IMPACT OF HETEROTROPHIC MICROBES AND UNIALGAL CONCENTRATE ON RACEWAY SYSTEM MANAGEMENT IN NURSERY PRODUCTION OF PENAEID SHRIMP

Thesis submitted in part fulfilment of the requirements for the degree of Master of Fisheries Science *in Aquaculture to the*
Tamil Nadu Veterinary and Animal Sciences University, Chennai.

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CERTIFICATE

This is to certify that the thesis entitled “Impact of heterotrophic microbes and unialgal concentrate on raceway system management in nursery production of penaeid shrimp” submitted in part fulfilment of the requirement for the award of the degree of Master of Fisheries Science in Aquaculture to the Tamilnadu Veterinary and Animal Sciences University, Chennai is a record of bonafide research carried out by Thiru. **HENRY RAJAR DINESH. S** under my supervision and guidance and that no part of this thesis has been submitted for the award of any other degree, diploma fellowship or similar titles. However, as per our University’s regulations, a portion of the thesis has been sent for publication in a peer reviewed journal and a copy of the manuscipt is enclosed

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LIST OF FIGURES

4.16.	Percentage composition of Gram +ve bacteria in raceway -2	
4.17.	Percentage composition of Gram -ve bacteria in raceway -2	
4.18.	Percentage of total species composition in raceway - 2	

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S. Henry Rajar Dinesh

Dedicated to ...

My Beloved Parents

ABSTRACT

Title	:	**"Impact of heterotrophic microbes and unialgal concentrate on raceway system management in nursery production of penaeid shrimp"**
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The effect of unialgal concentrate and heterotrophic microbial load on bioremediation with respect to ammonia control and overall growth and production of penaeid shrimp was assessed in the raceway farm complex established in Fisheries College and Research Institute, Thoothukudi. The trials were carried out with the shrimp species of *Penaeus monodon* and *Fenneropenaeus indicus.*

In trial 1 the raceways were stocked with *P. monodon* at two different stocking densities viz. 2000/m^3 and 1000/m^3. The final mean weight after 46 days rearing were 0.413 and 0.644 g for the stocking densities of 2000/m^3 and 1000/m^3 respectively. Survival, FCR, total biomass and specific growth were 51.6%, 1.68, 0.718 kg/m^3 and 0.131 respectively for the stocking densities of 2000/m^3, while for the stocking density of 1000/m^3, it recorded 82.0%, 1.79, 0.427kg/m^3 and 0.109 respectively. With regard to ammonia control, the increased algal density and microbial load established positive results By

maintaining the algal density (by addition of algal concentrate periodically) and microbial supplement (by fermentation) the ammonia level could be controlled within 0.06mg/l.

In trial 2, the raceways were stocked with *F. indicus* at two different stocking densities viz. 333/m^3 and 666/m^3. Nursery rearing was carried out for 53 days and the mean final weight obtained were 2.23 and 1.47g for the stocking densities of 333/m^3 and 666/m^3 respectively. Survival, FCR, total biomass and specific growth were 80.95%, 1.73, 0.60kg/m^3 and 0.053 respectively for the stocking densities of 333/m^3, while for the stocking density of 666/m^3, it recorded 61.1%, 1.87, 0.74kg/m^3 and 0.40 respectively. With regard to bioremediation, the lowest ammonia level noted was 0.13 mg/l, when the algal concentration and microbial load recorded the highest levels viz. 7.6 x 10^4 cells/ml and 5.9 x 10^4 respectively. The reduced levels of algal concentration and microbial load resulted in increased ammonia level. It was confirmed that the ammonia level was significantly reduced ($p>0.05$) with the increase of algal concentration and microbial load. The bacterial isolates identified in raceway include *Bacillus, Micrococcus, Staphylococcus, Arthrobacter, Lactobacillus, Vibrio* and *Pseudomonas* with the domination of *Bacillus* and *Vibrio.*

I. INTRODUCTION

Shrimp aquaculture is gaining considerable importance all over the world and it is considered to be an economically viable aquaculture sector in India among the various other aquaculture practices. However, several factors have limited the growth and expansion of the pond-based shrimp-farming sector in our country. One of the major problems facing the shrimp aquaculture sector is the poor survival of shrimp and production predictability when juvenile shrimps are stocked into growout ponds. Further, extensive and semi-intensive pond production systems are typically managed with high rates of water exchange and this production method raises environmental concerns regarding effluent discharge into receiving waters.

Another major concern is the limited growing season often found in temperate and subtropical regions. This factor restricts the overall production and usually allows only one crop per year. To cap it all several epizootic viral disease outbreaks have caused major damages to pond-reared shrimp allover the world. Such losses can be minimized through the adoption of technologies that enhances biosecurity and environmental control. Despite continuing problems with disease outbreaks and environmental concerns over effluent pollution and land usage, shrimp farming all over the world continue to expand.

In response to the above problems a management strategy has been developed to increase juvenile shrimp survival and production predictability in cost effective, indoor system capable of growing shrimp at moderately high

densities. Some recent research efforts in the US have been focusing on the use of biosecured green house enclosed raceways for intensive to super-intensive shrimp production that make efficient use of space, energy and labour. One such system is the raceways, which can be managed with zero to minimal water exchange thus greatly reducing the environmental impact due to effluent discharge and environmental regulation and user conflicts of coastal land and water can be addressed more effectively. Reduced water exchange not only minimize the potential negative environmental impact from effluent water, but also reduce the risk to their cultured stocks from contaminated incoming water. Biosecurity protocols also can be implemented to manage disease vectors. Green house enclosed systems also provide opportunities for inland culture operations using subsoil saline water and year round production can be achieved.

Recent studies have been focused on to develop a biosecure shrimp production system for nursery and grow out practices that lower the risk of viral disease outbreaks in cultured stocks while reducing the potential negative impacts from shrimp farm effluent waters. With biosecure zero water exchange systems, shrimp farmers have an opportunity to introduce a defined microbial community and specific algae such as *Chaetoceros spp.*, *Chlorella spp.*, etc. in to culture environment to work synergistically with the shrimp to maximize system productivity and stability.

Healthy algal bloom of marine diatoms is the preferred community in the system. It can also improve the shrimp growth performance by shading the bottom and preventing microalgae growth, removing toxic nitrogenous compounds, increasing shrimp appetite and probably by providing some undefined nutritional components. Diatom bloom is also beneficial for post larval growth and survival. Further, they improve the water quality by maintaining oxygen and pH in optimum level and ammonia level in control. Apart from that *Chaetoceros spp.,* also can be used to buffer against pH changes and mitigate potential ammonia toxicity.

Shrimp aquaculture operations generate metabolic waste products such as faeces, ammonia, uneaten feed etc. and this process put the system under continous stress. This would pave way for deteriorations in water quality, predisposing the shrimp stock to be attacked by pathogens. Among the various water quality parameters, ammonia is highly toxic to shrimp even at low levels. In high-density shrimp cultured ponds, ammonia is oxidized by biological filters to nitrite and to nitrate, which is virtually non- toxic.

Microorganisms are known to play an important role in nutrient recycling in any aquatic environment. Appropriate applications of microbes were shown to improve intestinal microbial balance,thus leading to improved food absorption and reduced pathogenic problems in the gastrointestinal tract. The bioremediation protocol includes mass production and application of appropriate culture, aeration during night, maintanence of good algal phytoplankton bloom

and monitoring ammonia and nitrite level. Bioremediation coupled with the introduction of closed culture system will be the next phase of development in the aquaculture industry world over.

In shrimp culture ponds, ammonia content is principally governed by the activities of algae and bacteria. Further, bacteria contribute significantly to the food web through the activities of the heterotrophic decomposers, enabling nitrogen and phosphorus to be recycled to stimulate primary production. Therefore managing microbial and algae composition within the conducive and optimum level is paramount important for the successful raising of shrimp crops in raceway system.

The present study was carried out with the following objectives :

i) To study the effect of microbial composition on the raceway system, for raising juvenile penaeid shrimp.

ii) Qualitative impacts of added unialgal concentration on the water quality management of raceway system.

iii) Performance of nursery production of penaeid shrimps in terms of growth, survival and health status.

iv) To assess the combined effects of unialgal concentration, microbial supplements, water quality parameters on the nursery production of penaeid shrimps in raceways.

II. REVIEW OF LITERATURE

2.1. Working principle of raceway culture system

Raceway system is described as a culture system with a high stocking density with zero or limited water exchange that ensures the biosecurity (Sturmer *et al*., 1992). Boyd and Clay (2002) recorded the basic principles and individual practices in the raceway systems which includes zero water exchange, lined ponds, mechanical aeration, water recirculation, sludge removal, high health shrimp and low protein feed. Samocha *et al.* (1993) provided the detailed description of the shrimp raceway system with bioremediation using diatoms.. Davis and Arnold (1998) described that raceways are ultra-intensive systems capable of supporting extremely high standing crops. Van Wyk (1999) found that raceway system obtain greatest profit making potential to those who utilize capital, labour and other inputs more efficiently.

For a better-controlled raceway system, particularly in temperate areas use of green house – enclosed intensive nursery system can facilitate production of more crops per year (Sturmer *et al*., 1992; Samocha and Lawrence, 1992). Rijn and Shilo (1989) demonstrated that the raceway system allows control over site limitation, improved environmental control, increased product availability and quality and facilitate the control of stock and effluent management. Moss (1994) developed a prototype of biosecure system for the intensive production of *L. vannamei.* Leung and Moss (1999) and Ogle and Lotz (1998) demonstrated that the major reason for the success of the raceway system is the high degree of biosecurity. Samocha *et al*. (2002) suggested that the losses due to

environmental degradation could be minimized through the adoption of raceway technology. He also noted that production of shrimp with reduced water exchange is another promising method to minimize monetary losses. Hopkins *et al*. (1995) stated that high shrimp yield was achieved without water exchange. Samocha *et al.* (2002) showed that the raceway system could support biomass load of *L. vannamei* and recorded survival rates as high as 90%. Michael and Andrews (1997) described the advantages of raceway system as i) higher stocking densities, ii) improved water quality, iii) reduced manpower, iv) ease of grading, v) ease of harvest and vi) precise health management.

Macia (1983) reported that pond flushing (exchange) normally remove phytoplankton, nitrifying bacteria and natural productivity that could have otherwise benefited the pond water quality. Hopkins *et al.* (1993) reported that reducing water exchange is possible without negatively affecting the shrimp growth and survival.

2.2. Description of raceway system with respect to size

Samocha *et al*. (2002) conducted trials with *L. vannamei* in raceways with three different sizes via 25,35 and 72m^3. He also used 10m^3 raceways for nursery rearing of *L. vannamei* with the components of settling box with foam fractionation, biological filter and secondary settling area. Arnold *et al.* (1990) described two different systems for the cultures of *L. vannamei* were 38m^3 (13.7 x 2.4 x 1.6m) and 28m^3(13.7mx2.4mx0.85m) for nursery rearing and 80m^3 (27.4x2.9x1.0m) for grow out phase. In 1998, Harbour Branch Oceanographic

Institution, USA set up two prototyped green houses measuring 9.2m^2 and 29.2 m^2 respectively. Each green house enclosed two 70.7 m^3 (4.4m x 26.8m x 0.60m) culture tanks, which occupied 90% of the area enclosed by the green houses.

2.3. Nursery rearing of penaeid shrimp

A culture system that incorporates only one transfer of shrimp from the nursery to the grow out pond is referred to as a two-phase grow out system (New and Rabanal, 1985; Lawrence and Huner, 1987; Felix and Samocha, 2000). The use of nursery is more common with semi-intensive and intensive farming system (Samocha and Lawrence, 1992). The nursery rearing technique for shrimp have been well studied by Samocha *et al.* (2002). Shrimp culture systems with a nursery phase increases control over to stock inventories, water quality and feed management (Samocha and Benner, 2001). Shrimp nursery systems provide for larger and harder shrimp to be stocked and facilitate a high number of crops per pond as a shorter period is needed to reach marketable size (Briggs and Brown, 1991; Fast, 1991). Use of nursery system can also prevent the spread of diseases as quarantine in nursery (Samocha *et al*., 2000 a; Samocha *et al*., 2001) and can also stock and maintain PLs in high density to reduce pressure on limited hatchery supplies (Samocha and Benner, 2001).

New and Singholka (1982) observed the advantages of nursery rearing. It include i) to obtain larger stocking materials, ii) to attain better survival in the grow out, iii) to utilize the feed efficiently in the nurseries through high stocking densities, iv) to detect the defects of stocking materials early, v) better size

uniformity, vi) better utilization of farm infrastructure, vii) improved risk management particularly biosecurity, viii) to produce stronger PL and ix) reduced feed wastage

2.4. Stocking density

A number of studies are available on the effects of stocking density for penaeid species (Ravichandran *et al.,* 1982). Increasing the stocking density of crustaceans in ponds usually cause problems with water quality and sediment deterioration (Aunimelech *et al.,* 1981; Wyban and Sweeney, 1989). Increasing density also increases the susceptibility of shrimp to diseases (Donbrousky *et al.,* 1988). In addition, increasing the density raised pressure on natural food resources (Hopkins *et al*., 1988) and reduced food conversion efficiency, which raised the total food costs. (New, 1987). The stocking densities of post larvae in the nursery phase varied from 350/m^2 to 1500/m^2 in different parts of the world (New, 1988). According to Parameswaran *et al*. (1992) there is no significant differences in growth and survival when stocking densities were maintained at 200, 300 and 400/m^2. Smith and Sandifer (1979) reported that for short term (30 days) holding of post larvae, densities above 2000 number / m^2 and for longer period of 60-90 days, lesser density (1000-1500/m^2) were reported to be reasonable . But, at density of 2000 PL/m^2, the growth and survival were not found to be affected up to 60 days when sufficient food, dissolved oxygen were made available (Sebastian *et al.,* 1992).

2.5. Growth and survival of penaeid species in raceway culture system

The overall survival in shrimp nursery system in Alabama was 75% (Moss 1994), in Latin America was 70-80% (Stern and Letellier, 1992) and in Texas was 90%(Samocha and Lawrance, 1992). Antonia *et al*. (2004) reported that the individual weight after 10 and 20 days nursery rearing was 0.018 g and 0.038 g respectively. CICTUS (1983) reported the mean weight after 20 days in nursery was 0.01-0.02 g. Growth and survival, which determine the ultimate yield, were influenced by a number of ecological parameters and managerial practices (Subrahmaniyam, 1973). Williams *et al*. (1996) reported an inverse linear relationship between stocking density and growth of *L. vannamei* and *L.setiferus* grown in an indoor recirculating system, and an inverse relationship between stocking density and survival of *L. setiferus.* Similarly Davis and Arnold (1998) recorded a negative effect of stocking density on growth and survival of *L. vannamei* reared in a recirculating raceway and Tseny *et al*. (1998) observed a similar effect of density on growth and survival of *P. monodon* in a recirculating system.

2.6. Heterotrophic microbes in raceway system

Studies have been carried out in shrimp culture pond waters for their heterotrophic bacterial count (Patrick 1978; Qing Yun *et al*., 1991). Similar works have also been done on the shrimps, cultured in Brazoria, Tex, (Vanderzant *et al*., 1973), and also in India (Hameed, 1993). Prem *et al.* (1996) reported that the maximum number of total heterotrophic bacteria in water was 3.22×10^5 cfu/ml.

Nwadiuto and Koske (2003) found that the bacterial load was lower in culture water than in shrimp gut. Jean *et al*. (1999) found higher aerobic bacteria in larval rearing phase using live prey feeding. Janahiram *et al*. (2000) reported the increase of bacterial count with respective increase of culture periods.

2.7. Microbes as bioremediating agent in raceway management

Bioremediation is a novel potent biotechnological approach which involves reduction of hazardous organic waste into environmentally safe levels through use of microorganisms (Thomas *et al.,* 1992). Few of the microorganisms, which help in this process in culture ponds are bacteria such as *Bacillus, Pseudomonas, Acinetobacter, Cellulomonas, Rhodopseudomonas, Nitrosomonas, Nitrobacter,* etc., (Krom *et al.*, 1995).

There are many reports on the bioremediation due to microbes in aquaculture systems. Some of the workers have found no benefits of bacterial augmentation in lowering total ammonia nitrogen concentrations or improving any other aspect of water quality (Boyd *et al.,* 1984; Funge-Smith and Hawthorn, 1996). In contrast to the above reports there are many reports supporting the fact of bioremediation in culture ponds (Eholich *et al.,* 1988; Moriarty, 1996).

Anon (1993, 1995) have indicated positive results on the bioremediation effect of microbes in shrimp ponds. Ehrlich *et al*. (1988) have reported positive effects of bacterial suspension and its ability to accelerate nitrification, decomposition rate of organic solids and to facilitate oxygenation. Felix and Samayakannan (2004 in press) reported that the use of fermented product in

shrimp (*P. monodon)* nursery raceway systems produced results in terms of controlling ammonia level throughout the crops. The reason for inefficiency of the bacterial mixtures used by Boyd *et al*. (1984) could be due to the product not containing a large enough number of the right strains of bacteria or the bacteria were not viable (Moriarty, 1996).

2.8. Algae as bioremediation agent

Austin *et al.* (1992) reported that micro algae (*Tetraselmis succica)* could inhibit the pathogenic bacteria. Information concerning the mechanisms of uptake of the two forms of nitrogen is limited (Flynn *et al.,* 1997) and most of these data have been derived from measurement of uptake systems in higher plants (Macduff *et al.,* 1987) rather than measurement of uptake systems in algae and bacteria. Low temperatures have a greater inhibitory effect on nitrate uptake than on ammonium uptake as documented previously in higher plant roots by several workers (Nedwell and Rutter, 1994). Several studies have confirmed that there is a preference for, or selection by small phytoplankton for ammonium (Mechling and Kilfam, 1983; Morita and Buck, 1974). The micro flagellate preference for ammonium was much greater than the preference for diatom (Harrison, 1983). *Chaetoceros spp.* has a capacity to assimilate ammonia and in this process it could improve the water quality (Avery and Aldrich, 1988). Samocha *et al*. (2002) conducted study with / without inoculation of the water with the diatom *Chaetoceros muelleri* and found some encouraging results on ammonia absorption. The form of inorganic nitrogen used most commonly by bacteria and algae are nitrate and ammonia (Wheeler *et al*., 1982). He further

reported that a large fraction of total ammonium (NH_4^+) uptake by bacteria might influence phytoplankton population structure and new production.

2.9. Water quality parameters in shrimp culture raceway systems

2.9.1. Salinity

Salinity is an important environmental factor which controls the growth and survival of *P.monodon* (Chakroborti *et al*., 1986). Effect of low salinity on the growth of post larvae and juveniles of penaeids in nurseries have been observed (Gunter, 1950). Venkataramiah *et al.* (1977) observed that in high-density culture, young shrimp could survive and grow in a wider salinity (8.5 to 17.0 ppt) than older shrimp. Mandal (1962) pointed out that, maximum release of ammonia occurred in controlled conditions at a salinity range of 10-20 ppt. Hudinaga (1942) observed that the lowest salinity required for the survival of egg, nauplius and zoea of *P. japonicus* was 27 ppt and the same for mysis and post larvae was 23 ppt. For L. vannamei, 5ppt (Samocha, 1993) and for pacific white shrimp, 0.5 ppt (Van Wyk, 1999) ideal for culture.

There is an increasing evidence to suggest that an environment with low salinity and high temperature or high salinity and low temperature in the optimal ranges would promote better growth condition. Nair and Krishnakutty (1975) observed that the growth rate of *F. indicus* was significantly higher in a salinity of 20 ppt for juvenile shrimps. *L. vannamei* grows naturally in salinities between 1-40 ppt (Menz and Blake, 1980). Rajyalakshmi (1980), recommended the salinity range of 27-34 ppt for *P. monodon.* Manik *et al*. (1978) and Navas and Sebastian (1989) reported that *P. monodon* could be cultured in low saline water. Samocha

et al. (2002) conducted short trails in raceways with *L. vannamei* and reported that pacific white shrimp can be raised in low salinity water without negative effects on growth and survival. The optimum range of salinity for the best growth and survival of *P. monodon* and *P. vannamei* were reported by Boyd (1989).

2.9.2. Water Temperature

Water temperature is an essential environmental factor to be monitored in the culture of *P. monodon* (Chen,1995). The temperature plays an important role in the distribution of heterotrophic bacteria. Hirono (1986) observed the slow growth of *L. vannamei* when the water temperature was maintained below 23^0c in raceway culture. He further explained that total mortality resulted when raceway water temperature gone below 13^0c. Samocha (1993) reported that high temperature in the raceways will result in severe stress, reduced growth and poor survivality. He further reported that temperature of 26^0C to 30^0C is considered to be optimum for maintaining shrimps in good condition in raceways. It was also reported that *P. monodon* may not withstand temperature below 12^oC (Cordover, 1989). Stables and Heales (1991) observed a maximum growth of *P*. merguiensis shrimp with respect to temperature. According to O' Brien and Sigemore (1994), optimum temperature is essential for the maximum growth and highest survival in *P. monodon* and *P. semisulcatus*. *P. monodon* has relatively higher tolerance for wide range of temperature (Liao, 1977).

2.9.3. Dissolved oxygen (DO)

The dissolved oxygen level of water has profound influence on the general metabolism and growth of cultivable shrimps. The oxygen requirement of *F.indicus* changes as it grows bigger and showing greater dependence on the oxygen content of water. Boyd and Pillai (1984) explained the influence of dissolved oxygen concentration on the survival, growth and reproduction of the cultivable shrimps. Chen and Zheng (1989) indicated that *P. monodon* die off when dissolved oxygen concentration is lower than 1.0mg/l.

Change and Ouang (1988) reported the critical level of low oxygen, following the die off in ponds, and subsequent decomposition of algal bloom. Chin (1988) pointed out that the maintenance of dissolved oxygen above 3.5 ppm was ideal for intensive shrimp farming. It has been established that dissolved oxygen would decrease with increase in temperature or salinity (Boyd *et al.,*1984). Impact of critical dissolved oxygen level on the growth of post larvae of *P. vannamei* and *P. monodon* has been studied by Tsai(1989). Low dissolved oxygen levels are the major limiting factors in intensive aquaculture (Boyd and Watten, 1989). Critically low dissolved oxygen levels can reduce growth, feeding and molting frequency (Clark, 1986). Egusa (1961) reported lethal level of dissolved oxygen in various penaeid including *P. japonicus, P. kerathurus P. schmitti* and *P. monodon* (Liao and Hung 1975). Samocha *et al.* (1993) reported the oxygen injection system should be used, when the dissolved oxygen decreases below 4 ppm. Ideally, the oxygen injection rate should be regulated to maintain the dissolved oxygen level above 5 ppm.

2.9.4. Water pH

Sankaranarayan *et al*. (1981) observed high pH during the premonsoon season and low pH during the monsoon season in shrimp ponds. Samocha (1993) explained that morning pH levels should provide adequate indication on the bacterial activity and biomass load in the raceway system. The increasing pH in the afternoon could be mainly due to photosynthesis. An optimum pH of 7.8-8.3 is ideal for *P.monodon*. Sarada and Pillai (1993) confirmed that pH values between 6.0 to 9.0 were not lethal to *P. indicus* (PL 5 onwards) in short term bioassay. Low pH can also reduce natural pond productivity (Boyd, 1982) and carbon source for photosynthesis. Apud (1985) observed that a pH of 5 or below in ponds caused mortality of penaeids.

Chakraborti *et al*. (1986) studied the growth rate of *P. monodon* with reference to pH variation. Tsai (1990) suggested pH values of below 4.8 or above 10.36 were lethal to penaeids. Mrithunjayan and Thampy (1986) brought out the effect of sudden variation in pH on the survival of *F. indicus* and *Metepenaeus spp.* The effect of water pH on the growth and survival of shrimp was explained by Boyd (1964) and Tsai (1989). Allan and Maguire (1992) studied the effect of pH and salinity on the survival, growth and osmoregulation in *P. monodon* (Colt and Armstriong,1981).

2.9.5. Transparency

Studies on the transparency of pond water have been made by several workers (Chin, 1988; Boyd, 1989). Samocha (1993) pointed out that the

transparency could provide a good estimate of the plankton population in raceway water. Salinity, temperature, light intensity, nutrient level, natural algal abundance are some of the factors influencing the transparency level in raceway system. Samocha *et al*. (2002) maintained the transparency of 20-25 cm for the growth of shrimps in raceway culture.

2.9.6. Ammonia (NH_3) and Nitrite (NO_2)

Ammonia and nitrite are toxic to the fish, crustaceans and molluscs in culture system (Colt and Armstrong, 1981). Samocha (1993) reported that the high ammonia level could increase disease susceptibility, damages gills and blood oxygen transport ability. Shrimp tolerance to ammonia varies with species, physiological condition and environmental factor. New and Singholka (1982) reported that unionized ammonia and ionized ammonia in the hatchery and rearing water should be less than 0.1 to 1mg/l respectively. Samocha *et al*. (2001) recorded that the nitrite level in raceway water was less than 1mg/l. The conservative levels of nitrite was 1.36 mg/l for post larvae and 0.11mg/l for nauplii (Chen *et al*., 1986) and for ammonia 1.15mg/l for post larvae and 0.13mg/l for nauplii (Chin and Chen ,1987). Liao (1975) reported that the long-term sub lethal exposure to ammonia might lead to depressed growth, survival in high stocking density culture. Thruston (1998) observed that the ammonia toxicity to aquatic animals was affected by temperature, pH, dissolved oxygen, and salinity. It was found that ammonia and nitrite increase exponentially both in the hatcheries and in the grow out ponds. Shrimp farms even with frequent water replacement showed an increase in ammonia toxicity at reduced levels of

dissolved oxygen (Chen *et al*., 1986). Therefore, the accumulation of ammonia and nitrite will have detrimental effects on shrimp rearing. Allen *et al*. (1990) observed the maximum acceptable range of ammonia by *P.monodon*. Kungvankij *et al*. (1985) reported that ammonia level should not exceed 1.5 ppm for NH_4 and 0.1 ppm for NH_3. Boyd (1989) reported that the concentration of ammonia between 0.4 and 2.31 mg/l is lethal to juvenile shrimp in nursery raceway system.

2.10. Interaction between water quality and bacterial flora

Bacteria in relation to environment was studied by Zobell (1941) and he recorded that a medium pH of 7.5 to 7.8 provided optimum conditions for better performance of marine bacteria. Bacterial counts in pond water and shrimps were found to be the lowest, where water temperature and salinity were high (Vanderzant *et al*., 1971).

It has been reported that during winter *Vibrio parahaemolyticus* occur commonly in bottom sediments and enters the water columns again when temperature increases (Kaneko and Colwell,1973). Tanaka *et al*. (1981) established a positive correlation between dissolved organic carbon and bacterial density. Kelly (1982) studied the effect of temperature and salinity on the occurrence of *V. vulnificus* in Gulf coast. Jawahar *et al*.(2003) found that the effects of season, temperature, nutrient concentration, depth and geographical location on the distribution of luminous bacteria.

III. MATERIALS AND METHODS

Details of the study area

The present study was carried out at the state-of-art Raceway Farm Complex facility recently established at the Fisheries College and Research Institute, Thoothukudi, with the funding support of DBT, New Delhi.

3.1. Nursery raceway system

3.1.1. Design layout of the raceway farm complex

The Raceway Farm Complex developed at the Fisheries College and Research Institute, Thoothukudi has the following components:

3.1.1.1. Green house facility

A green house was constructed using cast Iron pipe pillars, framing with gentle semicircle roofing of iron pipes coated with non-corrosive paint. The size of the green house was 20 x 10 m. The roofing was provided with of transparent FRP sheets (20%) and non-transparent FRP sheets (80%). The sidewalls were made up of green shading (75%) net material fixed to wooden frames.

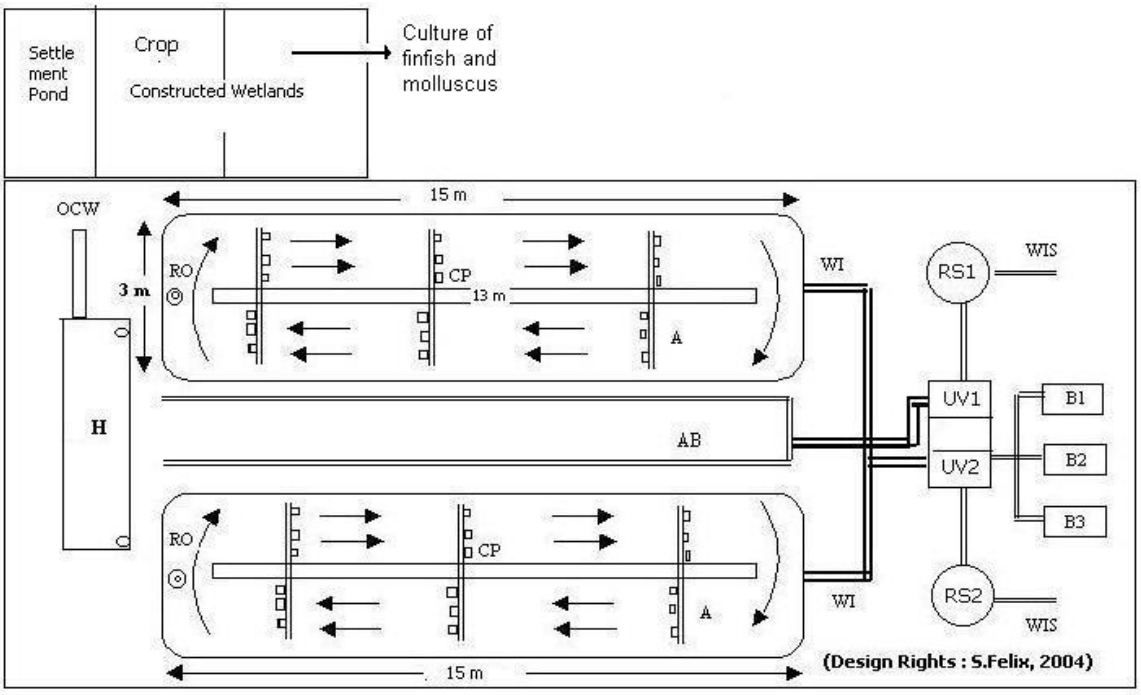

Fig. 1. Layout of Raceway System prototyped at Fisheries College and Research Institute, Thoothukudi, (Felix, 2004)

A	-	Airlift system
AB	-	Aeration line from blowers
CP	-	Central Partition (18mm)
B	-	Blower (5HP each)
H	-	Harvesting Tank
RO	-	Raceway water outlet connected to harvesting tank
OCW	-	Outlet to constructed wetlands
WI	-	Water inlet provision
WIS	-	Water inlet through Rapid Sand Filters
UV	-	Ultra violet sterilization of water
RS	-	Rapid Sand Filters (20000LPH)

3.1.1.2. Raceway tank

Dikes were formed for the raceways and after sufficient consolidation, fabricated precast cement slabs (50 mm thickness) were fixed on the bottom and sides of the raceway pond. The elongated raceways (15m long; 3 m width) with rounded end walls were lined with 700 GSM nylon fabric sheet. Water outlet drain provision was given towards the end of raceways. Bottom of the raceways was provided with adequate slope (0.5) towards the drain to allow easy draining of water. Outlet of raceways would be leading to the harvesting tank (3 X 1.5 X 2.1 m) and from where drain water was drained to constructed wetlands (CWs). The depth of the raceway was kept as one meter. Freeboard of 0.15 m was provided for the nursery raceways for producing a maximum of 2 - 3 g size shrimps.

3.1.1.3. Central partition in raceway tanks

Each raceway was provided with a 13 m long and 1 m deep central partition, made up of water proof marine plywood of 20 mm thickness and fixed on cross wooden beams with stainless steel bolts and screws. The height adjustment of central partition was also possible.

3.1.1.4. Airlift system

Water circulation in the raceways is depending primarily on airlift system. In this system, air is introduced 30 cm below the water level through a vertical PVC pipe which has a 90^{o} PVC elbow at the upper section. The air, lift the water on the pipe from bottom and sends it through the elbow. Air supply is ensured by

two 5 HP blowers operated alternatively every 3 hrs. Air pressure, airlift pipe diameter, submergence depth and the type of airlift system being used are among the major factors affecting the pumping rate and water circulation. In the raceways airlift systems are arranged in 6 sets, 3 on each side of a partition and each set was provided with 3 airlift pipes. The air lift pumps are fixed to a cross wooden beams with adjustable screws, which will allow to adjust the height of the air lift pumps in raceway.

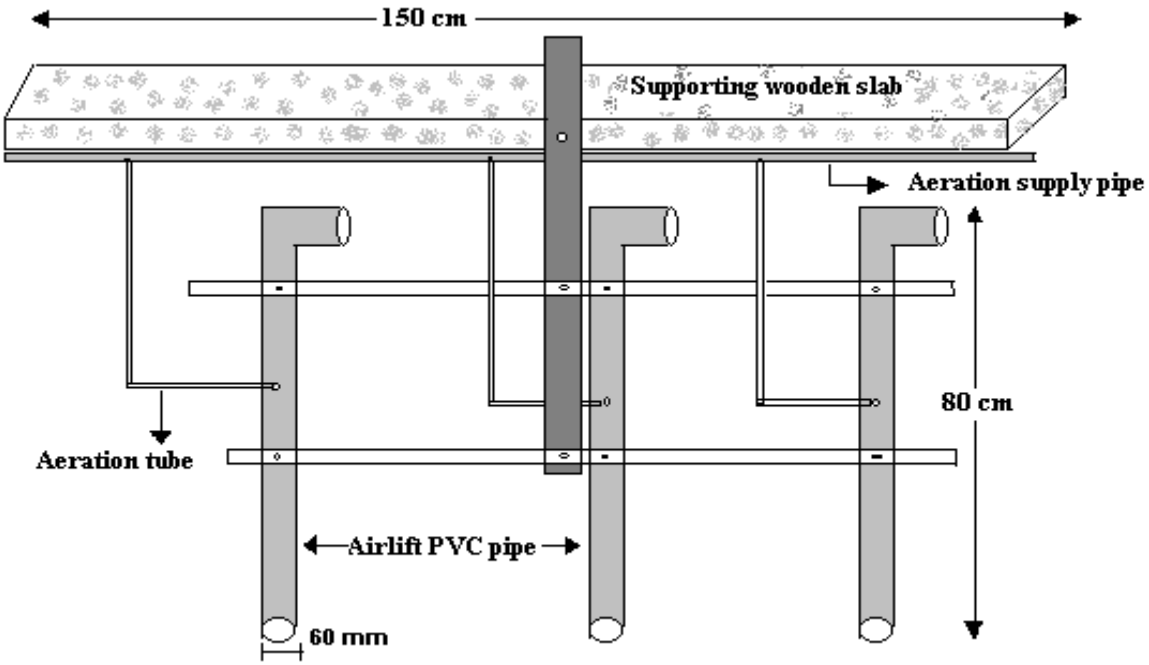

Fig. 3.2 : Longitudinal section through Airlift and the supporting structure

3.1.1.5. Rapid sand filter

A rapid sand filter (Water Co, Australia) with a filtration capacity of about 200m^3/h was provided for each raceways .The sand filter can be used to filter the incoming seawater as well as the raceway water .The multiport valve in the rapid sand filter is a multi positional valve with six operational modes such as, sand filter, backwash, rinse, circulation, waste and closed. The backwash and rinse

modes serve to maintain the sand filter in optimal conditions. The waste mode can be used to drain the water without using the external standpipe. The closed mode is a safety position to avoid accidental drainage through the multi port valve when the raceway is not in operation.

3.1.1.6. UV sterilizing system

Two numbers of UV sterilizers (Rainbow Lifeguard, CA) were installed to treat the incoming water to the raceways. UV sterilizers were connected in the water intake system and the filled water from the rapid sand filters was passed through the UV sterilizers.

3.1.1.7. Bore well

Raceway farm complex was provided with 3 bore wells to supply saline water of different salinities from different depths, to ensure round the year operation of raceways.

3.1.1.8. LDPE lined ‘storage’ and ‘mixing pond’

These tanks were constructed with the dimensions of 9.5 x 5 m each. The sides and the bottom were lined with LDPE sheet (250 GSM) to maintain the water quality and to arrest the water seepage. Water from all bore wells (saline water and freshwater) is received to the mixing pond where the required salinity is adjusted and from there it is pumped to the biological filter. The bio-filtered water is then stored in another LDPE lined storage pond. From the storage pond water is pumped (5 HP) to raceways through a rapid sand filter, an activated charcoal filter (20,000 LPH) and two U.V filters.

3.1.1.9. Biological filter

A cement concrete tank (5 m x 2 m) with central partition was constructed at the raceway farm complex for the purpose of serving as a biological filter. Over the false bottom, different filtration material layers were provided viz. big size gravels, medium size gravels, smaller gravels, activated charcoal, coral sand, coarse sand and fine sand. Each layer is separated by a nylon net material. The entire structure was kept under roof to avoid sunlight.

3.1.1.10. Acclimation tank

A few days before the scheduled post larvae arrival, acclimation tanks should be cleaned and disinfected. On the day of stocking, tanks should be filled with filtered seawater from the same raceway in which the post larvae are to be stocked. Water temperature and salinity should be adjusted according to the anticipated salinity and temperature in which the post larvae are transported.

3.1.1.11. Raceway outlets and filter pipes

The drain outlet of raceways was located towards the drain end, half way between the end of the partition and the raceway's end wall. The raceway water level can be controlled by a external standpipe positioned inside the harvesting tank. Each raceway should be provided with a different set of perforated filter pipes covered with following screen sizes 600, 800, 1000 and 2000 μm. Perforated filter pipes are mounted on the outlet to avoid losing shrimp during water drain. Filter pipes can be changed as the shrimp grow.

3.1.1.12. Harvesting tank

Towards the drain end of the raceways a harvesting tank (2x1.5 x 2) was provided for the purpose of water exchange, bottom waste clearing and for harvesting. Outlet drain pipes of both raceways were connected to harvesting tank, and outlet of harvesting tank in turn was connected to the constructed wet lands (CWs). During water exchange for waste removal and harvesting, 1000 to 2000 μm nylon net screen frame were used inside the harvesting tank (as per the size of shrimps) to avoid the escape of shrimps.

3.1.2. Raceway supporting facilities

3.1.2.1. Air blowers

Two numbers of 5 HP twin lobe air blowers (5 HP each) were installed in the raceway farm complex for the purpose of operating the airlift systems, which will enhance the dissolved oxygen level in raceways and to effect the continuous water circulation.

3.1.2.2. Generator

15 KVA generator (Kirloskar) is installed in order to operate the blowers, paddle wheel aerators, pumps and sand filters during the times of power failures.

3.1.2.3. Alarm systems during power failure

Simulation of power failure could immediately activate the alarm system. The alarm set is connected to field lab, which is at the centre of the farm site. When operating personals / technicians get the power failure alarm signals, they can switch on the generator.

3.1.2.4. Field laboratory

Routine water quality parameters like pH, dissolved oxygen, ammonia and nitrate, etc. were tested in field laboratory and also the routine health check up of shrimps, feed rationing, feeding schedule preparation etc., are under taken in the field lab.

3.1.3. Raceway crop management

3.1.3.1. Raceway filling and chlorination

Before filling the raceway tank, it was thoroughly cleaned and disinfected with a chlorine solution (30-100 ppm active chlorine). Raceways were filled with filtered seawater up to the required water level (0.75 m) one week prior to stocking. Water was drawn from three different subsoil bore wells having different salinity ranges and pumped to the mixing pond to adjust the required salinity. Then the water was passed through a biological filter, rapid sand filter and activated carbon filters and U.V. sterilizing system.

3.1.3.2. Feeding management

Feeding with dry feed started on the first day of stocking. Regular growth sampling for biomass evaluation was done weekly once or twice and feed ration revised. PL were fed six times a day containing 45% crude protein,5% fat,12% moisture and 4% ash. The particle size of the feed offered to PL should be increased according to the PL stage.

3.1.3.3. Stocking management

Trial: 1

Species stocked : *Penaeus monodon*

Stocking density : RW1- 2000/m^2

RW2- 1000/m^2

Trial: 2

Species stocked : Fenneropenaeus *indicus*

Stocking density : RW1 666/m^2

RW2 333/m^2

The shrimp seeds prior to stocking were randomly tested for WSSV (White spot syndrome virus) and MBV (Monodon baculo virus) using PCR (polymerase chain reaction technique) and M.G. staining technique.

3.1.3.4. Monitoring of population and growth

An important parameter for evaluating post larval performance in the nursery raceway system is its growth rate. The monitoring of population and growth was done in order to obtain accurate growth data for determining feed rations, population and health. To determine the growth, a random sample of about 100 post larvae should be collected from each raceway. Samples were taken from different parts of the raceway. The biomass and total number of shrimp in the sample were recorded to estimate the mean weight.

3.2. Indoor algal culture facility

Indoor unialgae section is an air-conditioned chamber, where ambient temperature was maintained at 22 – 24^0C. Walnes f/2 medium was used to culture *Cheatoceros calcitrans*. At regular intervals transfer of inoculam from lower to higher dilutions was carried out to sustain algal production in the indoor culture facility. For culturing indoor algae 50, 100, 250, 500 and 1000ml conical flasks with 4-liter LDPE pet jars and 20 liter capacity transparent buckets were used. Before addition of inoculams every stage of algal growth was carefully observed under microscope. Indoor algal culture, sterilization of seawater and glasswares were done by autoclaving or by heat sterilization using Titanium heater. Water needs to be cooled to optimum temperature before the addition of materials and inoculums. Initially pure inoculums of *Chactoceros calcitrans* was obtained from the Algal Culture Lab, CMFRI Thoothukudi and 2ml of inoculam was transferred to 20 ml flask containing filtered and sterilized seawater to serve as a stock solution to obtain $8.0x10^6$ cells / ml during 72 hrs incubation.

3.2.1. Small Flask culture

Small flask of 250 ml capacity was inoculated with 20 ml of stock culture and incubated for 48 hrs to obtain *Chaetoceros* cell density of 6.7 million cells / ml.

3.2.2. Big flask culture

200 ml of inoculam from small flask was transferred to 1800 ml nutrient medium and incubated for 2 days to obtain a cell density of 4 – 5.5 x 10^6 cells / ml.

3.2.3. Jar Culture

Cylindrical jars of 5l capacity were filled with 4.0 liters of filtered sterilized seawater and fortified with nutrient medium. Then the jar was inoculated with 500ml of inoculam obtained from big flask and incubated for 48 hrs.

3.2.4. Bucket culture

A bucket of 20l capacity filled with sterile seawater was enriched with nutrient medium and incubated with 4 l of stock culture obtained from the jar and incubated for 48 hrs.

3.2.5. Aeration in indoor unit

All the cultures starting from small flasks to buckets were continuously supplied with filtered air through aeration grid fitted in the indoor lab. Aeration was to prevent stratification of algal cells, to allow gas and heat transfer, light penetration, disperse or dissolve materials and prevent adherence of cells to the walls of culture vessel.

3.2.6. Light

Using flourescent tube continuous light in the range of 750 – 1000 lux was provided to all the culture vessels, including small flasks, big flasks, jars and buckets.

3.3. Out door algal culture

Outdoor algal culture was carried out in 500 - 1000 litres FRP tanks. Indoor produced *Chaetoceros calcitrans* diatom was inoculated to outdoor FRP

tanks and filtered water was fertilized with f/2 Walne's medium. After 24-48 hours depending on cell density, algae from FRP tanks were transferred to raceway tanks or limited water exchange growout (LWEG) ponds by pumping (0.5 HP). Cell density and quality were continuously monitored under microscope.

3.4. Algal cell counts

The apparatus used for algal cell counting was a haemocytometer with an improved Neubauer chamber. To determine the algal cell density in the suspension, the number of algal cells counted was divided by the larger grid area covered, multiplied by 10,000.

3.5. Analysis of physico- chemical parameters in raceway tanks

Samples from raceways for physico-chemical analysis were collected and estimated .The dissolved oxygen (DO) of water was recorded daily by Winklers titration method and the value expressed as mg/l. DO level was also simultaneously measured using [YSI- 055A USA]. The pH of the raceway tanks was measured with 0.1 accuracy electronic digital pH meter. In order to measure the water temperature mercury thermometer having measuring range of 0-100^{o}C was used. Transparency of water was measured by using a secchi disc. In ammonia estimation, the absorbance of indophenol blue complex was proportional to the ammonia present in the samples and was measured using a spectrophotometer [ELISA Reader: LAB SYSTEMS, Netherlands] at 630 nm and then compared with the standard graph to get the ammonia levels. For nitrite estimation, the pink azo dye complex in the sample was spectrometrically measured at 543 nm and compared with the standard graph. Salinity was

measured with Refractometer [ERMA,Japan] having 0-100 ppt range and by Harvey' titration method (APHA 1985).

Table 3. 1. Composition of the algal culture media and preparation

Sl. No.	Nutrient	Primary stock Solution (1 lt. of distilled water)	Working Stock Solution (1 lt. of distilled water)	Dosage (1 lt. of sea water)		
				f	f/2	f/4
1.	Nitrate & Phosphate	----	75 g. of Sodium nitrate and 5 g. of Sodium phosphate	2 ml	1 ml	0.5 ml
2.	Silicate	----	35 ml of Sodium silicate	2 ml	1 ml	0.5 ml
3.	Trace Metals	a) 10 gm of Copper sulphate b) 22 gm. of Zinc sulphate c) 10 gm. of Cobalt Chloride d) 180gm of Manganese chloride e) 6 gm. of Sodium molybdate	1 ml of primary stock solutions of a,b,c,d,e and 4.36 g of EDTA and 3.15 g of Ferric chloride	2 ml	1 ml	0.5 ml
4.	Vitamins	a) 20 gm. of Thiamine hydrochloride b) 100 mg. of Biotin c) 100 mg. of Cynacobalamine	5ml of Primary stock solution of a,b & c	2 ml	1 ml	0.5 ml

3.6. Microbial load assessment in raceway tanks

Materials

Water samples, chemicals/media, Glasswares and plasticwares.

Methods

3.6.1. Sterilization

The bacteriological media were steam sterilized at 121^{o}C for 15min, in an autoclave. Glasswares used for microbiological work were sterilized in a hot air oven at 160^{o}C for 2 hrs.

3.6.2. Preparation of samples for bacteriology

3.6.2.1. Water

The water samples after proper shaking were serially diluted to a required level.

3.6.3. Total plate count

Appropriate dilutions were prepared from the water and plated on Zobell marine agar by spread plate technique in duplicate. Serial dilutions of water samples were made with sterile diluents containing 2% Nacl. Appropriate dilutions were inoculated on to Zobell marine agar and TCBS agar plates for quantifying the bacteria by spread plate technique. All the plates were incubated at 37^{o}c for 48 hrs (APHA, 1976; Collins *et al.,* 1989). Counts were done at 48 hrs after incubation, and those tubes counts falling between 30-300 were taken for the calculation of THC.

3.6.4. Purification and identification of bacteria

Bacteria with distinct colony morphology from the plates and those counts, which were falling between 30-300, were randomly selected, picked and streaked repeatedly

on TSA agar to get pure culture. About 50 cultures were purified for qualitative analysis .The purified colonies were then maintained on TSA plates at room temperature (32^{o}c) and subjected to standard morphological, physiological and biochemical tests following the methods of Collins *et al.* (1989). All the isolates were identified up to genus level using the keys of Oliver (1982). The percentage composition of microbes in each raceway was estimated and recorded.

3.7. Preparation of fermented product for raceways

A specific yeast based fermented product was also added to the raceways to sustain the beneficial microbial population in the raceways to maintain them as a heterotrophic bacterial based system.

Table 3.2. Composition of the fermented product

Sl. No.	Ingredients	Quantity
i.	Wheat flour	1000 g
ii.	Sugar	500 g
iii.	Jaggery	500 g
iv.	Yeast	10 g
v.	Water	20 litres

The above composition was mixed with 20 l of water and kept overnight in aeration for incubation. The extract of this was added to the raceway at regular intervals or whenever the ammonia build up was noticed beyond the optimum level.

IV. RESULT

TRIAL I

4.1. Nursery rearing of *P. monodon* in raceway culture system

The *P. monodon* seeds for the stocking were procured from a hatchery, after carrying out the required PCR test.

The mean initial weight of post larvae used for raceway rearing was 0.001g. Raceway rearing was carried out for a period of 46 days at two different stocking densities viz 2000/m^3 and 1000/m^3 in raceway 1 and 2 were adopted respectively. The growth and production details of shrimp obtained from the two raceways with different stocking densities were recorded along with water quality parameters, microbial load algal concentration, etc.

Table 4.1. Stocking details of raceways

Raceway Tank No.	DOC (Days)	Stocking Density (Nos./m^3)	Numbers stocked (Nos.)	Mean initial weight (g)
1.	46	2000	60000	0.001
2.	46	1000	30000	0.001

4.2. Water quality parameters in raceway tanks

The physical parameters such as temperature, pH and transparency were recorded daily in the raceways. The mean values of these parameters for every 5 days were estimated and presented in Table 4 and 5. It was observed that the temperature was varying between 28 and 31^oC, which was possible due to the temperature control roofing structures provided on the green house for the raceway system. The pH was

maintained within the optimum ranges in raceway systems. While the RW1 recorded pH ranging between 7.9 and 8.5, the RW2 recorded 8.0 and 8.6, maintained in conducive condition with respect to pH for rearing shrimps throughout the crop.

The chemical characteristics include salinity, dissolved oxygen (DO), and nitrite (NO_2) and the observed parameters were presented in Table 4 and 5. As the raceways were stocked with *P. monodon* the salinity was maintained at 25 ppt. Due to the presence of three bore wells of different salinities maintaining the salinity at 25 ppt or close to that range was possible in the raceway culture systems. The dissolved oxygen levels in the raceway system were recorded daily at 9 am using an on farm DO meter (YSI 550A, USA), which was calibrated against Winkler titration weekly. While the DO did not drop below 4.7 mg/l in raceway-I, it was around 4.3 mg/l in raceway no.2, which were well above the minimum required dissolved oxygen for the raceway system. The nitrite level of the raceway culture system were recorded weekly and it was observed that the nitrite level was ranging from 0.06 to1.9mg/l in raceway no.1 and it was below 0.06 to 0.5 in raceway no.2. The graphical representation of recorded data are shown in figure (1,2,3,4,5,6).

Table 4 .2. Water quality parameters recorded daily in raceway 1

DOC (days)	Temp (^{0}C)	Salinity (ppt)	Transparency (cm)	pH	DO * (mg/l)	Nitrite (mg/l)
1-5	29.0±0.4	25.0± 0.0	38±2.1	8.5±0.1	5.2 ± 0.18	0.06
6-10	30.0±00	25.5 ± 00	29±1.0	8.4±0.01	5.2 ±0.10	0.07
11-15	28.1±0.6	25.3 ±0.4	28±0.94	8.4±0.04	4.7 ±0.62	0.07
16-20	31.2±0.2	25.5 ±0.0	25±0.5	8.2±0.05	4.8 ±0.25	0.39

21-25	29.0±0.0	25.8 ±0.2	21±1.4	8.2±0.04	5.5 ±0.16	1.1
26-30	28.2±0.2	25.5 ±0.5	24±0.0	8.1±0.01	5.5 ±0.20	1.9
31-35	29.3±0.6	25.1 ±0.2	25±0.47	7.9±0.08	5.1 ±0.16	1.1
36-40	28.5±0.5	24.7 ±0.2	27±2.5	7.9±0.05	5.2 ±0.10	1.6
41-45	29.7±0.5	25.1 ±0.2	29±1.47	8.24±0.2	5.3 ± 0.21	0.9

* Time of measurement : 9 AM

Table 4.3. Water quality parameters recorded daily in raceway 2

DOC (days)	Temp (^oC)	Salinity (ppt)	Transparency (cm)	pH	DO * (mg/l)	Nitrite (mg/l)
1-5	29.0±0.4	25.6 ± 0.2	36±2.6	8.6±0.08	5.0 ± 0.09	0.07
6-10	30.0±00	25.5± 0.0	32±0.0	8.5±0.05	5.2 ±0.10	0.06
11-15	28.1±0.6	26.0±0.4	31±1.8	8.5±0.04	4.5 ±0.12	0.07
16-20	31.2±0.2	24.7±0.2	26±0.0	8.5±0.05	4.3 ±0.30	0.08
21-25	29.0±0.0	25.1±0.2	21±1.2	8.5±0.04	5.4 ±0.10	0.40
26-30	28.2±0.2	25.3±0.2	21±1.0	8.5±0.04	5.4 ±0.07	0.20
31-35	29.3±0.6	25.8±0.2	25±2.0	8.0±0.04	4.9 ±0.28	0.10
36-40	28.7±0.5	24.5±0.5	28±2.0	8.1±0.05	5.2 ±0.05	0.50
41-45	29.7±0.5	25.4±0.3	30±3.2	8.3±0.05	5.4 ± 0.33	0.30

* Time of measurement : 9 AM

4.3. Quantitative analysis of bacterial load

The observed total heterotrophic count (THC) of raceway tank 1 and 2 were furnished in Table 6. The colony forming units per ml (cfu/ml)of raceway tank 1 was ranging from 9.2 x 10^3 to 5.4 x 10^4 cfu/ml. The minimum count recorded was 9.2 x 10^3 on the midway of culture and the maximum count was 5.4 x 10^4 on the 11^{th} DOC of culture. In raceway tank –2 the range was between 7.8 x 10^3 to 7.4 x 10^4 cfu/ml. While the minimum count was recorded during the initial phase of raceway culture, the highest count was recorded during the midway of culture.

Table 4.4. Microbial load recorded in raceway 1 and 2

Days of Culture	Microbial Load (cfu/ml)	
	Raceway Tank 1	**Raceway Tank 2**
3	18 x 10^4	1.9 x 10^4
7	94 x 10^3	78 x 10^3
11	54 x 10^4	78 x 10^3
15	92 x 10^3	78 x 10^3
19	21 x 10^4	19 x 10^4
23	90 x 10^3	13 x 10^4
27	22 x 10^4	74 x 10^4
29	16 x 10^4	22 x 10^4
35	22 x 10^4	21 x 10^4
39	34x10^4	30x10^4
43	44 x 10^4	28 x 10^4

4.4. Bioremediation effect of algae and bacteria in controlling ammonia in raceway culture systems

From the figures (3,4,5 and 6) it was evident that the ammonia level could be controlled by specific addition of algae and microbial supplements in raceways. The lowest ammonia level was recorded when the algae density and microbial load were high. It was further noticed that the concentration of ammonia was increasing whenever the algae density and microbial load were decreasing. Abrupt alteration in any one of the parameters viz. algae density and microbial load could affect the ammonia level in the system. The statistical analysis (Tables 7,8,9 and 10) confirmed that the ammonia concentration in raceway systems reduced significantly ($P<0.05$) with respect to the addition of algae and microbial load.

Table 4.5. Analysis of variance for algal conc. vs ammonia conc. in raceway 1

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	2.11E+10	1	2.11E+10	188.7663	1.2E-11	4.35125
Within Groups	2.24E+09	20	1.12E+08			
Total	2.34E+10	21				

Table 4.6. Analysis of variance for algal conc. vs ammonia conc. in raceway 2

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.84E+09	1	6.84E+09	17.9134	0.000501	4.413863
Within Groups	6.88E+09	18	3.82E+08			
Total	1.37E+10	19				

Table 4.7. Analysis of variance for THC vs ammonia conc. in raceway 1

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.84E+09	1	6.84E+09	17.9134	0.000501	8.285497
Within Groups	6.88E+09	18	3.82E+08			
Total	1.37E+10	19				

Table 4.8. Analysis of variance for THC vs ammonia conc. in raceway 2

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.06E+09	1	4.06E+09	10.67414	0.004843	4.493998
Within Groups	6.08E+09	16	3.8E+08			
Total	1.01E+10	17				

Table 4.9. Application dosage of *Chaetoceros calcitrans* to raceway tanks

DOC (weeks)	**Algal quantity in each raceway (litre)**	**Transferred algal cell density (cells/ml)**
1	20	$7.2x10^6$
2	20	$7.8x10^6$
3	20	$8.5x10^6$
4	20	$9.1x10^6$
5	20	$6.5x10^6$
6	20	$8.2x10^6$
7	20	$7.6x10^6$

Table 4.10. Application level of fermented product to raceway tanks

DOC (weeks)	Quantity of fermented product transferred to each raceway (litre)
1	10
3	10
5	10
7	10

4.5. Growth performance of *P. mondon* in raceways

The growth pattern observed in this study was shown in table 12. After 46 days of culture the mean body weights observed were 0.4142 and 0.645 g for the stocking densities of 2000/m^3 and 1000/m^3 respectively.

The feed input and production details were presented in Table.13 At the stocking density of 2000/m^3, the shrimp utilized 0.717kg feed and yielded to the turn of 0.427 kg of net biomass per m^3, where as at 1000/m^3, the utilized feed was 1.226 and the yield was at the rate of 0.7175 kg/m^3. The FCR of the two treatments were found to be at 1.68 and 1.79 for the stocking densities of 2000/m^3 and 1000/m^3 respectively.

The biogrowth parameters were estimated and presented in Table 13. While the survival rates recorded as 51.6% and 82% for the stocking densities of 2000/m^3 (raceway-1) and 1000/m^3 (raceway-2), the mean weight increment of individual shrimps for RW1 and RW2 were estimated as 0.4132 and 0.644 respectively for 46 days of raceway rearing. The specific growth rates (SGR) were recorded as 0.008g and 0.014g for the raceway-1 and raceway-2 respectively. The other parameters such as survival rate, biomass production and FCR also were estimated and recorded (Table.13)

Table 13 : Bio-growth parameters recorded for Trial – 1 in raceway (Species : *P. monodon)*

Sl. No	Experiment code	Stocking Density (/m^3	Initial mean weight (g)	Final mean weight (g)	Weight increment (g)	SGR	Survival (%)	Biomass produced (kg)	FCR
1	T_1 R_1	2000	0.001	0.414	0.413	0.131	51.6	0.427	1.68
2.	T_2 R_2	1000	0.001	0.645	0.644	0.109	82.0	0.718	1.79

TRIAL- 2

4.6. Nursery rearing of *F. indicus* in raceway culture system

The seeds for the stocking were procured from a hatchery, after carrying out the required PCR test. The mean initial weight of post larvae used for raceway rearing was 0.1368g and 0.1257g for the stocking density of 333/m^3 and 666/m^3 respectively. Raceway rearing was carried out for a period of 53 days. The growth and production details of shrimp obtained from the two raceways with different stocking densities were recorded along with water quality parameters, microbial load, algal concentration, etc.

Table 4.12. Stocking details of raceways

Race way Tank No	DOC	Stocking density (Nos./m^3)	Numbers stocked	Mean initial weight (g)
1.	53	333	10000	0.1368g
2.	53	666	20000	0.1257g

4.7. Water quality parameters in raceway tanks

The physical parameters include temperature, pH and transparency were recorded daily in the raceways. The mean valves of these parameters for every 5 days were estimated and presented in Table 14 and 15. It was observed that the temperature was varying between 24.4 to 27.1^oC,maintaining the temperature with in the permissible level possible because of green house roofing structure. The pH was maintained within the optimum ranges in raceway systems. While the RW1 recorded pH ranging between 7.9 and 8.1, the RW2 recorded 7.9 and 8.3, which was the conducive range of pH for rearing shrimps in raceway system. The mean transparency level in the

raceways ranged between 18.2 and 39 cm. It showed that the value goes up to 18.2 may be due to algal bloom.

The chemical characteristics include salinity, dissolved oxygen (DO), and nitrite(No_2) The observed parameters were presented in Table. As the raceways stocked with *Fenneropenaeus indicus* the salinity was maintained at 18 ppt. Due to the presence of three borewells of different salinities maintaining the salinity at 18 ppt or close to that range was permissible in the raceway culture systems. The dissolved oxygen level in the raceway system were recorded daily at 9.0a.m using D.O meter (YSI 550A USA) which was calibrated against Winkler titration weekly. While the DO did not drop below 7.3mg/l in raceway no. 1, it was around 7.8mg/l in raceway no.2, which were well above the minimum required DO for the raceway system. The nitrite level of the raceway culture system were recorded weekly and it was observed that the nitrite level was ranging from 1.2 to 0.002 mg/l in raceway no.1 and it was below 0.9 mg/l in raceway no. 2.

4.8. Quantitative analysis of bacterial load

The observed total heterotrophic count (THC) of raceway tanks were furnished in Table 15. The colony forming units per ml (cfu/ml)of raceway tank 1 was ranging from 1.6×10^4 to 1.3×10^5 (cfu/ml). The minimum count recorded was 1.6×10^3 on the initial phase of culture and the maximum count was 1.34×10^5 on the end of culture. In raceway tank 2 the range was between 4.6×10^4 to 1.5×10^5 cfu/ml. While the minimum count was recorded during the midway of raceway culture, the highest count was recorded during the initial phase of culture.

Table 4.13. Microbial load recorded in raceway tank 1 and 2

Days of Culture	Microbial Load (cfu/ml)	
	Raceway Tank 1	Raceway Tank 2
3	$1.6x10^4$	$7.1x10^4$
7	$8.7x10^4$	$6.2x10^4$
11	$4.5x10^4$	$1.5x10^5$
15	$2.2x10^4$	$1.2x10^5$
19	$9.6x10^4$	$4.6x10^4$
23	$9.6x10^4$	$1.0x10^5$
27	$7.2x10^4$	$8.0x10^4$
29	$8.3x10^4$	$8.2x10^4$
35	$1.1x10^5$	$7.9x10^4$
39	$1.3x10^5$	$8.6x10^4$
43	$1.1x10^5$	$8.8x10^4$
45	$1.3x10^3$	$7.6x10^4$
49	$7.8x10^4$	$5.5x10^4$

4.9. Bioremediation effects of algae and bacteria in controlling ammonia in culture of *F. indicus*

From the figures 17 and 18, it was evident that the ammonia level could be controlled by addition of algae and microbial supplements in raceways. The lowest ammonia level was recorded when the algae density and microbial load were at optimum level. It was further noticed that the concentration of ammonia was increasing

whenever the algae density and microbial load were decreasing. Abrupt alterations in these parameters viz. algae density and microbial load did affect the ammonia level in the system. The statistical analysis (Tables 16) confirmed that ammonia concentration in raceway systems reduced significantly ($P<0.05$) with respect to the addition algae and microbial load.

Table 4.14. Ammonia vs THC and algae density recorded in raceway 1 and 2

Duration (Weeks)	Ammonia (mg/l)		THC (cfu/ml)		Algae density (cells/ml)	
	RW 1	RW 2	RW 1	RW 2	RW 1	RW 2
1	0.48	0.28	$5.1x10^4$	$6.6 x10^4$	$8.4 x10^4$	$8.4 x10^4$
2	0.61	0.69	$3.3x10^4$	$10 x10^4$	$9.0 x10^4$	$6.7 x10^4$
3	0.80	0.82	$8.8x10^4$	$9.6 x10^4$	$3.9 x10^4$	$1.7 x10^4$
4	0.21	0.17	$9.6x10^4$	$8.1 x10^4$	$5.2 x10^4$	$3.3 x10^4$
5	0.16	0.22	$12x10^4$	$8.2 x10^4$	$6.0 x10^4$	$3.9 x10^4$
6	0.13	0.13	$10x10^4$	$8.2 x10^4$	$7.6 x10^4$	$5.4 x10^4$
7	0.13	0.13	$5.9x10^4$	$4.4 x10^4$	$7.6 x10^4$	$5.7 x10^4$

Table 4.15. Analysis of variance for algae conc. vs ammonia conc. in raceway 1

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	6.2E+10	1	6.2E+10	169.9663	1.05E-17	7.17057
Within Groups	1.82E+10	50	3.65E+08			
Total	8.03E+10	51				

Table 4.16. Analysis of variance for algae cont. Vs ammonia conc. in raceway 2

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	3.92E+10	1	3.92E+10	171.5454	5.67E-19	7.093149
Within Groups	1.32E+10	58	2.28E+08			
Total	5.24E+10	59				

Table 4. 17. Analysis of variance for THC. vs ammonia conc. in raceway 1

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.67E+10	1	4.67E+10	62.78031	2.12E-08	7.721269
Within Groups	1.93E+10	26	7.43E+08			
Total	6.6E+10	27				

Table 4.18. Analysis of variance for THC. vs ammonia conc. in raceway 2

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Between Groups	4.67E+10	1	4.67E+10	121.2383	4.64E-12	4.170886
Within Groups	1.16E+10	30	3.86E+08			
Total	5.83E+10	31				

Table 4.19. Application dosage of *Chaetoceros calcitran* to raceway tanks

DOC (weeks)	Algae quantity in each raceway (litre)	Algal cell density (cells/ml)
1	20	$8.9x10^6$
2	20	$7.1x10^6$
3	20	$6.8x10^6$
4	20	$9.5x10^6$
5	20	$8.3x10^6$
6	20	$7.0x10^6$
7	20	$8.1x10^6$

Table 4.20. Application level of fermented product to raceway tanks

DOC (weeks)	Quantity of fermented product Transferred to each raceway
1	10
3	10
5	10
7	10

4.10. Qualitative microbial flora of raceway 1 and 2

The qualitative flora of raceway water was observed on the midway of culture was presented in the table The composition of Gram positive and Gram negative bacteria in raceway tank 1 was 67% and 33% respectively. Among the Gram +ve group, *Bacillus* contribute 35% of the total population and *Staphylococcus* and *Arthrobacter* contributing 5%. Among the Gram -ve bacteria *Vibrio* was dominant contributing 60% of the total population. Generally *Bacillus* and *Vibrio* are the dominating group of contributing 23.3% and 20% of the total population. The

composition of Gram positive and Gram negative bacteria in raceway tank 2 was 70% and 30% respectively. Among the Gram +ve group, *Bacillus* Contribute 26% of the total population and *Staphylococcus* and *Arthrobacter* contributing 10%. Among the Gram-ve bacteria *Vibrio* was dominant, contributing 67% of the total population. Generally *Bacillus* and *Vibrio* are the dominating group of contributing 26% and 16% of the total population.

4.11. Growth performance of *F. indicus* stocked in raceways

The growth pattern observed in this study was shown in figure: After 53 days of culture the mean body weights obtained were 2.23 and 1.47g for the stocking densities of 333/m^2 and 666/m^2 respectively.

The feed input and production details were presented in Table 23. At the stocking density of 333/m^3, the shrimp utilized 1.03 kg feed and yielded to the turn of 0.6 kg of net biomass /m^3, where as at 666/m^3, the utilized feed was 1.38 and the yield was at the rate of 0.74 kg/m^3. The FCR of the two treatments were found to be at 1.73 and 1.87 for the stocking densities of 333/m^3 and 666/m^3 respectively.

The bio growth parameters were calculated and presented in Table 23. While the survival rates recorded were 80095 % and 61.1% for the stocking densities of 333/m^3 (Raceway-1) and 666/m^3 (Raceway-2), the mean weight increment of individual shrimps for RWl and RW2 were estimated as 2.0093 and 1.244g respectively for 53 days of raceway rearing. The specific growth rates (SGR) were recorded as 0.53 g and 0.40 g for the raceway-1 and raceway-2 respectively. The other parameters such as survival rate, biomass production and FCR also were estimated and recorded Table 23.

Table 23 : Bio growth parameters recorded for Trial – 2 in raceway (Species : *F.indicus)*

S.No	Experiment code	Stocking Density (/m^3)	Initial mean weight (g)	Final mean weight (g)	Weight increment(g)	SGR	Survival (%)	Biomass produced (kg)	FCR
1	T_1 R_1	333	0.136	2.23	2.009	0.053	80.95	0.60	1.73
2.	T_2 R_2	666	0.125	1.47	1.344	0.040	61.1	0.74	1.87

V. DISCUSSION

5.1. Water quality management in nursery raceway

5.1.1. Temperature

Temperature is the vital factor and changes in the physiological responses is possible due to temperature variation(Sastry and Vargo, 1977). With the rise of ambient temperature the basal metabolic rate increases and vice versa in aquatic animals. Cordover (1989) observed that *P.monodon* could not withstand temperature below 12^{o}C. While reports of Liao (1988) stated that water temperature above 31^{0}C increases the risk of disease outbreak. Samocha and Benner (2001) suggested relatively low temperature of 23^{0}C is ideal for nursery raceway culture. Chin (1988) recommended a temperature level of 26 to 32^{0}C for better growth and survival of shrimps. In the present study, it was observed that temperature in raceways were with in the recommended temperature of Chin (1988). It was clear that the temperature maintained in the raceway was not a limiting factor. Aeration and water exchange could monitor the variation of temperature with in the favorable ranges.

5.1.2. Salinity

The salinity range of 27-34 ppt was reported to be suitable for the culture of penaeid shrimp (Hudinaga,1942;Cook and Murphy,1969). Nair and Krishnakutty (1975) observed that high salinity (30 ppt) was needed for juvenile shrimps. Effect of low salinity on post larvae has been reported by Gunter (1950). Samocha (1993) reported that the salinity of 5 ppt for *L. vannamei* was ideal for raceway culture. Van Wyk (1999) reported that low salinity i.e. almost 0.5 ppt is

conducive for culturing pacific white shrimp in raceways. Samocha and Benner (2001) maintained the salinity of 20 ± 2ppt for the culture of *L. vannamei*. Stick on to the above reports salinity range of 17.0 to 18.2 was maintained for culturing *P. monodon* and 17.2 to 18.6 ppt for *F. indicus* under raceways.

5.1.3. Dissolved oxygen concentration

Dissolved oxygen exerts a tremendous effect on growth and production of shrimps directly by respiration and indirectly through its impact on sulphate reduction. Boyd and Pillai (1984) explained the influence of dissolved oxygen concentration on the survival and growth of the cultivable shrimps. Millamena (1990) found depressed growth and survival of *P. monodon*, when the dissolved oxygen decreased from 6 to below 3 ppm. Bart and Arnold (1992) suggested the oxygen range of 4.2-8 ppm as the ideal dissolved level for high density culture. Samocha and Benner (2001) maintained the dissolved oxygen concentration above 3.5 mg/l throughout the cycle. Dissolved oxygen levels recorded in the present study was well within the favourable range as reported by Samocha and Benner (2001) and Bart and Arnold (1992). In the two trials the day-to-day change of dissolved oxygen was very narrow indicating better water stability.

5.1.4. Water pH

The pH of culture water is usually not a direct threat to shrimps because it seldom exceeds the value of 9.0 in the water column (Boyd, 1989). A pH range of 6.8 to 8.7 was recommended as optimum for shrimp culture (Chin, 1988). Samocha *et al.* (1993) reported the morning pH was an indication on the

bacterial activity and biomass load in the raceway system. Bart and Arnold (1992) maintained the pH of 7.2-8.0 for penaeid shrimp culture. Samocha and Benner (2001) reported the pH of slightly alkaline condition is ideal for penaeid culture in raceways. In the present study, the pH ranges recorded for both the trials were highly comparable. The pH was varied between 7.9- 8.5, which was with in the permissible range as indicated by the above said authors.

5.1.5. Transparency

Turbidity in shrimp ponds occur primarily due to plankton and suspended matter of inorganic and organic origin. Samocha *et al.* (1993) pointed out that the transparency is good estimate of the plankton population in raceway water. Boyd (1989) reported that secchi disc reading of 40-60 cm was optimum for shrimp ponds. Samocha *et al*. (2002) maintained the transparency of 20-25 cm in nursery raceway culture. Chin (1988) reported 30 cm is optimum for intensive shrimp farming. In the present study the transparency was varied between 18-38 cm. During the latter period of culture the transparency of the ponds decreased upto 18 cm due to the increased algal concentration. However, it did not affect the growth and survival of shrimps. Even though the variation was wide it was with in the permissible range.

5.1.6. Ammonia and Nitrite

The ammonia in ionized state is highly toxic, normally leading to poor growth in shrimps (Boyd, 1989; Tsai, 1988). Samocha *et al.* (1993) reported that the high ammonia level could lead to disease outbreak. Samocha *et al*. (2002)

reported that *L. vannamei* could tolerate up to the ammonia level of 10.1mg/l. Chin and Chen (1987) reported that conservative levels of nitrite and ammonia in culture waters were 1.36 and 1.15mg/l respectively. In the present study it was observed that the nitrite level in the raceway shoot up to 1.9 mg/l. The increase in nitrite level observed may be due to the absence or inadequate level of nitrite-oxidizing bacteria, *Nitrobacter sp.* in the raceways. The maximum ammonia level noticed was however only 0.8mg/l

5.2. Growth and survival of penaeid species in raceway culture system

The general observation is that when the survival rate decreased, the stocking density decreased. This was supported by Davis and Arnold (1998); Williams *et al*. (1996) and Sandifier *et al. (*1987). In the present study growth rate was fluctuating for different stocking densities. In the first trial (*P. monodon)* the growth per week was 0.06g and 0.1g for the stocking densities of 2000/m^2 and 1000/m^2 . In the second trial *(F . indicus*) the growth rate per week was 0.17g and 0.27g for the stocking densities of 666/m^2 and 333/m^2. Samocha *et al*. (2002) observed that shrimp production could be increased by 70-80% by adopting nursery raceway culture. Fast (1991) reported the survival rate of 85-95% at the end of the nursery raceway culture. Samocha *et al*. (2001) noticed that survival was decreasing from 89.5% to 44.8% when the stocking density increased from 165/m_3 to 1018/m^3. Whereas in the present study shows that the survival was as high as 82% in *P. monodon* trial and 80% in *F. indicus* culture. Somocha and Benner (2001) reported the mean final weight of 0.12g after 34 days rearing in the raceway, but the yield was as high as 5.6kg/m^3, with the

estimated FCR of 0.8.In this study the final weight was higher than those reported by Samocha and Benner (2001). In trial I (*P. monodon*) the final weights were 0.414g and 0.645 g for the stocking densities of 2000/m^3 and 1000/m^3 respectively with the holding period of 43 days. In the second trial (*F. indicus*) the final weights were 2.23g and 1.47g for the stocking densities of 333/m^3 and 666/m^3 respectively, with the holding period of 55 days. The calculated FCR valves for the first trial were 1.68 and 1.79 for the stocking densities of 2000/m^3 and1000/m^3, respectively, where as in the second trial the estimated FCR values were 1.73 and 1.87 for the stocking densities of 333/m^3 and 666/m^3. Chien *et al*. (1989) reported that for the production of 1kg shrimp as high as 43m^3 of water was required in an intensive pond system. Hopkins and Villaton (1992) found only small correlation between estimated water usages per unit weight of shrimp. Hopkins *et al*. (1995) reported that high shrimp yield could be achieved without water exchange (7,000 kg/ha/crop). Since the main principle of the system was zero water exchange, the water required for the shrimp production was very low. Davis and Arnold (1998) concluded that *P. setiferus* grow faster in nursery raceway than of *L.vannamei*. From the comparison of two trials, the second trial with less stocking density paves final weight higher compared with the first trial.

5.3. Algae as bioremediation agent

James and Sweeney (1993) reported that the diatom is the preferable community in the pond ecosystem. It can also improve the shrimp performance by shading the bottom and removing toxic nitrogenous compounds. Mechling *et*

al. (1983) and Morita *et al.* (1974) reported that certain phytoplankton groups exhibit preference for ammonia. Avery and Aldrich (1988) observed that *Chaetoceros spp.* improve the water quality by assimilating ammonia for their cell wall development. Samocha *et al.* (2002) reported that nitrogenous waste from the raceway water was removed by algae and aquatic plants. Wheeler *et al*. (1982) explained that the inorganic nitrogen used most effectively by algae were nitrate and ammonia. Samocha *et al*. (2001) demonstrated that increase of algal concentration from 2.5 to 3.6x 10^5 cells/ml reduced the ammonia level considerably. Samocha *et al*. (2002) explained the addition of monoculture of *Chaetoceros muelleri* resulted in ammonia reduction control in nursery raceway system.

The present study dealt with the contribution of algae through bioremediation technique in managing the ammonia level in the raceway. It was noticed that lower than usual algal density resulted in higher ammonia concentration. This study confirmed ammonia level could be reduced significantly ($P<0.05$) with the addition of algae and microbial supplement in shrimp culture raceways.

5.4. Microbes as bioremediation agent in raceway system

Microorganisms, which are helpful in raceway system in the bioremediation process in shrimp ponds include bacteria such as *Bacillus, Pseudomonas, Acinetobacter, Cellulomonas, Rhodopseudomonas, Nitrosomonas, Nitrobacter,* etc., (Krom *et al* ., 1995). In the present study also,

the bacterial isolates identified were *Bacillus, Lactobacillus*, *Micrococcus, Staphylococcus, Vibrio* and *Pseudomonas*. Among the isolates *Bacillus* was a dominated bacterial isolate followed by *Vibrio spp.*

In contrast to our reports, some of the workers have found no benefits of bacterial flora in lowering total ammonia nitrogen concentrations (Boyd *et al.,* 1984). The reason may be due to the fact that the commercial products they tried might not have right strains of bacteria (Moriarty,1996). Yeast normally produce polyamines and some of these strains have a strong adhesion potential to intestinal mucous, an important condition for probiotic activity (Tovar *et al.,* 2002). He also noticed that that survival of larvae was considerably increased due to the effect of such products. Urono *et al.* (2002) demonstrated that yeast was characterized by decomposition of organic polymers such as proteins and reducing sugars. Felix and Samayakannan (2004.in press) reported that the use of yeast based fermented product in shrimp nursery raceway systems could control ammonia within the optimum levels throughout the crop duration. In this study we added yeast based fermented product (composed of yeast, wheat bran, sugar, jaggery, etc.,) to maintain the raceway system in heterotrophic condition.

In the present study we observed that the increased nitrite level might be due to the absence or inadequate level of nitrite-oxidizing bacteria, *Nitrobacter sp.* in the raceways. Frank *et al*. (1991) reported that whenever the concentration of nitrifying bacteria decreased, the heterotrophic bacteria were using mainly ammonium instead of nitrate as a source of nitrogen. Apart from that the trial

was carried out in rainy season, and this may also be the reason for increased ammonia and nitrite level recorded in the nursery raceways.

5.5. Total heterotrophic bactereia (THB)

Total heterotrophic bacteria in the first trial ranged between 7.8 x 10^3 to 7.4x10^4, while the count was ranged from 1.6x10^4 to 1.5 x 10^5 cfu/ml for the second trial. Jean et al. (1998) found the higher heterotrophic bacterial count in early phase of culture. In contrast to above report, our study showed that the higher THB count reported on the final phase of culture. There are reports supporting the findings of our results including Janahiram *et al*. (2000) reported the increase of bacterial load with respective increase of culture periods. Fonseka (1990) reported a total heterotrophic count of 5.0 x 10^2 to 8.8 x 10^3/ml from shrimp culture ponds in Srilanka. Qing yun et al. (1991) reported a relatively higher counts ranged from 1.2 x 10^5 to 7.6 x 10^5 / ml from the culture ponds of Indonesia.

5.6. Qualitative composition of bacterial flora

In the present study the bacterial colonies identified were Bacillus, *Lactobacillus*, *Micrococcus, Staphylococcus, Vibrio* and *Pseudomonas*. Among the isolates *Bacillus* and Vibrio combinely dominated up to 50% of the total population. Vanderzant *et al*. (1971) documented that microbial flora of shrimp pond water was usually dominated by *Coryneforms, Flavobacterium spp, Mornxella spp* and *Bacillus spp* . Dalmin *et a*l. (1997) reported that *Vibrio, Pseudomonas, Aeromonas* and *Corynebacterium* comprised the major portion of

micro flora of shrimp pond water. The present study showed that gram-positive population contributing more than 50% of total isolates. According to Otta *et al*. (1999) Vibrio accounted for more than 50% of the bacterial flora in various shrimp farms, whereas in this study it was around 20% of the total bacterial composition.

VI. SUMMARY

To assess the impact of unialgal concentrate and microbial load on nursery raceway rearing of penaeid shrimp with special reference to ammonia control, the present study was carried out. The adoption of raceways in rearing of penaeid shrimps has become inevitable due to the risk involved in culturing the shrimp in semi-intensive and intensive system owing to unforeseen and frequent disease outbreak which are mainly due to environmental degradation particularly by water quality. In addition, there is a necessity for overcoming disease problems without altering the aquatic environment. Further, raceways help to increase the survival of juvenile shrimps at the early phase of culture. The best alternative to resolve these problems is adoption of eco-friendly raceways for rearing of penaeid shrimp.

Experiments were carried out at the raceway farm complex recently established at Fisheries College and Research Institute, Thoothukkudi.

In trial 1 the raceways were stocked with *P. monodon* at two different stocking densities viz. 2000/m^3 and 1000/m^3 in raceway tank No. 1 and 2 respectively. The mean initial weight of post larvae used was 0.001g. After 46 days of rearing the final mean weight obtained were 0.413 g and 0.644 g for the stocking densities of 2000/m^3 and 1000/m^3 respectively. From the result it showed that the increasing stocking density has a tremendous effect on growth of the shrimps. Survival and FCR of 51.6% and 1.68 respectively were recorded with a total biomass of 0.718 kg/m^3 for the stocking densities of 200/m^3. The specific growth recorded was 0.131g. While for the stocking densities of 1000/m^3,

total biomass produced was 0.427g with survival and FCR of 82.0% and 1.79 respectively. The specific growth recorded was 0.109g.

With regard to ammonia control, the increased algal density and microbial load indicated positive results regarding control of ammonia. Abrupt changes in these factors resulted in increased ammonia level. By maintaining the algal density (by addition of algae periodically) and microbial load (by fermentation) the ammonia level could be controlled within 0.06mg/l. The highest ammonia level noted was 0.25 mg/l with respective algal density and microbial load at 4.5×10^4 cells/ml and 3.8×10^4 cfu/ml respectively, where as the lowest level recorded at 8.1×10^4 cells/ml and 8.3×10^4 cfu/ml respectively

In trial 2, the raceways were stocked with *F. indicus* at two different stocking densities viz. $333/m^3$ and $666/m^3$ in raceway tank No. 1 and 2 respectively. The mean initial weights of post larvae used were 0.136g and 0.125g. Nursery rearing was carried out for 53 days and the mean final weight obtained were 2.23 and 1.47g for the stocking densities of $333/m^3$ and $666/m^3$ respectively. From the results it showed that the survival was higher (80.95%) in low stocking densities i.e. $333/m^3$ and it was around 61.1% for the stocking densities of $666/m^3$. The biomass produced, SGR and FCR for the first tank were 0.60 kg/m^3, 0.053 g and 1.73 respectively. For the second raceway the biomass, SGR and FCR were 0.74kg/m3, 0.040 and 1.87 respectively.

With regards to bioremediation the lowest ammonia level noted was 0.13 mg/l, when the algal concentration and microbial load recorded 7.6×10^4 cells/ml and 5.9×10^4 respectively. The reduced levels of algae concentration and

microbial load resulted in increased ammonia level. It was confirmed that the ammonia level was significantly reduced ($p>0.05$) with increase of algae concentration and microbial load. The bacterial isolates identified in raceway include *Bacillus, Micrococcus, Staphylococcus, Arthrobacter, Lactobacillus, Vibrio* and *Pseudomonas* with the domination of *Bacillus* and *Vibrio.*

VII. REFERENCES

Allan, G.L. and Maguire, G.B., 1992. Effects of pH and salinity on survival, growth and osmoregulation in *Penaeus monodon* (Fabricus) *Aquaculture*. **107:** 33 – 47.

Allan, G.L., Moriarty, D.J.W. and Maguire, G.B., 1990. Effects of pond preparation and feeding rate on production of *Penaeus. Monodon,* Fabricus, water quality, bacteria and benthos in model farming ponds. *Aquaculture*. 329-349.

Anon, 1993. Bioremediation tecEhrlich, K.F.,Cantin,M.C. and Horsfall,F.L., 1988. Bioaugmentation:Biotechnolhnology for shrimp culture Does it work?.Asian shrimp news; **16(4):** 3.

Anon, 1995. Water treatement booster profits for Indonasian shrimp farmers, *Fish farming internationa*l **22(12):** 10-11.

Antonia, G.D.Y., David, B.R. and D. Allen, 2004. Influence of nursery period on the growth and survival of *Litopenaeus vannamei* under pond production conditions. *J. World. Aquacu. Soc.,* **35(3):** 357-365.

APHA., 1976. Compendium of methods for microbiological examination of foods. Speck. M.L (eds.) APHA, Newyork, 70p

Apud. T.D., 1985. Extensive and semi-intensive culture of prawn and shrimp in the Philippines, Tabuly, Primnera TH, Hobrern JA, etc. Proceedings of the 1st International conference on the culture of penaeid prawn shrimps. Phillipines IIoilo. SEAFDEC *Aquaculture Development*. 105-113.

AQUA COP, 1990. Freshwater prawns. In Aquaculture, Vol. 1, Gilbert Barnabe (Ed.),. Ellis Haqwood Ltd., England, 501-508.

Arrignon, J.C.V., Huner, J.V., Laurent, P.J., Griessinger, J.M., Lacroix, D., Gondovin, P. and M. Antarnd, 1990. Warm water crustaceans. Macmillan publication, London, p.46-91.

Austin, B.E., Bandet. and Stabie,M., 1992. Inhibition of bacterial fish pathogens by *Tetraselmis suecica*. *Journal of Fish Disease*. **15:** 53 – 61.

Avery, D.J. and Aldrich, D.V. 1988. Ammonia – ammonium nitrogen uptake by C*haetoceros gracilis* with applications in shrimp mariculture. *J. World Aquacult. Soc.,* **19:** 15A.

Avnimelech,Y., Lacher, M., Rarch, A. and O. Zur. 1981. A method for the evaluation of condition in a fish pond sediment. *Aquaculture.* **23:** 361-365.

Bart. R. and Arnold, C.R., 1992. The intensive culture of *Litopenaeus vannamei* Boon in a recirculating raceway systems. *Jour. World Aqua.,* **23(2):** 146-153

Boyd, C.E and Clay. J.W., 2002. "Evaluation of Belize Aquaculture,Ltd., A super intensive shrimp Aquaculture system". NACA, WWF and FAO consortium program on shrimp farming and the Environment. 1 – 17.

Boyd, C.E., 1989. Water quality management and aeration in shrimp farming, Fisheries and allied Aquaculture Departmental Series No.21, Alabama Agricultural Experimental Station, Arburn University, p. 70.

Boyd, C.E., Hollerman, W.D., Plumb J.A. and Saeed, M., 1984. Effect of treatment with a commercial bacterial suspension on water quality in channel cat fish ponds. *Prog. Fish. cult.,* **46:** 36 – 40.

Boyd,C.E. and. Watten B.J., 1989, Aeration system in aquaculture Rev. *Aquaculture.* **3:** 425 – 472.

Boyd,C.E.,1982. Water quality management for warmwater fish culture. Development in aquaculture and Fisheries science. Elsevier, Amsterdam, **9:** 318

Boyd. C.E. and Pillai. V.K., 1984. Water quality management in aquaculture. Central Marine Fisheries Research Institute. Special Publication. No.. **22,** pp 97.

Briggs, M.R.P. and J.H. Brown. 1971. Intensive rearing of post larval *P. monodon* in concrete nursery tank system. *Jour. of the world Aqua. Soc***., 22(3):** 16A

Bright S., Lakshmanaperumalsamy, P. and Chandramohan A., 1998, Bacterial flora of pond reared *Penaeus indicus* (Milne Edwards*), J. Aqua Trop.,* **13(2):** 133-142.

Catedral, F.F., Gerochi, D.D., Quibuyen, A.T. and Casalmir, C.M., 1977. Effects of nitrite, ammonia and temperature on *Penaeus. monodon* larva Q. Res. Rep. Southeast Asian Fisheries Development Center, 3rd Quarter**. (3):** 9 - 12.

Chakraborti R.I, Halder, D.D., Das N.K., Mandal S.K. and Bhomic M.L., 1986. Growth of *Penaeus monodon*(Fabricus) under different environmental conditions, *Aquaculture*. **151 (4):** 190-194.

Chang, W.Y.B. and H. Ouyans, 1988. Dynamics of dissolved oxygen and vertical circulation in fishponds. *Aquaculture*. 74: 263-276.

Chen, S.N., 1995. Currant ststus of shrimp aquaculture in Taiwan. In. Swimming through troubled waters. Proc. Of the special session on shrimp farming. C.L. Browdy and J.S. Hopkins (eds,). World Aquaculture Soc., 29-34.

Chien, Y.H., Liao,I.C. and Yang, C.M. 1989. The evolution of prawn growout systems and their management in Taiwan. In: K. Murray (eds)/ Aquaculture Engineering Technologies for the future. Hemisphere publ. New York, 143-168.

Chen, J.C., Chin. T.S. and Lee, C.K., 1986. Effects of ammonia and nitrite on larval development of the shrimp, *Penaeus monodon.* In : Maclean, J.L., Dizon. L.B. and Hosillos. L.V., (eds.). The First Asian Fisheries Forum, Asian Fisheries society, Manila. The Philippines. 657 – 662.

Chen, Yuwang and Zheng, Hemzhang, 1989. Water quality deterioration and its importance in Prawn culture *J. oceanogr*. **8(1):** 17 – 22.

Chin, J.S. and J.C. Chen, 1987. Acute toxicity of ammonia to larvae of the tiger prawn, *Penaeus monodon*, *Aquaculture*, **66:** 247 – 253.

Chin,Y.N.,1988. Water Quality management for intensive prawn ponds. In: Technical considerations for management and operation of intensive prawn farms, pp 102 – 128.

Clark, S.N., 1986. Inhibition of moulting in Penaeus semisulcatus (De Haan) by longterm hypoxia. Aquaculture**. 52:** 253-254.

Clay brook, D.L., 1983. Nitrogen metabolism, In: mantel, L.H., (ed.), The Biology of Crustacean, Vol. 5 Internal anatomy and Physiology of regulation. Academic Press, New York, 166 – 213.

CICTUS. 1983. Shrimp culture “unidad experimental peasio” Centro de investigaciones cienficas y technologicas de la universidad sonoro, Mexico

Clifford, H.C., 1985. Semi intensive shrimp farming. In: Texas shrimp farming manual, Chamberlain, G.W., Haby, M.G. and Miget, R.J., (eds.), Texas agricultural extension service, Corpus Christ, 15.

Cohen,J.M., Samocha,T.M., Gandy, R.L., Jones,E.R.,Fox,I.M. and A.L. Lawrence. 2001. The culture of *Llitopenaeus vannamei* under bio-secured and zero-exchange conditions. *Aquaculture:* 130A

Collins, C.H. and Lyne, P.M. (1989). Microbiological Methods. 4th edn. Butterworths. London. 409.

Colt, J.E. and Armstrong, D.A., 1981. Nitrogen toxicity to crustaceans, fish and molluscs. In: Allen, L.J., Kinney E.C., (Editors). Proceedings of the Bio – Engineering symposium for fish culture. Fish culture section, American Fisheries society, Northeast society of conservation Engineers, Bethesda, M.D, pp. 34 – 47.

Cook, H.L. and Murphy M.A., 1966. Rearing penaeid shrimp from eggs to post larvae proc. 19th Ann. Conf. S.E.A.H Game and fish Comm. **19:** 283-288.

Cordover, R., 1989. Prawn farming. The Australian way. Australian Aquaculture magazine, **3(7):** 8-10.

Dalmin, G., Purushothaman, E., Kannapiran, E., Sankar, G. and K. Srinivasan, 1996. Microbiology of a shrimp pond with special reference to total heterotrophic, coliform and beneficial bacteria. Paper presented at National seminar on water quality issues in aquaculture system held at Fisheries Cillege and Research Ins., Tuticorin, Abstract No. WQ1. 03.

Davis, A.D. and Arnold, C.R., 1998. The design, management and production of a recirculating raceway system for the production of marine shrimp. *Aquaculture Engineering.* **17:** 193 – 211.

Donbrovsky,A., Paynles, J.L., Sambhi,S.K., Atherton,I.G. and Leaster,R.J.G., 1988. Observation on the ultra structure of baculovirus in Australian *Penaeus monodon* and *Penaeus mergiunsis. Jour. Mar. Freshwater.Res.* **39:** 743-749.

Durairaj, S., Chinnasamy G., Sayed. M. and Mohammed. 1983. A bacteriological study of the natural flora of edible oysters, *Crasostrea madragensis*. *Fish Technol.,* **20:** 111 – 114.

Ehrlich,K.F., Cantin,M.C. and Horsfall,F.L., 1988.Bioaugmentation: Biotechnology for improved aquaculture production and environmental protection. In: Aquaculture engineering technologies for the future. The institution of chemical engineers, Rugby,U.K. Hemisphere publishing corporation. A member of the taylor and francis group, Newyork, Washington, London, 329-341

Farmantarmaian, A. and Moore, R., 1978. Seasonal thermal aquaculture I- Effects of temperature and dissolved oxygen on survival and growth of *Macrobrachium rosenbergii. Proc. World Maricu. Soc*.: 55-66.

Fast, A.W., 1991. Marine shrimp Pond grow out conditions and strategies: A review and prognosis *Aquatic sciences***. 3:** 357 – 400.

Egusa, S., 1961. Studies on the respiration of the kurima shrimp, *Penaeus japonicus,* Preliminary experiments on its oxygen consumption. *Bull. Jap. Soc. Sci Fish.*, **27:** 650-659.

Felix, S. and Samocha, T.M., 2001. Third generation shrimp farming technology to revive shrimp farms in India. Infofish International, 4 : 50-55.

Felix.S. and Samayakannan, M., (2004). Biosecured and improved penaeid shrimp produciton through organic nursery raceway system in India. *Aquaculture Asia* (in press).

Flynn, K.J., Fasham,M.J.R. and C.R. Hipkin. 1997.Modelling the interaction between ammonium and nitrate uptake in marine phytoplankto. Phil. Trans. Res. Soc.London. **352:** 1-22.

Fonseka, T.S.G., 1990. Microbial flora of pond cultured prawn. Papers presented at 7th session of the Indo Pacific fish. Comm. working party on Fish technology and marketing. Bangkok. Thailand, 24 -31.

Foster, L.R.M. and Beard, T.W., 1974. Experiments to assess the suitability of nine species of prawns for intensive cultivation. *Aquaculture***. 3:** 355-368.

Frank, T., Verhagen,M. and Laanbrock, H.J., 1991. Competition for ammonium between nitrifying and heterotrophic bacteria in dual energy limited chemostats. *Applied and Environmental Microbiology*, **57(11):** 3255-3263.

Funge- Smith,S. and Hawthorn,S., 1996. The effect of bacterial remediation products on the water quality inthr presence of shrimp pond sediments. *World Aquaculture Society*, 133A

Gunter, G., 1950. Seasonal population changes and distributions as related to salinity of certain invertebrates of the Texas coast. including the commercial shrimp. Publs. Ints. Mar. Sci., Unive Tex., **1(2):** 7 – 51.

Hameed, A.S.S., 1993. A study of the aerobic heterotrophic flora of hatchery reared eggs, larvae, post larva of *Penaeus indicus*, *Aquaculture.* **117:** 195-204.

Harrison, W.G. 1983. The time course of uptake of inorganic and organic nitrogen compounds by phytoplankton from the eastern Canadian Arctic: a comparison of temperate and tropical populations. *Limnol. Oceanogr*. **28:** 1231-1237.

Hirono, Y., 1986. Shrimp farm management in Ecuador, in proceedings of the Southeast Asia shrimp farm management workshop Dean Akiyama (Edi) American Soybean Association.

Hopkins, J.F., Baird, M.L., Grados O.G., Maieri P.P., sandifer, P.A. and Stokes A.D., 1988. Impacts of intensive shrimp production on the culture pond ecosystem. *J. world Aquacult Soc.* **19 (1)** : 37A.

Hopkins, J.S. and J. Villaton. 1992. Synopsis of industrial panal input on shrimp pond management. In: J. Wyban (eds.), Proc. Of the special sesson on shrimp farming. Louisiana. *World Aquaculture. Soc.* 138-143.

Hopkins, J.S., Devoe, M.R., Holland, A.F., Browdy, C.L. and A.D. Stockes. 1995. Environmental impacts of shrimp farming with special reference to the situvation in the continental United Statews. *Esturies* 18: 25-42.

Hudinaga, H. 1942. Reproduction, development and rearing of *Penaeus Japonicus* Bate. Jpn. *J. zool*. **10:** 305 – 393.

James.W and Sweeney. J. 1993. Intensive shrimp production in round ponds. *CRC Handbook of mariculture*. Vol. **1:** 275 – 288.

Janakiram P., Jayasree L. and Madhavi R., 2000. Bacterial abundance in modified extensive and semi-intensive shrimp culture fond of *Penaus monodon*. *Indian Jour mar. Sci.* **29:**319-323.

Jawahar, A., Shanmugam, S.A., Planoappan, R. and K. Dhevendran., 2003. Distribution and abundance of luminous bacteria with special reference to shrimp farming activities. *Indian Jour. of Mar. Sci.,* 32(3): 208-213.

Jean L.M., Yves V., Oliver G. and Dominque, P., 1998, Shrimp rearing: Stocking density, growth, impact on sediment, waste output and their relationships studied through the nitrogen budget in rearing ponds. *Aquaculture.***139:** 55-62.

Kaneko, T. and R.R. Colewell, 1973. Ecology of *Vibrio parahaemolyticus* in Chesapeake bay. *Microb. Ecol*.**4:** 135-155.

Kelly, M.T., 1982. Effect of temperature and salinity on Vibrio (Benackae) vulnificus occurrence in gulf coast environment. *Appl. Environ. Microbiology*, **44 (4):** 820 – 824.

Krom,M.P., Ellner,S., Rijn,J.V. and Neori,A., 1995.Nitrogen and phosperous cycling and transformation in a prototype non- polluting integrated mariculture system. Eliat, *Israel. Mar. Ecol.prog.Ser.,* **118(1-3):** 25-36

Kungvankij, P., 1985. Biology and culture of sea bass (*Lates calcarifer*). Paper submitted to the second international conference on warm – water Aqua culture, Fin fish, Bright young University, Laie, Hawaii, 5-8

Lawrance, A.L. and Huner, J.Y., 1987. Penaeid shrimp culture in the united states: a brief overview stressing species, seed production and grow out. In: Sinderman, C.J.(eds.), Reproduction, maturation and seed production of culture species. United states, Dept. of Commerce. NOAA Technical report. 31-41.

Leung, P.S. and Moss, S.M., 1999. Economic assessment of a proto type biosecure Shrimp grow out facility. In controlled and Biosecure production system : Proceedings of a special session – Integration of shrimp and chicken models (R.A. Bullis and G.D. Pruder, eds.), The Oceanic Institute, Hawaii, USA , p 97 – 106.

Liao,I.C., 1977. A culture study of grass prawn, *Penaeus monodon* in Taiwan-the problems and prospectus. *J. Fish. Soc*. **5 (2):** 11-29.

Liao,I.C., 1988. Feasibility study for alternative culture species in Taiwan *Penaeus penicillatus*. *Jour. of the world Aqua. Soc*. **190:** 227.

Liao.L.C. and Huang,T.L., 1975. Experiments on propagation and culture of prawns in Taiwan. In: Coastel aquaculture in the indo- pacific region. Pillay.T.V.R., (ed.), Fishing News books, Faraham, England-368.

Macduff, J.H., Hooper, M.J. and A. Wild. 1987. The effects of root temperature on growth and uptake of ammonium and nitrate by Brassica napus. *Jour. Exp. Bot.,* **38:** 53-66.

Macia P.P. 1983 Penaeus shrimp pond grow out in panama. In: J.P. M.C. Vey , *CRC Handbook of Mariculture* Volume 1, crustacean Aquaculture , CRL Press., Bola Raton, FLUSA. 169-178.

Mandal, L.N., 1962. Nitrogenous fertilizers for brackish water ponds. Ammonia or nitrite form. *Indian Jour. Fish.* **9(1A):** 123-134

Manik, R., Kahar, R. and Tiensegrusmee, B., 1978. The culture of shrimp, *Penaeus monodon* in low saline pond. *Bull. Brakish water Aquaculture Development Center*. **41( ):** 288-292.

Mechling, J.A. and S.S. Kilfam.1983. Temperature effects on silicon limited growth of the lake Michigan diatom *Stephanodiscus minultus* (Bacillariophyceae).*Jour. Phycol*., **18:** 119-205.

Menz. A. and B.F. Blake. 1980. Experiments on the growth of *Penaeus vannamei* Boon. *Jour. Experi. Mar. Biol. and Ecol.* **48:** 99 – 111.

Mevel, G. and Chamroux, G., 1981. A study on nitrification in the presence of prawn (*Penaeus monodon)* in marine closed system. *Aquaculture*., **23:** 29 – 43.

Michael P.M., and Andrew L., 1997. Southern Regional Aquaculture Centre : In Pond raceways. Pg : 1-8.

Moriarly, D.J.W., 1996. Microbial Biotechnology a key ingredient for sustainable aquaculture. *INFOFISH international.*, **4/96:** 29-33.

Millamena, O.M., 1990. Organic pollution resulting from excess feed and metabolic build up: effect on *Penaeus monodon* postlarvae, Aquacul. Eng**., 9:** 143

Morita, R.Y. and G.E. Buck. 1974. Low temperature inhibition of substrate uptake, In R.R.Colewell, andR.Y. Morita (ed.), Effects of ocean environment on microbial activities. University Park Press, Baltimore. 124-129.

Moss S.M., 1994.Growth rates, nucleic acid concentrations and RNA IDNA ratios of juvenile white shrimp *P.vannamei* . Boone feed different algal diets. *Jour. Experi.Mar. Biol. and Ecol* ., **182:** 193-204.

Mrithunjayan, P.S., and Thampy, D.M.,1986 Leaching of Soil from the bunds as a factor responsible for the lowering of pH of water in brackish water ponds. Proc. symp. Coast. Aquaculture**, 4:** 1131 – 1135.

Nair, S.R.S. and Krishnankutty, M. 1935. Note on the varying effects of salinity on the growth of the juveniles of *Penaeus indicus* from cochin backwater. Bull. Dept. Mar. Sci. Univ. Cochin, **7 (1):** 181 – 184.

Nwadiuto,E. and Koske,Y., 2003. Analysis of bacteria associated with gut of healthy wild penaeid shrimps. A step towards effective probiotic in aquaculture. *J.Aqua .Tropics*, **18(4):** 275-286.

Navas. K.A. and Sebastian, M.J., 1989, Effects of low salinities on the survival and growth of *Penaeus monodon* Fabricus *Indian .Fish.,* **36 (3):** 257-261.

Nedwell,D.B. and M. Rutter. 1994. Influence of temperature on growth rate and competition between two psychrotolerant Antartic bacteria: low temperature diminishes affinity for substrate uptake. *Appl.Environ. Microbiol.,* **60:** 1984-1992.

New M.B. and Singholka S., 1982. Freshwater Prawn during in: Manual for the culture of *Macrobrachium rosenbergii* food and agricultural organization of the United nations. Rome. 116.

New M.B., 1987. Food and feeding of fish and shrimp. *A manual on the preparation and presentation of compound feeds for shrimp and fish.*

New, M.B., 1988. Fresh water prawns: Status of Global Aquaculture, 1987. NACA Tech. Manyal 6. UNDP/FAO RAS/86/047, Bangock,,Tahiland, 58pp

New,M.B. and H.R, Rabanal, 1985. A review of the status of penaeid aquaculture in south East Asia. In. Rothlisberg, P.C., Hill, B.J. and Staples, D.J., (eds), Second Australian Prawn seminar. 307-326.

O' brien, C.A.and Sigemore, R.K., 1979. Distribution of the luminous bacteria *Vibrio harveyi* in a semi tropical esturine environment. *Appl. Environ .Microbiol.,* **38:** 928-933.

Ogle, J.T., and J.M. Lotz, 1998. Preliminary design of a closed, biosecure shrimp grow out system us marine shrimp Farming Program Biosecurity Workshop. The oceanic Institute, Makapnu point, Hawii.

Oliver, J.D., 1982. Taxonomic scheme for theidentification of Marine bacteria. *Deep sea Research.* **22(6A):** 795-798.

Otta S.K., Indran, K., Tanro, P. and Karunasager, I., 1999. Microbial diseases of shrimp. Review on *Indian Journal of microbiology.* **38:** 113-125.

Paquotte,P., Chin,L., Martin, J.L.M., Lemos,E.,mStern, M. and Tosta, G., 1998. Intensive culture ofshrimp *Penaeus vannamei* in floating cages: Zootechnical, economic and environmental aspects. *Aquaculture.* **164:** 151-166.

Parameswaran, S., Monbodh, M. and K. Hawgibhay, 1992. Aquaculture of The Fresh Water Prawns. E.G. Silas (Ed). P.10-22 . Proc*. Natl. Symp on fresh-water prawns*, *Macrobrachium spp* KAU. Trissur.10-22.

Patrick, F.M., 1978. The use of membrane filtration and marine agar 2216 E to enumerate marine heterotrophic bacteria, *Aquaculture.* **13:** 369 – 372.

Prem A., Petterson J.K. and Ayyakkannu K., 1996. Monitoring of a shrimp culture system with special reference to vibrio and fungi. *Indian Journal of Marine Sciences.* **22:** 253 – 258.

Qing Yun, S., Lu Ying. and Syn Xiuqin., 1991. Experimental reports on quantitative distribution of microbes in culturing environment of *Penaeus, orientalis* in show growing country, Shandong, Province. J. Ovan, G. Huanghni Bohai seas Huangbohai Haiyang. **9 (3):** 64 – 68.

Rajyalakshmi, T., 1980. Manual on Brackish water Aquaculture *Bull. Cent. Inland Fish Res.Inst*. India , **31:** 126.

Ravichandran, P., Verghese, P.U. and Rajalakshmi. T., 1982. Effect of stocking density and size of *penaeus monodon* (Fabricus) post larvae in nursery rearing experiment. In. Proc. Symp Coastel Aquaculture Cochin, India Part I Prawn culture Mar Biol. Assoc. India, Cochin. 412-413.

Rijn, J.V., and M. Shilo., 1989. Environmental factors in fish culture systems. In: M. Shilo and J. Sarig, (eds). Fish culture in warmer systems: Problems and Trends, CRC Press, Bola Raton, FL USA. 163-179.

Samocha,T.M., Hamper, L., Emberson, C.R., Davis, A.D., McIntosh, D., Lawrence, A.L.and Van wyk, P.M., 2002. Review of some recent developments in sustainable shrimp farming practices in Texas, Arizona and Florida. *J. Appl. Aquacult.,* **12(1);** 1-42.

Samocha, T.M., A.L.Lawrence and W.A. Bray. 1993. Design and operation an intensive nursery raceway system for Penaeid shrimp. In: J.P.McVey, and J.R. Moore, (eds.), *CRC Handbook of Mari culture,* Vol. I Crustacean Aquaculture. CRC Press, Boca Raton, FL, USA. 173-210.

Samocha, T.M., Benny, R., 2001. Use of intensive nursery systems in commercial shrimp production operation in Texas. Industry Briefs Vol. **7(1):** 1-3.

Samocha, T.M. and Lawrence, A.L., 1992. Shrimp nursery systems and management. In: wyban. J., (Eds,). Providing of the special session on shrimp farming. *World Aquaculture society.* Baton Ronge. USA. 87 – 105.

Samocha, T.M., L.Hamper, C.R. Emberson, A.D. Davis, A.Balafa's, D.McIntosh, A.L. Lawrence, P.G. Lee & G.F.Wood. 1999. Potential tools for development of a sustainable and biosecured shrimp farming industry. Proceedings of the fifth Ecualorian Aquaculture Conference. October 28 – 30, 1999. Expoplaza, Guayaquil, Ecuador.

Samocha, T.M., Blacher, T.and E. Estrada. 2000a. High-density nursery of *Litopenaeus vannamei* in raceway system with limited water discharge in white spot infected area. Paper presented at the 4th Latin American Aquaculture Congress and Exhibition, 2000, ATLAPA Convention Center Panama City, Panama.

Samocha, T.M., Blacher, T., Cordova, J. and A. Dewind. 2001. Use of intensive nursery raceway system with limited water discharge to improve production of *Litopenaeus vannamei* in white spot infected area in

Ecuador. Abstract of a paper presented at the Aquaculture 2001, Annual Meeting of World Aquaculture Society, 2001, Orlando, FL.

Sandifer, P.A. and smith I.I.J. , 1976 . Effects of population density on growth and survival of *Macrobractium rosenbergii* reared in recirculating water management systems. Proc. 6th Annu. Workshop word mariculture SOL, seattle , W.A. 27-31.

Sandifer, P.A., Hopkins, J.S. and Stocks, A.d., 1987.Intensive culture potential of Penaeus vannamei. *J.World. Aquacu. Soc.,* **18(2):** 94-100

Sankaranarayanan, V.N., Kumaran,S., Balasubramanian,T., Rosamma Stephen and Parampunnayil,S.V., 1982. Studies on the Enviornmental conditions of tidal ponds in the Ramanthurth Island (Cochin). Proc. *Sym. Coast. Aquaculture.* 1: 362 – 368.

Sarada, P.T and Pillai, K., (1993). Studies on the effects of the temperature and pH on the post larvae of *Penaeus. monodon*. (H.Milne Edwards.) CMFRI *spl.publ*. **53:** 30-36.

Sastry, A.N. and Vargo, S.L., 1977. Variation in the physiological responses of crustaceans larvae due to temperature. In: F.J. Vernberg. A. Calabrese. F.P.Thurberg and W.B. Vernberg (eds.), Physiological responses of marine biota to pollutants. Acadamic Press, Newyork, 401-424.

Sebastian, C.D., Mathew,G. and K.C. George, 1992. Intensive farming of freshwater Prawn in Kerala – A preliminary study. In freshywater Prawns E.G. Silas (Ed) Proc. Natl. Symp. *On Freshwater Prawns*, *Macrobrachium spp* KAU Trissur.206-209

Shijueno.K., 1945. Shrimp culture in Japan. Association for International Technical Promotion, Tokyo, Japan, 153.

Simon C.E. and Wade W., 1994. Comparison of raceway and cylindroconical tanks for brackish water production of juveniles. Florida Red Tilapia under high stocking densities. *Aqua. Engineering.* **139:** 59-69.

Smith V.H.1993, Implications of resource ration theory for microbial ecology, *Limnol Oceang.,* **38:** 239 – 249.

Smith, T.I.J. and P.H. Sandifer, 1979. Development and potential of nursery systems in the farming of Malaysian prawn *Macrobrachium_rosenbergii*. *Proc.World Maricult. Soc.,* **10:** 369-389.

Spotte, S., 1970. Fish and invertebrate culture: water management in closed system: First Edition, wiley,Newyork: 144.

Stainer, R.Y., Aderlberg, E.A. and Ingrham, J.L., 1976. General microbiology, Maemillan, London, 71.

Stables, D.J. and Heales, D.S. 1991. Temperature and salinity optima for growth and survival of juvenile banama prawns, *Penaeus merguinsis*. Jour. of Exp. Mar. Bio**., 154:** 251-275.

Stern, S. and E. Letellier. 1992. Nursery systems and management in shrimp farming in Latin America. In: Proceedings of Special session on Shrimp Farming. World Aquaculture Society Annual meeting. Aquaculture'92. 22-25 May. Baton Rouge, Louisiana, USA. 106-109.

Sturmer,L.L., Samocha, T.M. and Lawerence, A.L.1992.Intensification of penaeid nursery systems. In: A.W. Fast and L.G. Lester, (eds,). Culture of marine shrimp : Principles and practices. Elsevier, Amsterdam. 323-346.

Subramanyan, M., 1973. Experimental studies on growth in *Penaeus monodon* Fabricius. Proc. seminar on mariculture and mechanised Fishing. Madras, India, 1973, 70-73

Tanaka, N., Sugiyama.M. and Ohwada.K., 1981. Bacterial population and their growth rates in fish rearing tank with running water. *Bull. Natl. Res. Inst. Aquaculture*. **2:** 65 – 71.

Thruston, R.V., Russo, R.C. and Vinogradov, G.A., 1981 Ammonia toxicity to fish effect of pH on the toxicity of the un – ionized ammonia species. *Environ Sc – Technol.*, **15 (7):** 837 – 840.

Thomas, W.H., and Dodson, N.N. 1974, Effects of interactions between temperature and nitrate supply on the cell division rates of two marine phytoflagellates. *Mar. Biol*., **24:** 213-217.

Thurston, R.V., 1998. Some factors affecting the taxicity of ammonia to fishes. In proceedings of the third us USSR symposium of the effects as pollutants, upon equatic ecosystem (ed, Swain, W.R and Shannon. V) . Borok, USSSR . 118-137

Tovar, D., Zambonino, J., Cahu, C., Gatescoupe F.J., Vazquez R. and Lesel. R. 2002. Effects of live yeast incorporation in compound diet on digestive enzyme activity in seabass (Dicentrarchus labrax) larvae. *Aquaculture.* **204:** 113- 124.

Tsai, C.K., 1989. Water quality management, Proceedings of South east Asia shrimp farm management workshop, Philippines, Indonesia, Thailand, July 26 Aug 11. 56 – 63.

Tsai, C.K., 1990 water quality management In: Proce. South Cast. Asia Shrimp Farm management workshop (ed. D.m Akiyama) Phillipines, Indonesia, Thailand, 26th July. 11 August 1989, Amercian Soyabean Association, Sigapore. 56-63.

Tseng, K.F., H.M. Su. and M.S. Su., 1998. Culture of *Penaeus monodon* in a recirculating system. *Aqua. Engineering*. **17:** 138-147.

Tucker, C.S. and Lloyd, S.W., 1984. Phytoplankton. communities in channel cat fish ponds. *Hydrobiologia*. **112:** 137 – 141.

Urono, N., Sasak, E., Veno, R., Namba, H. and Uhida, Y., 2002. Bioremediation of fish cannery wastewater with yeast isolated from drainage canal. *Marine Biotechnology.* Vol. **4(6):** 559 – 564.

Vanderzant, C., Cobb. B.F. and C.A. Thomson, 1973. Microbial flora, chemical characteristics and shelf life of four species of pond reared shrimp *J. milk food technol*., **36 (9):** 443 – 446.

Vanderzant, C., Nickelson, R. and P.W. Judkins 1971. Microbial flora of pond reared brown shrimp *Penaeus azetecus*. *Appl. Mirobiol*., **33 :** 916 –929.

Van Wyk, P. 1999. Farming Marine shrimp in Freshwater: An economic Development strategy for Florida in Proc. Production of marine shrimp in freshwater recirculating aquaculture systems. Florida Dept. of Agri. And Consumer Services. Bob Crawford. Tallahasse, USA 1- 32.

Venkataramanan , L.V., Becker, W. E., Khanum, P.M. and Mathew, K.R., 1977. Short term feeding of algae *Scenedesmus acutus* processed by different method: growth pattern and histopathological studies*. Nutr. Rep. Int.,* **16:** 231-240.

Westin,D.T. 1974. Nitrite and nitrate toxity to Salmonoid fishes. *Prog.Fish Culture.* **36 (2):** 86-89.

Wheeler, P.A., Gilbert, P.M., and McCarthy J.J. (1982). Ammonia uptake and incorporation by Chesapeake Bay phytoplankton: short term uptake kinetics. *Limnol. Oceanogr*., **27**: 1113 – 1128.

Wickins, J.D., 1976. The tolerance of warm water prawn to recirculated water. *Aquaculture*. **9(11):** 19 – 37.

Williams,A.S., Davis, D.A. and C.R. Arnold. 1996.Density dependent growth and survival of Penaeussetiferus and Penaeus vannamei in a semi closed recirculating system. *Jour. World Aquacu. Soc.,* **27(1):** 107-112.

Wyban,J.A. and Sweeney, J.N., 1989. Intensive shrimp growout trial in a round pond. *Aquaculture*. **76:** 215-225.

Zobell, C.E., 1941. Studies on Marine bacteria (1) the cultural requirements of heterotrophic Species*. J. Mar. Res.,* **4:** 42 – 75.

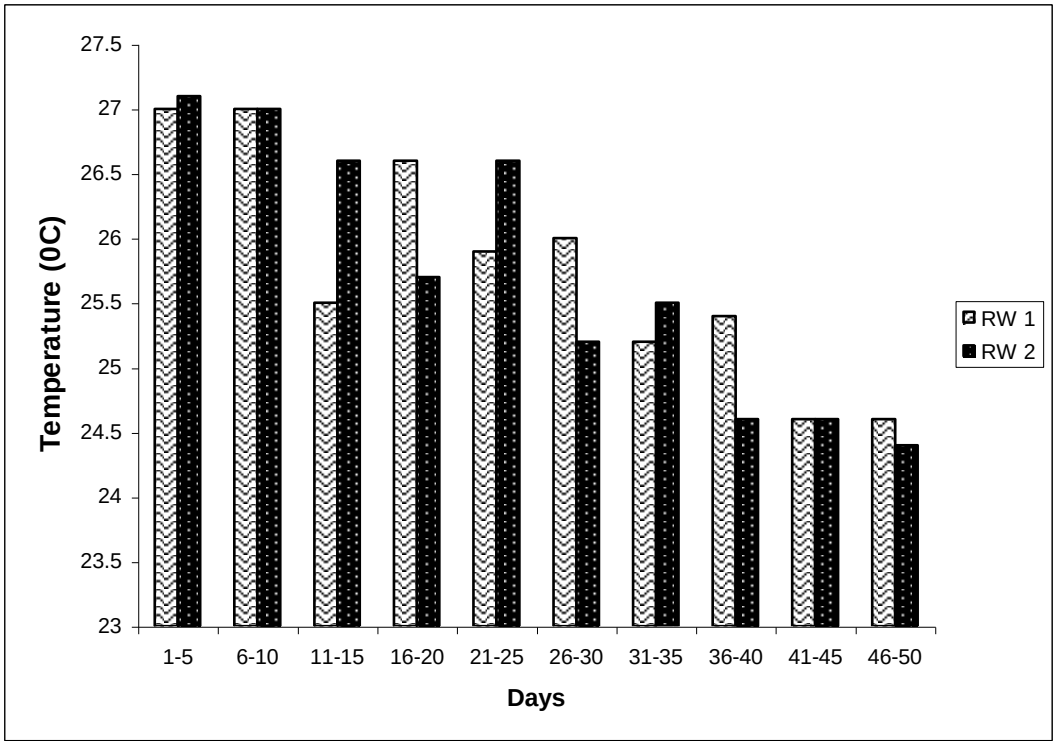

Fig. 4.5. Temperature recorded in raceway 1 and 2

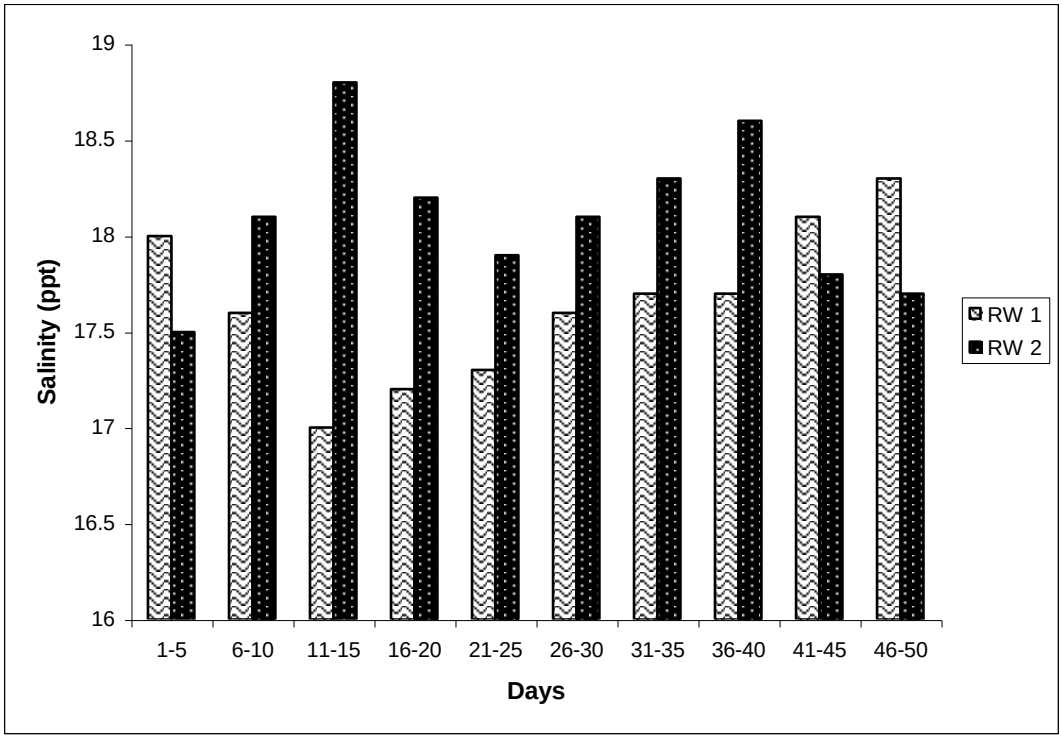

Fig. 4.6. Salinity recorded in raceway 1 and 2

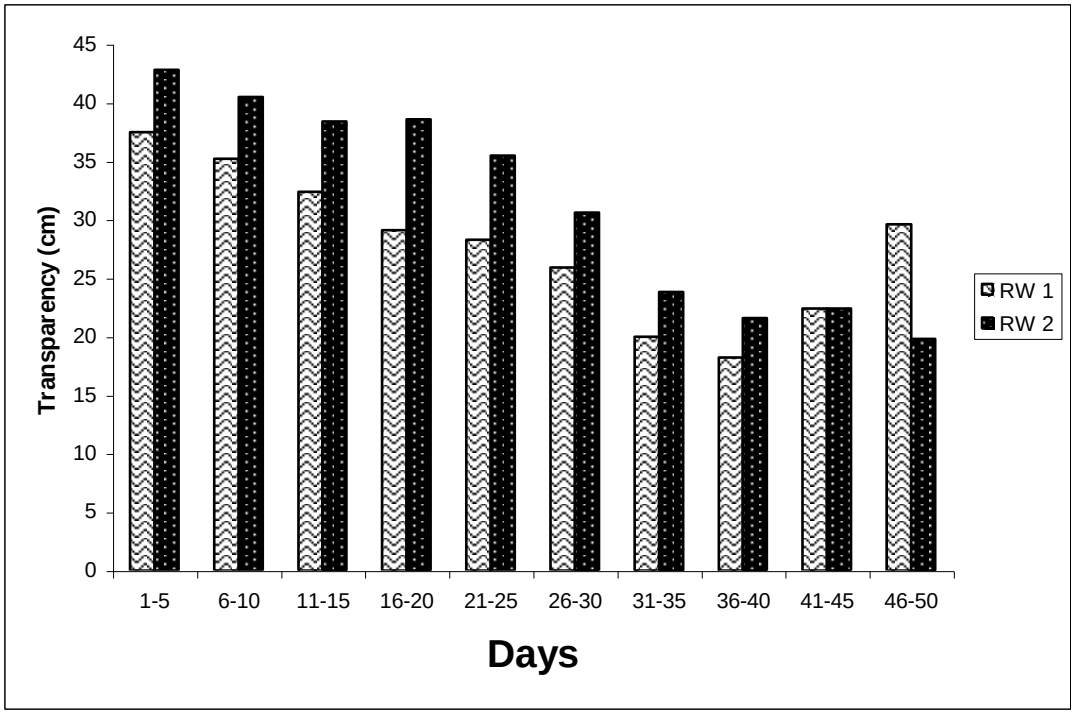

Fig. 4.7. Transparency recorded in raceway 1 and 2

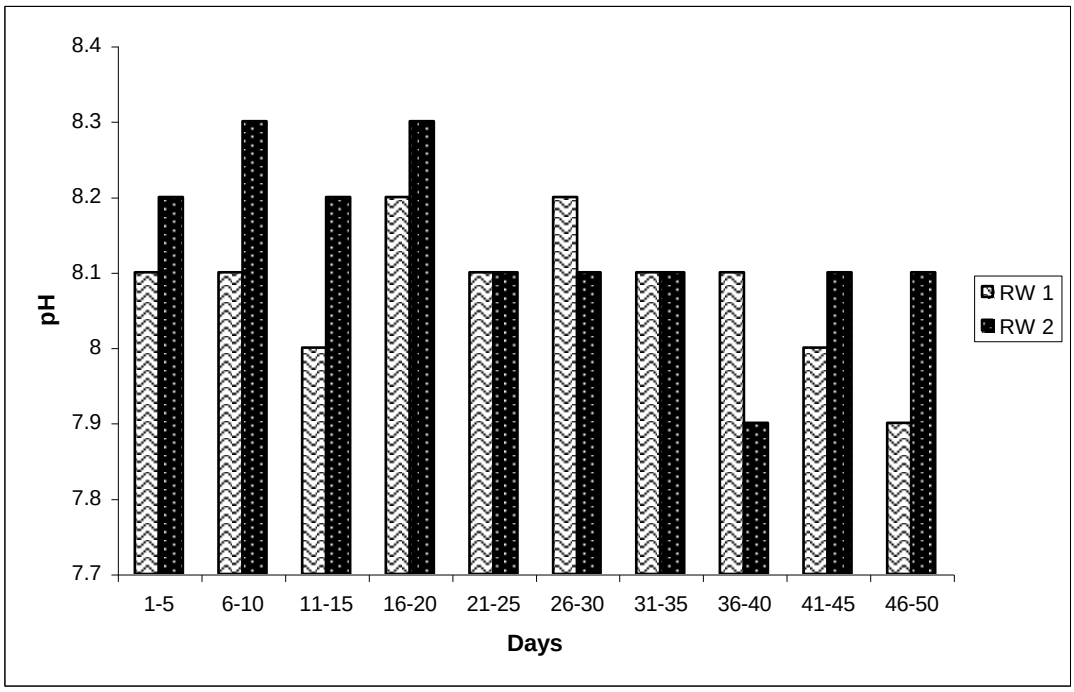

Fig. 4.8. pH recorded in raceway 1 and 2

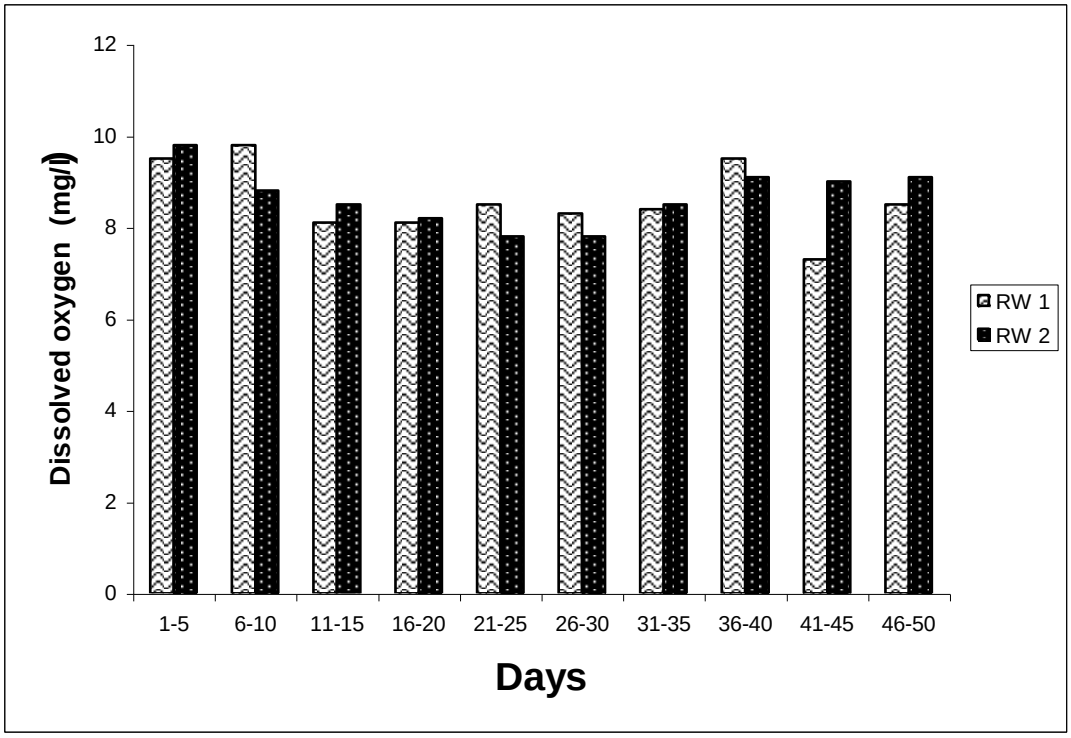

Fig. 4.9. Dissolved oxygen recorded in raceway 1 and 2

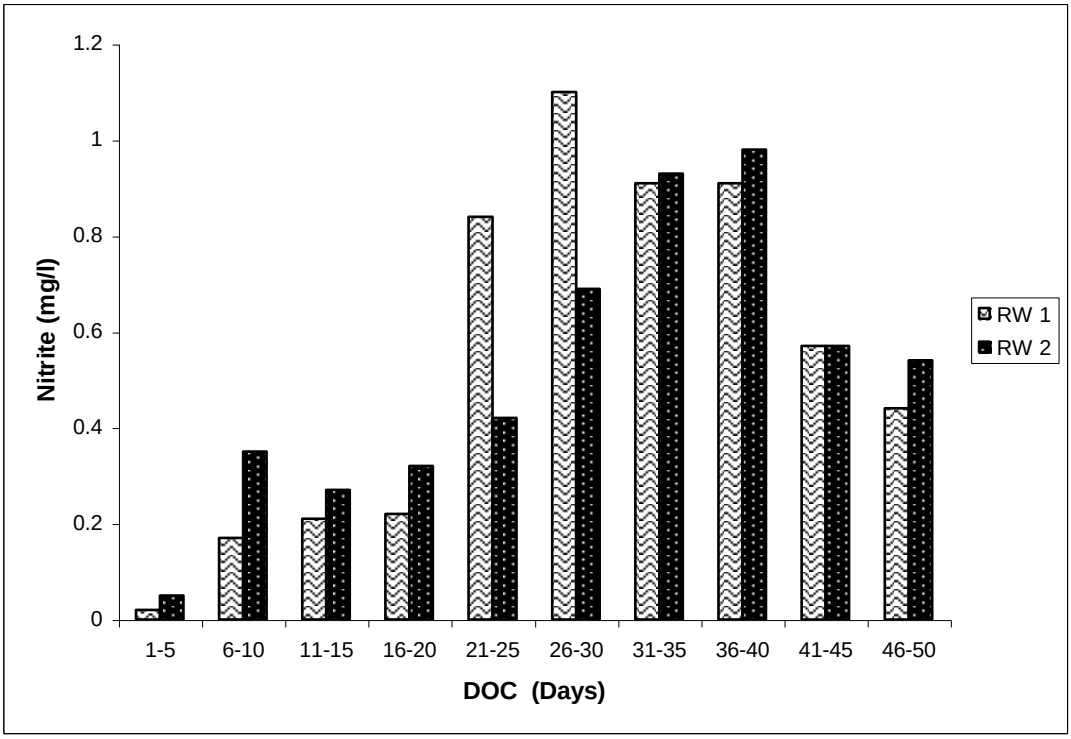

Fig. 4.10. Nitrite recorded in raceway 1 and 2

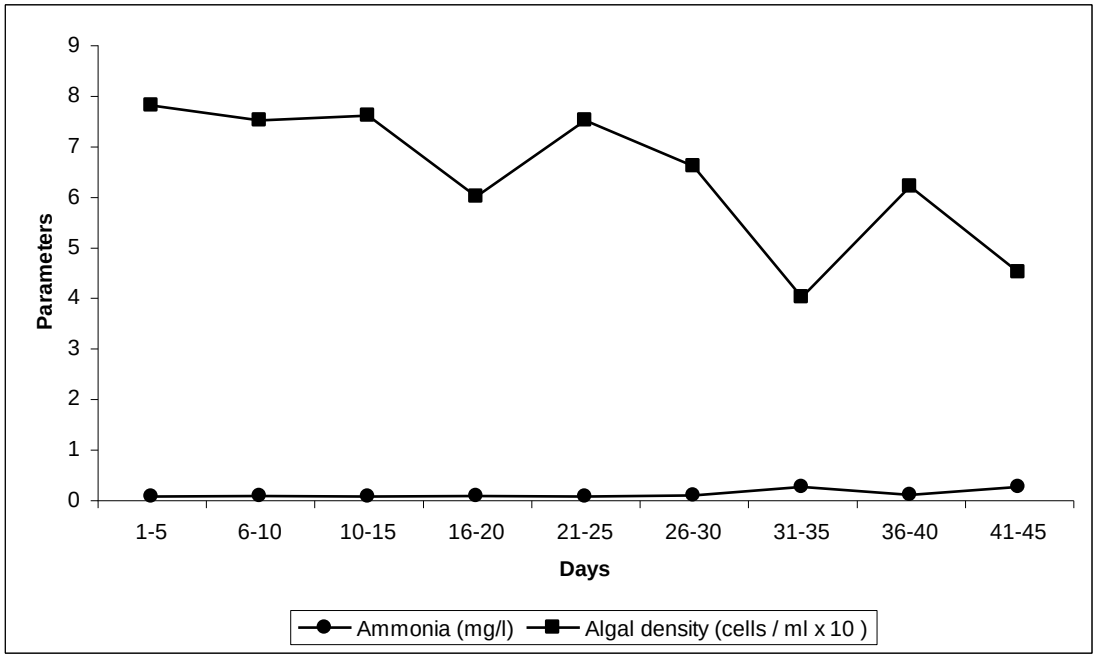

Fig. 4.1. Ammonia vs. algal concentration in raceway 1

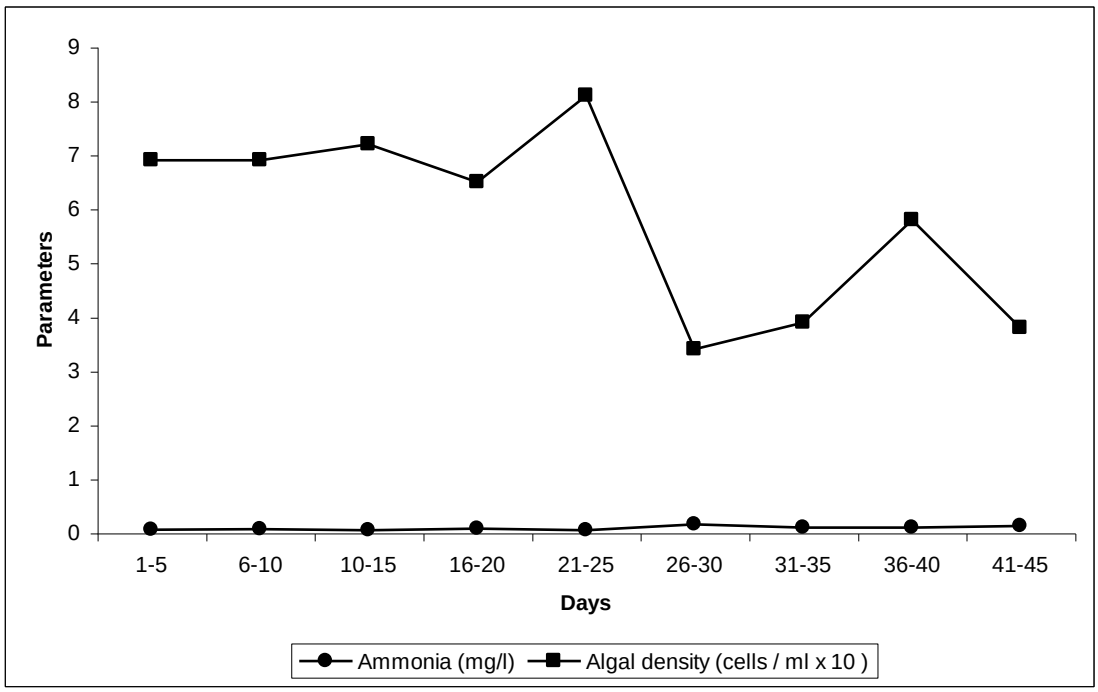

Fig. 4.2. Ammonia vs. algal concentration in raceway 2

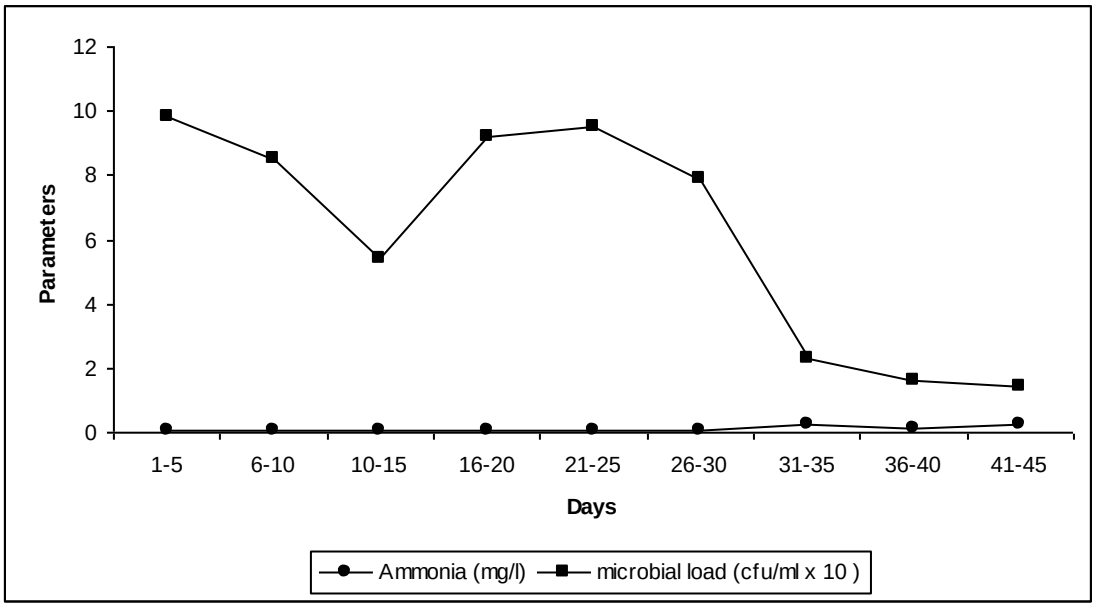

Fig. 4.3. Ammonia vs. microbial load in raceway 1

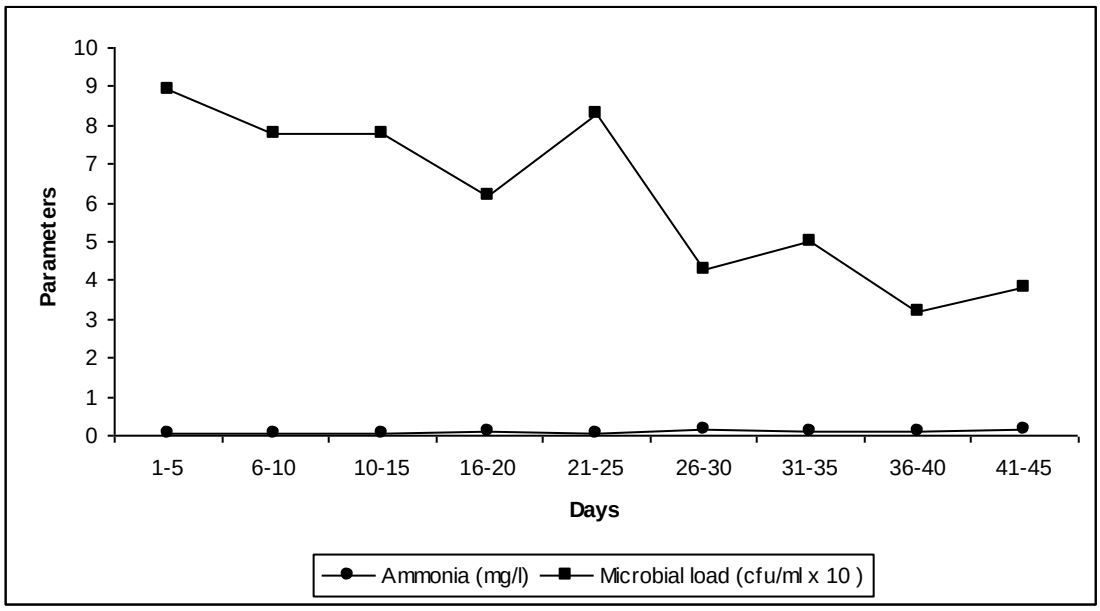

Fig. 3.4. Ammonia vs. microbial load in raceway 2

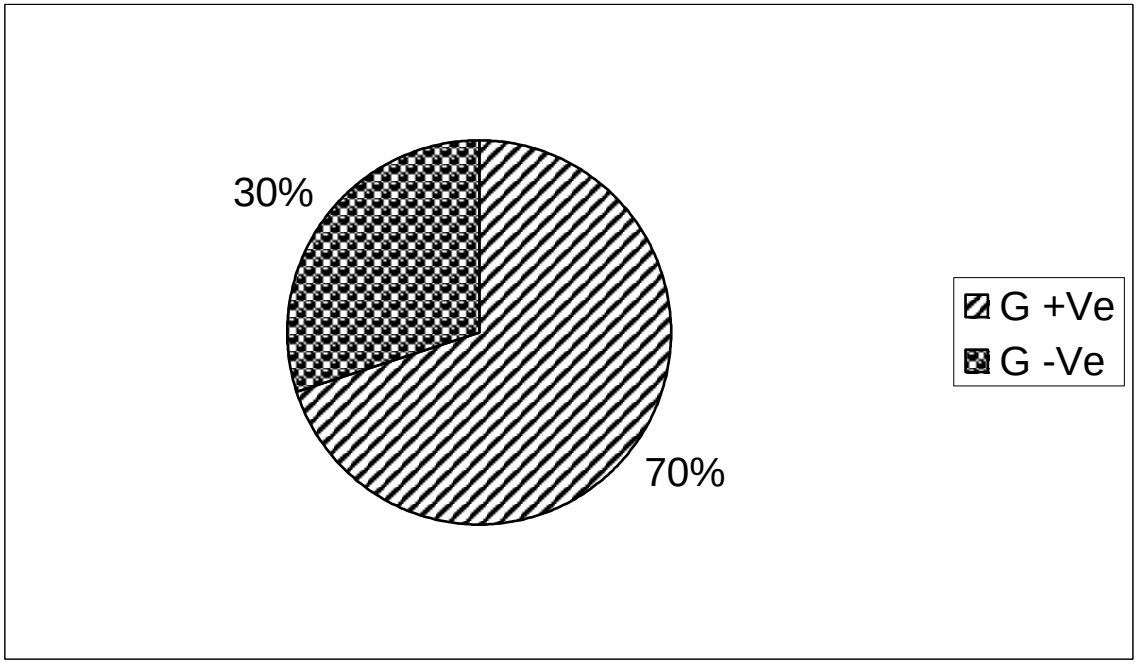

Fig. 4.11: Percentage composition between Gram +ve and -ve bacteria in raceway 1

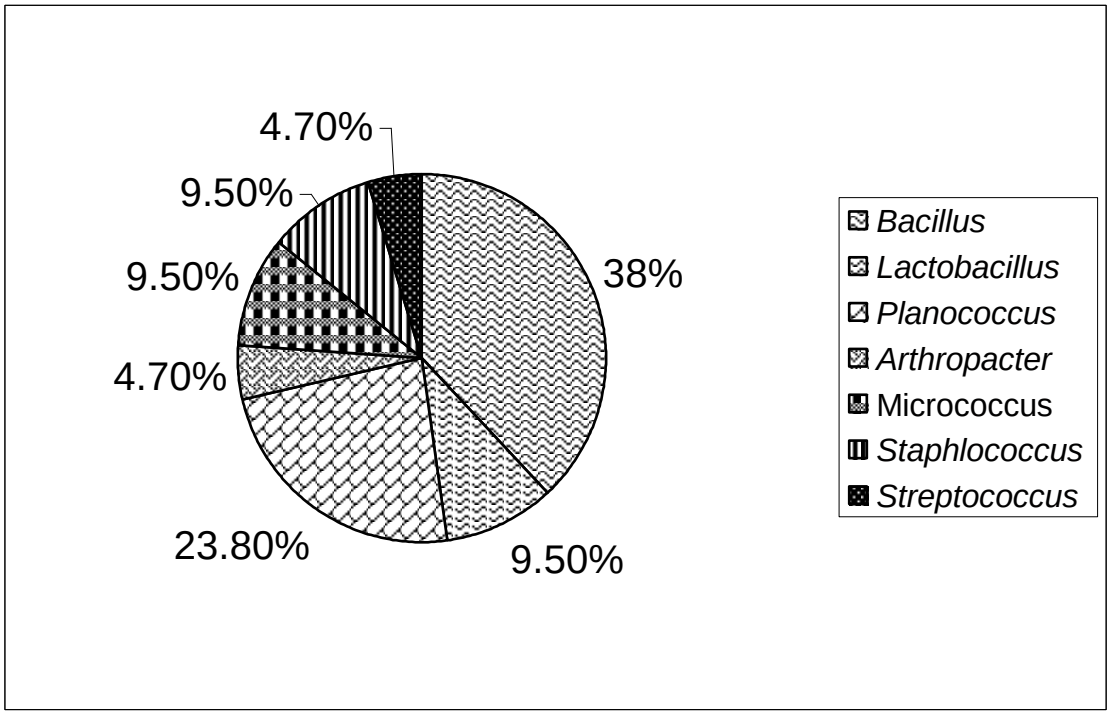

Fig. 4.12 : Percentage composition of Gram +ve bacteria in raceway 1

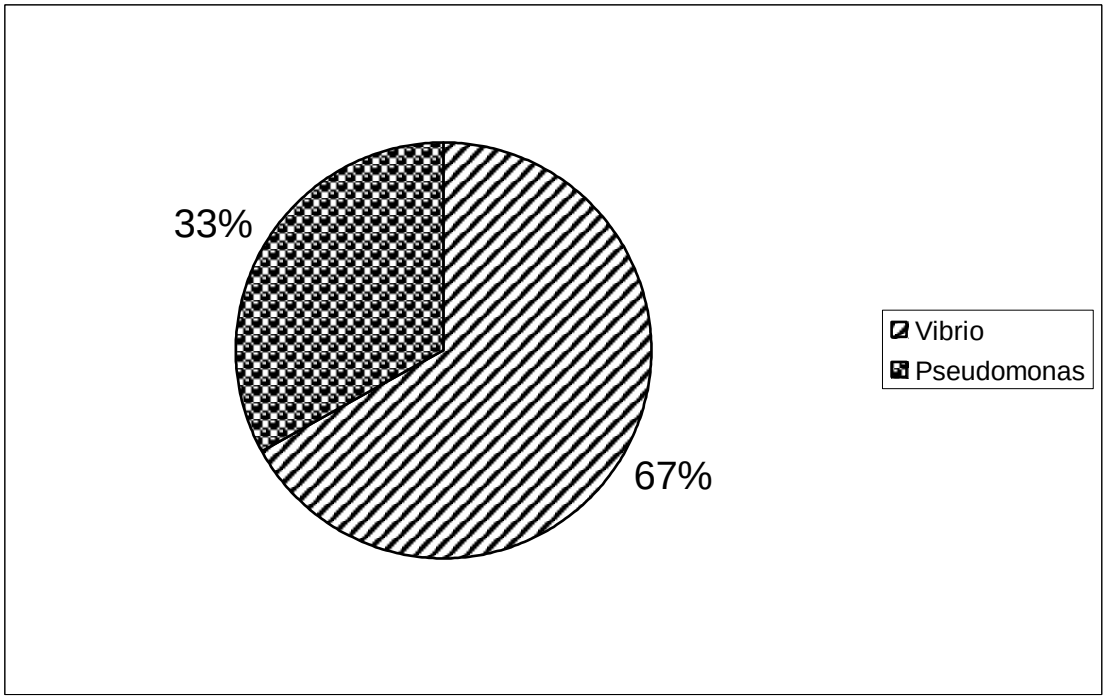

Fig. 4.13 : Percentage composition of Gram –ve bacteria in raceway 1

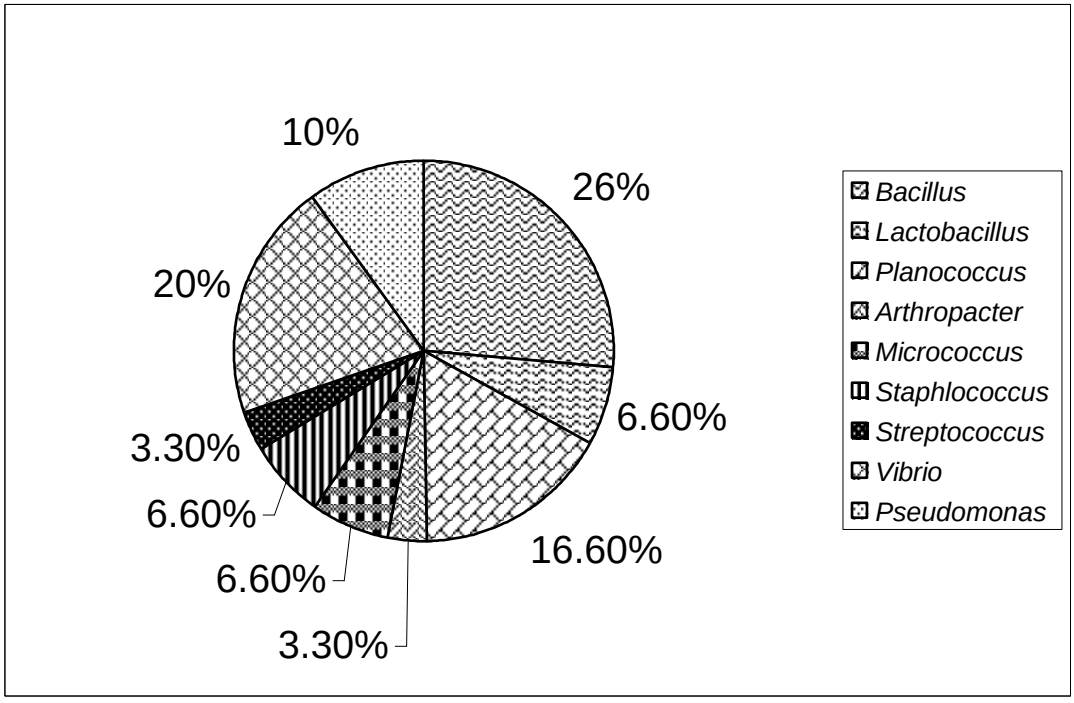

Fig. 4.14 : Percentage of total species composition in raceway 1

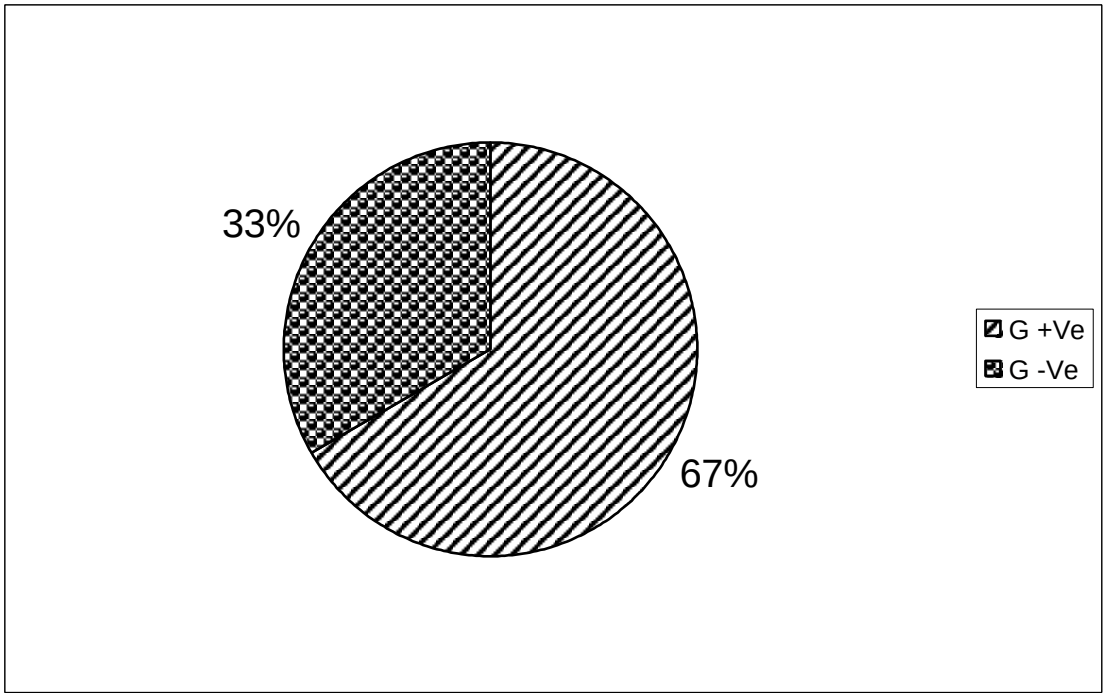

Fig. 4.15 : Percentage composition of Gram +ve and –ve bacteria in raceway 2

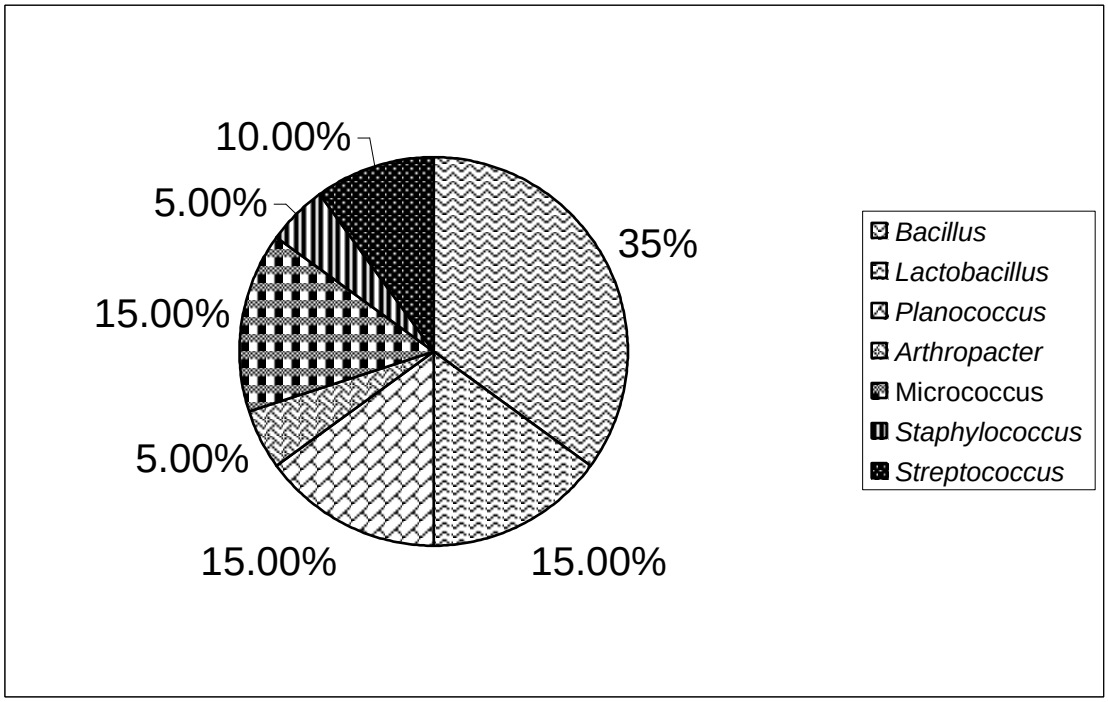

Fig. 4.16 : Percentage composition of Gram +ve bacteria in raceway 2

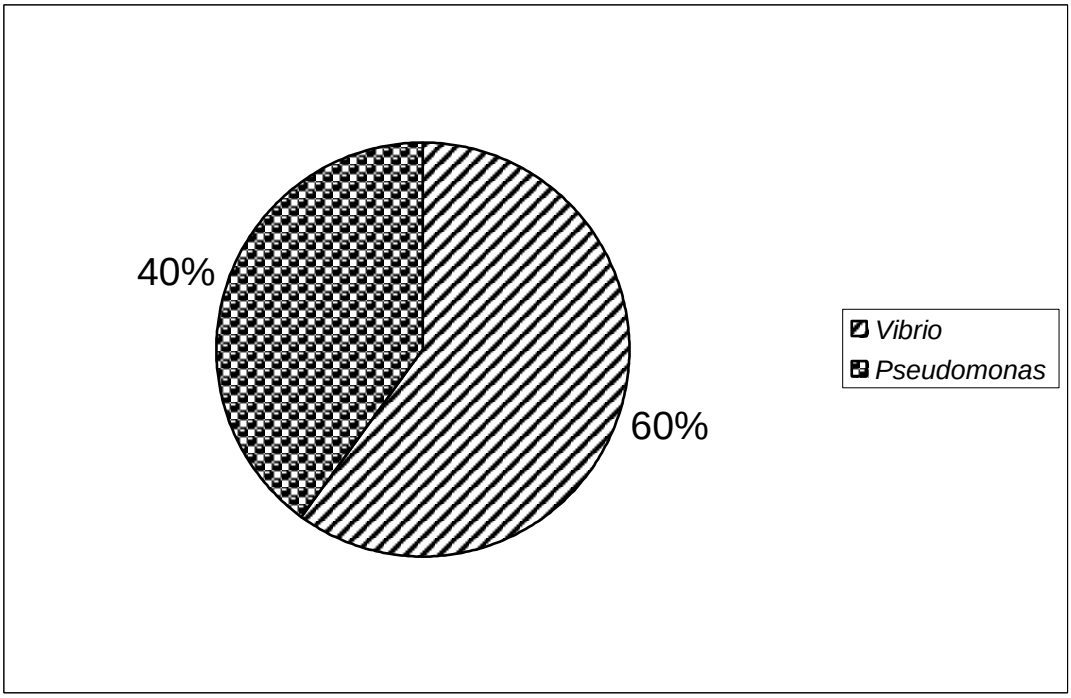

Fig. 4.17 : Percentage composition of Gram –ve bacteria in raceway 2

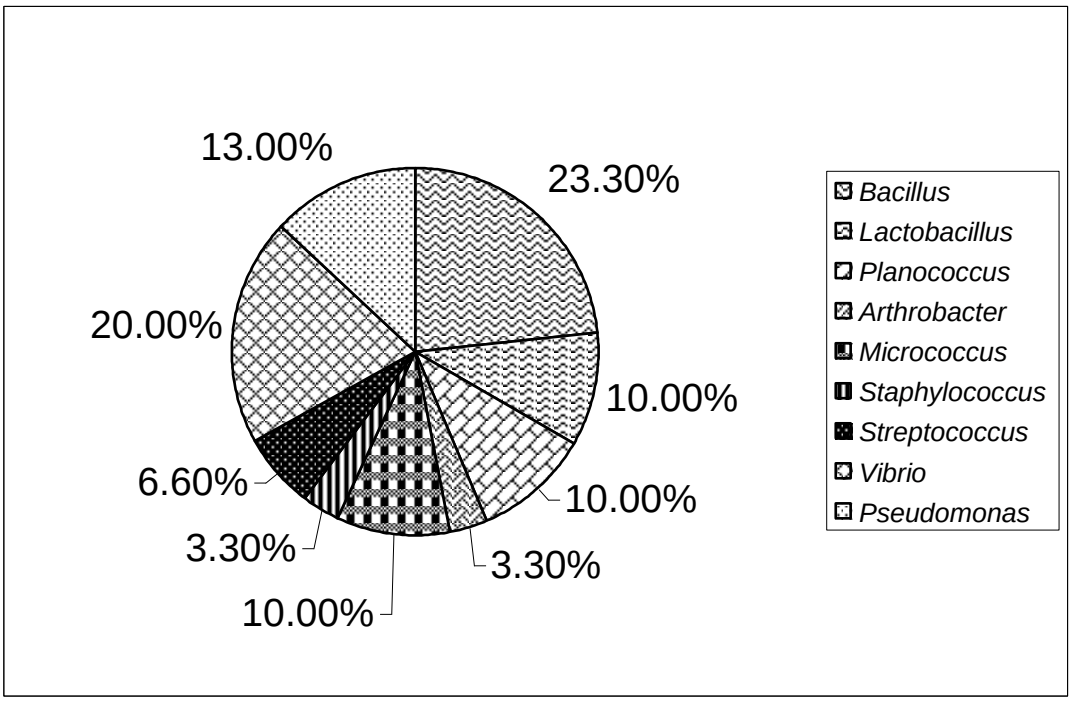

Fig. 4.18 : Percentage of total species composition in raceway 2