation**

**Soil culture:** Initially the pH of the pond soil was checked and was found to be between 6.0 and 6.5. Required quantity of lime [Ca $(OH)^2$] was applied to increase the pH to 7.2 and the bottom was tilted for oxygenating. The total amount of lime applied was 600 kg per pond. After a week of soil preparation, water from Vellar estuary was pumped in with the help of a 10 HP (Kirloskar) pump.

**Water culture:** The water was pumped in directly from the source and filtered in an 80 μ mesh filter bag. Initial fertilization was done using cow dung at the rate of 100 kg $ha^{-1}$ and inorganic fertilizers in the ratio of 10:2 (N:P) for fish culture. For shrimp culture, fertilizers were not applied but dosages of dolomite were added regularly throughout the culture period. Within a period of one week, plankton development could be observed, mainly represented by diatoms.

**Stocking:** The fish seeds of 5-7 g individual weight were collected from the wild and required numbers were sorted out and stocked in the ponds at the rate of 7000 and 8000 numbers of *Mugil cephalus* and *Chanos chanos*, respectively in 1.3 ha. For shrimp culture, the seeds were purchased from a reputed hatchery and were stocked at a density of 7.7 shrimps $m^{-2}$. In the control pond, the shrimps were stocked at the same density.

**Feeding:** Initial feeding was done using rice bran and groundnut oil cake and during the later stages, a commercial fish feed (Higashimaru feeds India Ltd.) was used. The feed was given at 5% of the body weight regularly. During the early stages, bottom algae and animalcules (Lab lab) were allowed to grow, as they serve as an important natural food to the milk fishes. For shrimp culture, feeding was done using CP feed (Charoen Pokhpand Aquaculture India Pvt. Ltd., Chennai). The feeding schedule was based on the feed chart provided by the manufacturing company. Blind feeding was done for the first 30 days. Later, the feeding was adjusted based on the check tray observation and sampling. Four check trays/pond were installed. The daily feed ration was divided for 4 feedings, 25% for morning (6.00 am), 20% for noon (12.00 pm), 30% for evening (6.00 pm) and 25% for night (1.00 pm) feeding, respectively. The feed was broadcast from the dyke during the initial phase and boat feeding followed during the later stages. The same practice was followed in the control pond also.

**Sampling:** Sampling was done every fortnight for fish culture and once in 10 days for shrimp culture, during early hours of the day with a cast net. Five hauls were made in the pond. The animals caught per haul and their individual weights were recorded. Healthiness, survival rate, Average Body Weight (ABW) and Average Daily Growth (ADG) of the animals were estimated through the samples. The diameter of the cast net used for sampling was 3.3 mts. The area of the net was calculated with 60% efficiency of coverage at the bottom.

**Water exchange:** Water exchanges were done at the rate of 10-15 cm $day^{-1}$ on a weekly basis for both the cultures and the control experiments.

**Water quality assessment:** The physicochemical parameters monitored in the present investigation included temperature, salinity, pH, transparency, dissolved oxygen, ammonia, nitrite, nitrate, phosphate and silicate by standard methods (Strickland and Parsons, 1972).

**Microbial analysis:** In this study, sediment and water samples from fish, shrimps and the control experiments separately, were analyzed. Water and sediment samples were taken from the pond with the help of presterilized bottles and polythene bags. The colleted samples were transferred to the laboratory immediately and analyzed within an hour of collection to avoid possible contaminations. Five tube three dilution MPN (Most Probable Number) method was followed for all the samples. For water sample, 10 mL of unfiltered water was transferred to double strength Alkaline Peptone Water (APW). Then, 1 and 0.1 mL aliquots were transferred to a single strength APW. For analysis of sediment samples, approximately 10 g of sediment was added to 90 mL of water blank and from the water blank 10, 1 and 0.1 mL aliquots were transferred to double strength and single strength APW, respectively (Five dilution in five tube method) (Food and Drug Administration, 1984) (FDA bacteriological manual).

**Biometric studies:** In the present study, characters of *Penaeus monodon* of cultured and wild males and females were studied separately by all possible combinations using the linear regression techniques and correlation coefficient.

## RESULTS AND DISCUSSION

In general, the difficulty in reducing the concentration of pathogenic bacteria in shrimp ponds by conventional chemical disinfection, other effective means such as biological control should be explored. The additions into the environment of bacterial microorganisms that serve as antagonists of the target pathogens and the manipulation of the environment in such a way that beneficial microorganism are favored to proliferate. Examples of these approaches are the use of probiotics and crop rotation, respectively. Crop rotation is a type of "sanitation" practice and is an integral part of plant health management. A sanitation practice should reduce the initial inoculum to a sufficiently low level so that the normal development of disease will not reach a high level to cause appreciable yield loss, provided unusual influx was avoided (Berger, 1977).

**Water quality assessment:** In fish culture, the water quality can be defined as the suitability of water for the survival, growth and production of fish. Hence to ensure a good water quality, the water quality parameters were monitored and accordingly, regular water exchange was done during the present experiment.

**Temperature:** Temperature was found to vary between 28.5 and 32.4°C and 24.3 and 28.1°C during fish and shrimp culture, respectively. It ranged between 29.6 and 32.9°C and 25.6 and 29.9°C in control ponds (experiment 1 and 2), respectively (Fig. 1-2). The water temperature is a major environmental factor that affects the growth and survival of any aquatic organism. In the present fish culture experiment, the water temperature of 28.5-32.4°C had no harmful effect. In the case of warm water fishes the maximum metabolic activity was seen at 30-35°C (Beamish and Dickie, 1967). In the light of this, temperature was favourable for the stocks in the pond with minor variations between unit times.

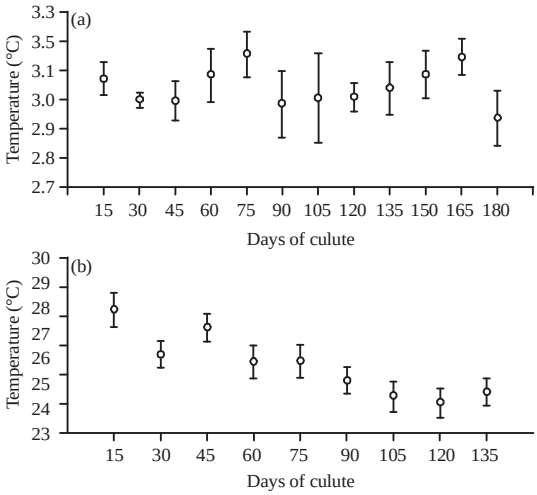


Fig. 1(a-b): Variations in temperature during (a) Fish culture experiment and (b) Shrimp culture experiment

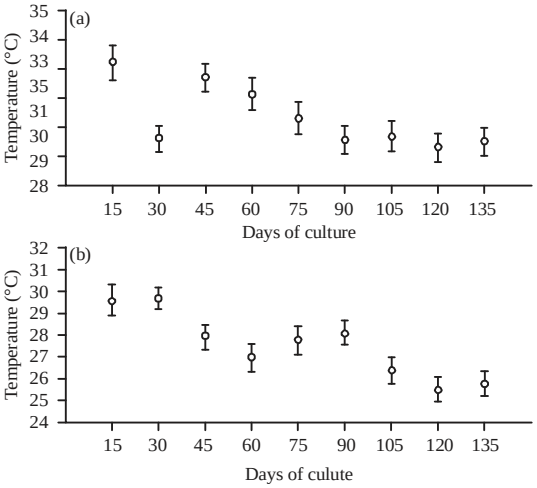


Fig. 2(a-b): Variations in temperature during shrimp culture control (a) Experiment 1 and (b) Experiment 2

**Salinity:** During fish culture, salinity ranged from 26-42 ppt. the same ranged between 10-19 ppt during the shrimp culture experiment. In the control experiments 1 and 2, it ranged from 28.7-44 and 12-21 ppt, respectively (Fig. 3-4). The master environmental factor of salinity, during the experiment was ranging from 26-42 ppt. In spite of salinity values higher than 35 ppt in certain occasions, there was no adverse effect since the mullets and milkfishes can tolerate wide range of salinities as euryhaline species (Bardach *et al*., 1972). The food intake, growth rate, food conversion and protein synthesis have been found to be affected by ambient oxygen level (Medale, 1985).

**Transparency:** Transparency reduced from 95-55 cm during fish culture experiment and 95-58 cm during shrimp culture experiment. In control experiment 1, it reduced from 90-49 cm and in control experiment 2, it reduced from 85-55 cm (Fig. 5-6).

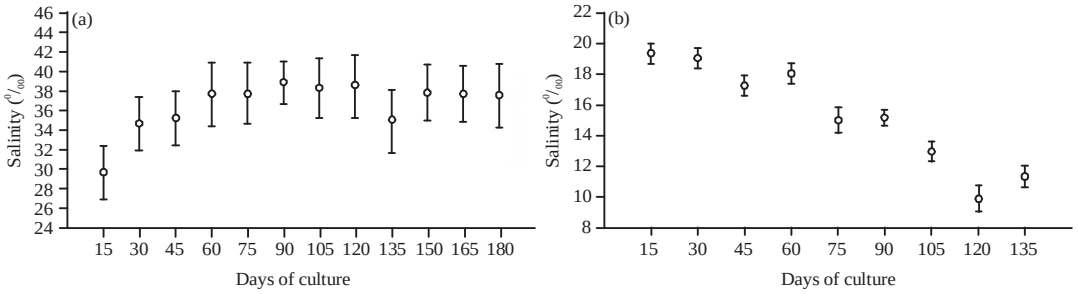


Fig. 3(a-b): Variations in salinity during (a) Shrimp culture experiment and (b) Fish culture experiment

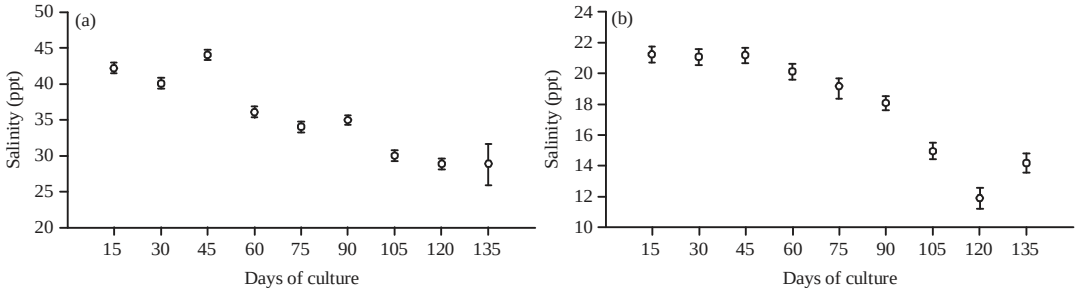


Fig. 4(a-b): Variations in salinity during shrimp culture control (a) Experiment 1 and (b) Experiment 2

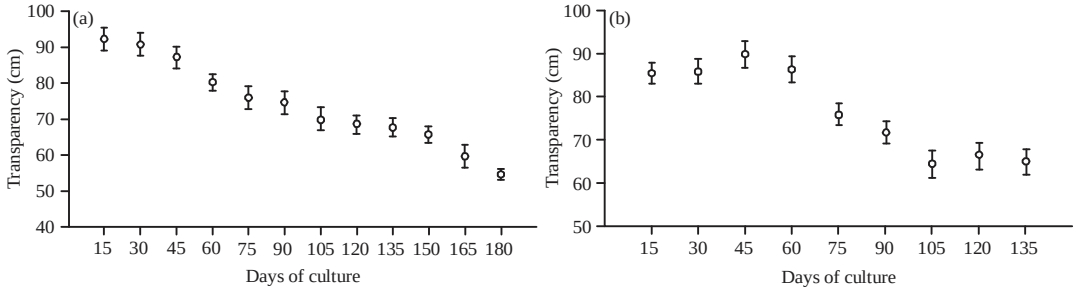


Fig. 5(a-b): Variations in transparency during (a) Fish culture experiment and (b) Shrimp culture experiment

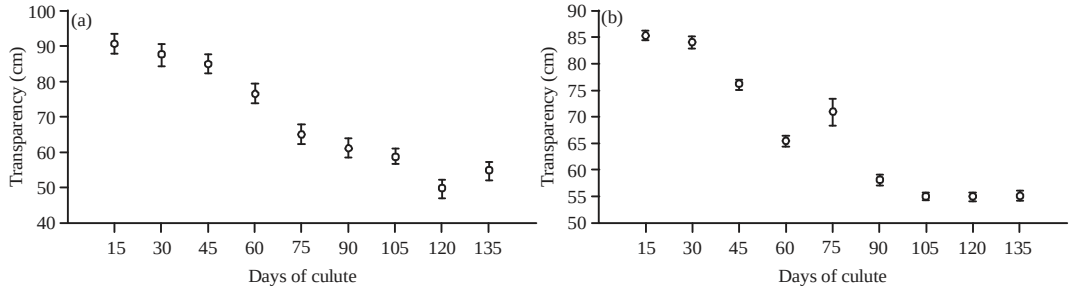


Fig. 6(a-b): Variations in transparency during shrimp culture control (a) Experiment 1 and (b) Experiment 2

**Dissolved oxygen:** The dissolved oxygen content ranged from 4.1-6.2 mg $L^{-1}$ during fish culture and 4.2-6.1 mg $L^{-1}$ during shrimp culture. In the control experiments, the values were from 4.2-5.9 and from 4.6-6.1 in 1 and 2, respectively (Fig. 7-8).

**pH:** The pH values ranged between 7.2 and 8.1 during fish culture and 7.1 and 8.6 during shrimp culture. In the control experiment 1, the values varied between 7.6 and 8.4 and in the control experiment 2, it ranged between 7.5 and 8.3 (Fig. 9-10). In the present experiment, the pH varied from 7.2-8.1, which had positive effect on the activity of the fishes, as also reported by Huet (1975) who observed that the pH range of 7.0-8.0 (neutral or slightly alkaline) is best for fish culture. The present finding is in close agreement with those of Huet (1975).

**Ammonia:** The values of ammonia during fish culture increased from 0.30-1.18 ppm. During shrimp culture, the values increased from 0.52-1.03 ppm. The values ranged from 0.61-1.13 ppm and from 0.70-0.99 ppm in the control experiments 1 and 2, respectively (Fig. 11-12).

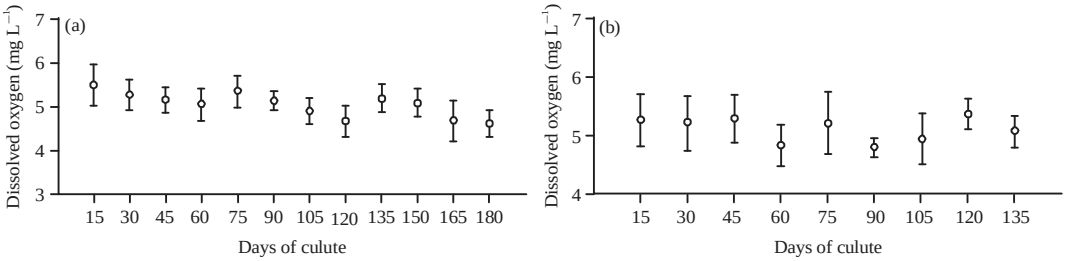


Fig. 7(a-b): Variations in dissolved oxygen during (a) Fish culture experiment and (b) Shrimp culture experiment

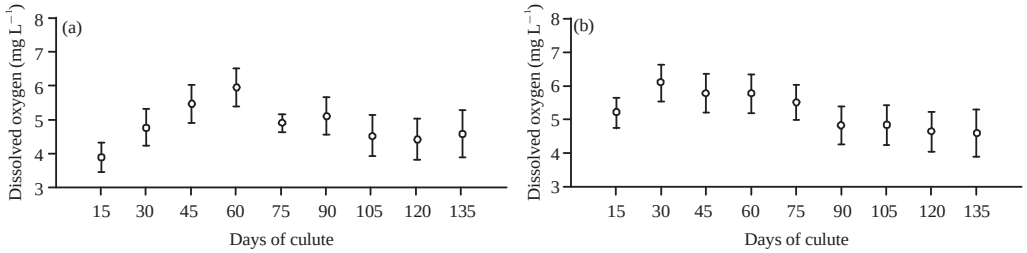


Fig. 8(a-b): Variations in dissolved oxygen during shrimp culture control (a) Experiment 1 and (b) Experiment 2

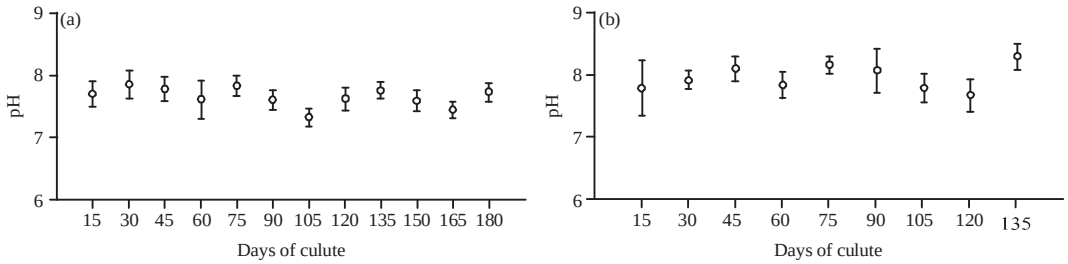


Fig. 9(a-b): Variations in pH during (a) Fish culture experiment and (b) Shrimp culture experiment

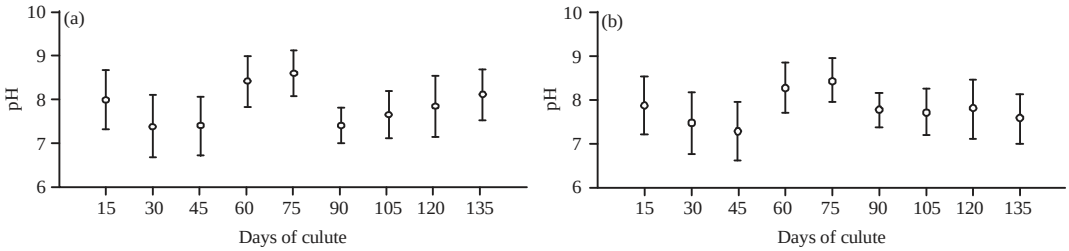


Fig. 10(a-b): Variations in pH during shrimp culture control (a) Experiment 1 and (b) Experiment 2

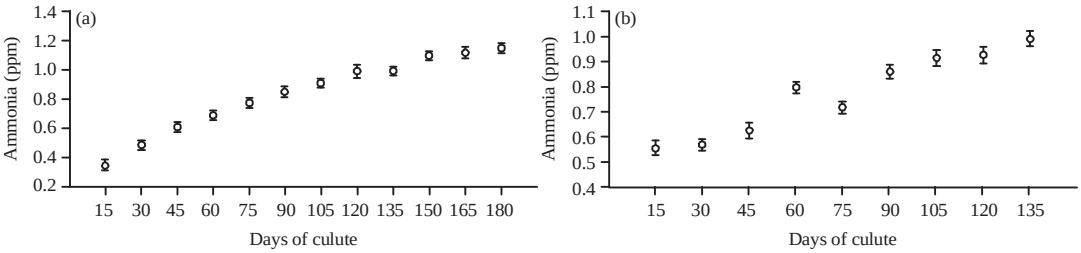


Fig. 11(a-b): Variations in ammonia during (a) Fish culture experiment and (b) Shrimp culture experiment

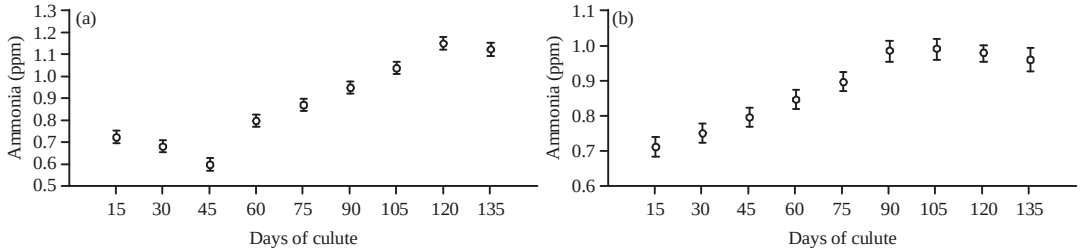


Fig. 12(a-b): Variations in ammonia during shrimp culture control (a) Experiment 1 and (b) Experiment 2

**Nitrite:** During fish culture, the values of nitrite ranged from 0.0022-0.0170 ppm and the values were from 0.0024-0.0119 ppm during shrimp culture. In the control experiments 1 and 2, the values were between 0.0026 and 0.0123 ppm and from 0.0034-0.0131 ppm, respectively (Fig. 13-14).

**Nitrate:** Nitrate values ranged between 0.0035 and 0.0157 ppm during fish culture and 0.0035 and 0.0125 ppm during shrimp culture. The values were from 0.0039-0.0130 ppm and from 0.0029-0.0136 ppm in control experiments 1 and 2, respectively (Fig. 15-16).

**Phosphate:** The values of phosphate ranged from 0.0017-0.0116 ppm during fish culture and the same fluctuated between 0.0030 and 0.0098 ppm during shrimp culture. In the control experiment 1, the values ranged from 0.0028-0.0097 ppm and in the control experiment 2, the values were between 0.0031 and 0.0102 ppm (Fig. 17-18).

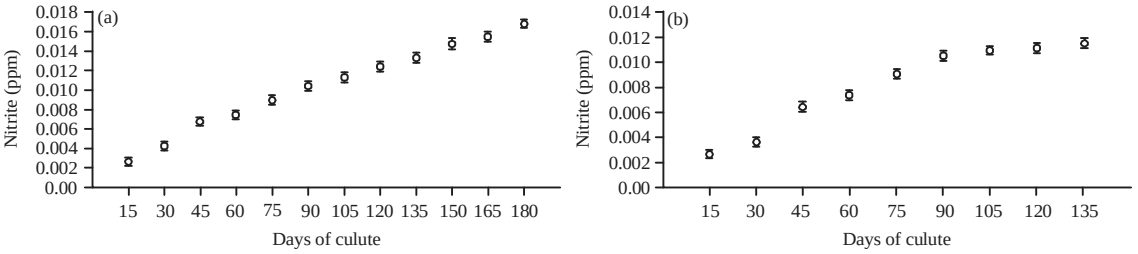


Fig. 13(a-b): Variations in nitrite during (a) Fish culture experiment and (b) Shrimp culture experiment

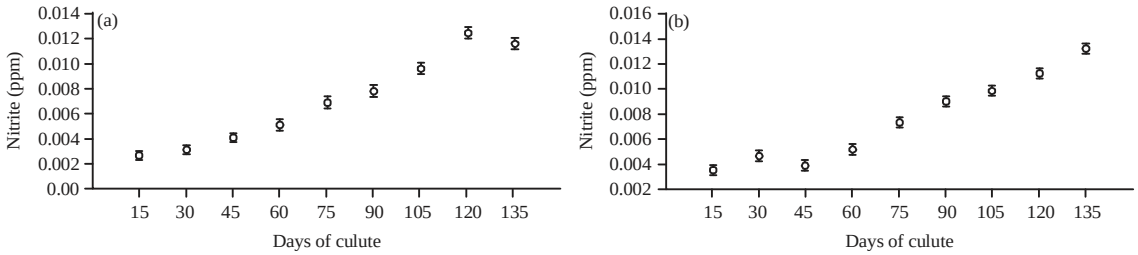


Fig. 14(a-b): Variations in nitrite during shrimp culture control (a) Experiment 1 and (b) Experiment 2

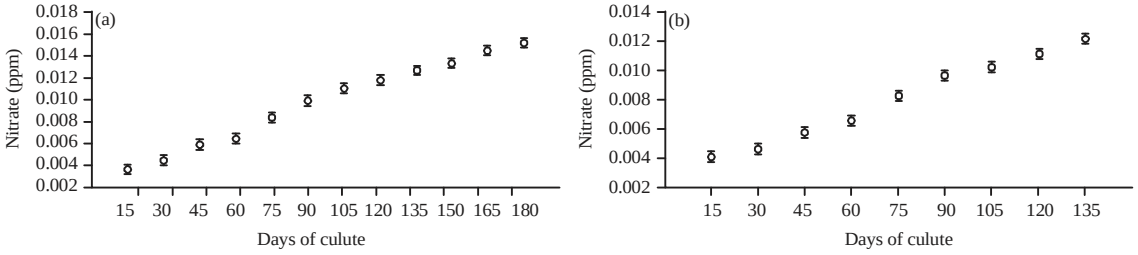


Fig. 15(a-b): Variations in nitrate during (a) Fish culture experiment and (b) Shrimp culture experiment

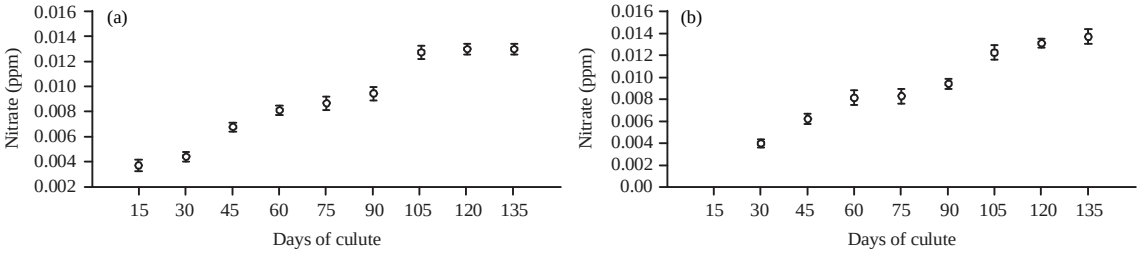


Fig. 16(a-b): Variations in nitrate during shrimp culture control (a) Experiment 1 and (b) Experiment 2

**Silicate:** Silicate ranged between 0.0042 and 0.0128 ppm during fish culture and 0.0065 and 0.0130 ppm during shrimp culture. The values were from 0.0061-0.0134 and 0.0059-0.0129 ppm in the control experiments 1 and 2 (Fig. 19-20).

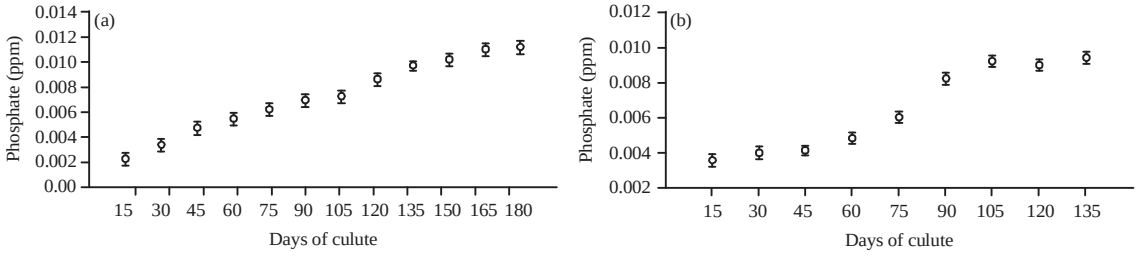


Fig. 17(a-b): Variations in phosphate during (a) Fish culture experiment and (b) Shrimp culture experiment

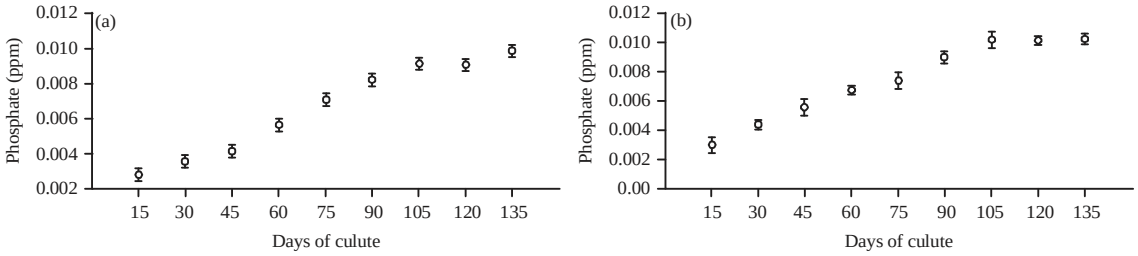


Fig. 18(a-b): Variations in phosphate during culture control (a) Experiment 1 and (b) Experiment 2

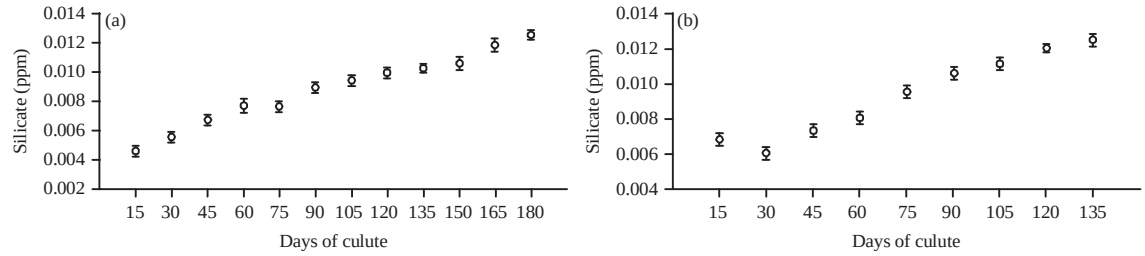


Fig. 19(a-b): Variations in silicate during (a) Fish culture experiment and (b) Shrimp culture experiment

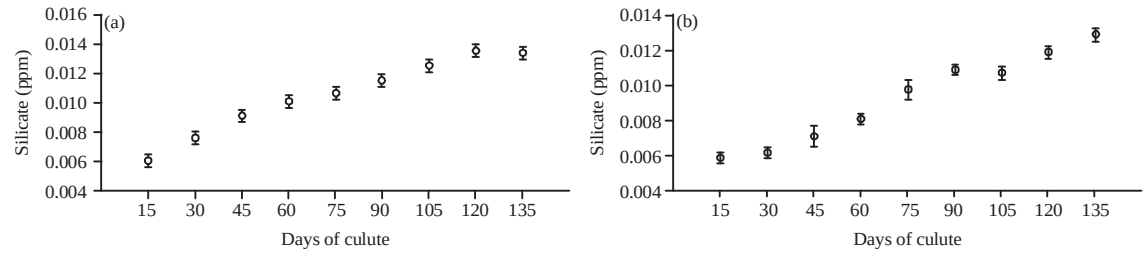


Fig. 20(a-b): Variations in silicate during shrimp culture control (a) Experiment 1 and (b) Experiment 2

**Microbial analysis:** The results from the microbial analysis showed that the population of sucrose positive bacteria both in the water and sediment samples increased during the fish culture experiment ($3.3\times10^2$-$1.1\times10^3$ MPN/100 mL and $3.8\times10^2$-$1.4\times10^3$ MPN/100 mg in water and

sediment, respectively). The sucrose negative bacterial population was more or less stable throughout the culture period (2.6×102-4.6×102 MPN/100 mL and $5.6\times10^2$-$8.4\times10^2$ MPN/100 mg in water and sediment, respectively). During the shrimp culture experiment, there was a gradual reduction in the population of sucrose positive bacteria ($4.5\text{x}10^2$-$3\times10^2$ MPN/100 mL and $6.2\times10^2$-$3\times10^2$ MPN/100 mg in water and sediment samples, respectively) and an increase in the population of sucrose negative bacteria ($3.3\text{x}10^2$-$1.1\times10^3$ MPN/100 mL and $4.6\times10^2$-$1.3\times10^3$ in water and sediment samples, respectively). In the control experiment 1, the population of sucrose positive bacteria in the water sample was found to be reducing from $3.2\times10^2$-$2.3\times10^2$ MPN/100 mL, whereas the population of sucrose negative strain increased from $4.3\times10^2$-$1.4\times10^3$ MPN/100 mL. In sediment samples, the sucrose positive bacterial populations ranged between $2.4\times10^2$ and $3.4\times10^2$ MPN/100 g. The sucrose negative populations increased from $4.8\times10^2$-$1.5\times10^3$ MPN/100 g. In the control experiment 2, the population of sucrose positive bacteria in the water sample was found to be reducing from $3.4\times10^2$-$2.4\times10^2$ MPN/100 mL, whereas the population of sucrose negative strain increased from $4.8\times10^2$-$8.6\times10^2$ MPN/100 mL. In sediment samples, the sucrose positive bacterial populations ranged between $2.6\times10^2$ and $3.6\times10^2$ MPN/100 g. The sucrose negative populations increased from $6.1\times10^2$-$1.6\times10^3$ MPN/100 g. The control experiment 2 was terminated on 96th DOC due to high mortalities due to vibriosis.

The microbial analysis during the present study indicates that there was an increase in the population of sucrose positive bacteria, mainly *Vibrio*, during the fish culture experiment, which may be attributed to the addition of carbon rich diets, such as rice bran and groundnut oil cake and in the case of the shrimp culture experiment, the populations of sucrose negative bacteria increased. In the experimental pond 2, the pathogenicity of sucrose negative strains went to the extent of termination of the experiment. Paclibare *et al.* (1998) stated that *Vibrio harveyi* has two major biotopes, namely the sucrose positive and sucrose negative forms. Most of the pathogenic strains of *V. harveyi* of shrimp are sucrose negative while the sucrose positive strains are benign and even used as probiotics (Owens *et al*., 1996). Paclibare *et al*. (1998) observed that the sucrose positive vibrios usually dominate ponds of healthy tilapia and also noted that, crop rotation of shrimps and tilapia reduces disease incidence in shrimp culture. The greater phylogenetic differences between the culture organisms used in crop rotation, the better sanitary effects have been experimented (Francis and Clegg, 1990). In this experiment, the shrimp crop rotated with mullets and milkfishes, which belong to different orders within the animal kingdom, resulted in rewarding crop, in the same culture system.

**Performance of the cultured organisms:** In the crop rotation experiment, the total production of fishes (*Mugil cephalus* and *Chanos chanos*) was 1308.6 kg in 180 days. The ABW of *Mugil cephalus* and *Chanos chanos* was 108 and 153 g, respectively. In shrimp culture in the same pond after fish culture, the production of shrimp was 2557.5 kg with a survival of 75% in 130 days with an ABW and ADG of 34.1 and 0.26 g, respectively. In the control pond (continuous shrimp culture), shrimp production during the first and second crops was 1696.5 and 1043.1 kg, respectively with a corresponding survival rate of 56 and 57%. However, in the control pond, during the second crop, culture could not be extended beyond 90 days, due to bacterial problems. Motoh (1981) found 64% survival rate of *P. monodon* at the stocking density of 32,000 $ha^{-1}$ of 105 days culture and the culture system was improved extensive. Recently, Roy *et al.* (2013) reported that, the average survival rate of *P. monodon* in all the ponds under T1 and T2 was 56.30 and 55.20%, *Macrobrachium rosenbergii* was 71.35 and 69.95% and *Catla catla* was 75.5 and 75.20%, respectively when assessing the crop rotation practices.

The performance of shrimp in experimental pond, after the crop of fish culture, was remarkably good. While, comparing the performance of shrimps in the experiment pond and control pond, experimental shrimp pond yield was interesting with 695.3 kg of more production, similarly survival rate was 25% higher, ABW was 2.9 g higher and ADG was 0.16 g higher for 90 days of culture. These results clearly establish the advantages of crop rotation with fish than continuous culture of shrimps in the same system. Further, it is to be stressed that in the experimental pond, shrimp culture was possible up to 130 days due to previous fish culture, while, the shrimp crop could not continue beyond 90 days in the continuous shrimp culture pond due to severe bacterial disease and mortality.

## CONCLUSION

Crop rotation in shrimp aquaculture is worth exploring and may prove feasible in view of the recent findings on the host specificity/preference of certain strains of *Vibrio harveyi*. The results of the study clearly establish and concluded that the advantages of crop rotation with fish than continuous culture of shrimps in the same system. Further, it is to be stressed that in the experimental pond, shrimp culture was possible upto 130 days due to previous fish culture, while, the shrimp crop could not continue beyond 90 days in the continuous shrimp culture pond due to severe bacterial disease and mortality.

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