FISH DRIVEN BIOTURBATION AS A VIABLE AQUACULTURE TECHNOLOGY FOR INCREASING PRODUCTION AND IMPROVING ECOLOGICAL SUSTAINABILITY OF GIANT TIGER SHRIMP *PENAEUS MONODON* (FABRICIUS)

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by
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Fish Driven Bioturbation as a Viable Aquaculture Technology for Increasing Production and Improving Ecological Sustainability of Giant Tiger Shrimp *Penaeus Monodon* (FABRICIUS)

Ph.D. Thesis under the Faculty of Marine Sciences

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Dedicated to My Beloved Parents,
Sisters and Husband

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Declaration

I, Joyni Jacob M., do hereby declare that the thesis entitled **"Fish Driven Bioturbation as a Viable Aquaculture Technology for Increasing Production and Improving Ecological Sustainability of Giant Tiger Shrimp Penaeus Monodon (FABRICIUS)"** is a genuine record of research work done by me under the supervision of Dr. B. Madhusoodana Kurup, Vice Chancellor, Kerala University of Fisheries and Ocean Studies, Panangad, Cochin-06 and has not been previously, formed the basis for the award of any degree, diploma, associateship, fellowship or other similar title of any University or institution.

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Abbreviations

ANOVA - Analysis of variance

CFU - Colony Forming Units

Chl-*a* - Chlorophyll-*a*

CMFRI - Central Marine Fisheries Research Institute

CUSAT - Cochin University of Science and Technology

EOM - Easily Oxidized Matter

H_2S - Hydrogen Sulphide

MPEDA - Marine Products Export Development Authority

NO_2^--N - Nitrite-Nitrogen

NO_3^--N - Nitrate-Nitrogen

P. monodon - *Penaeus monodon*

PL - Post Larvae

PO_4^{3-}-P - Phosphate -Phosphorus

S.D - Standard Deviation

SIF - School of Industrial Fisheries

TAN - Total Ammonia Nitrogen

THB - Total Heterotrophic Bacteria

TOC - Total Organic Carbon

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Chapter 1

INTRODUCTION AND REVIEW OF LITERATURE

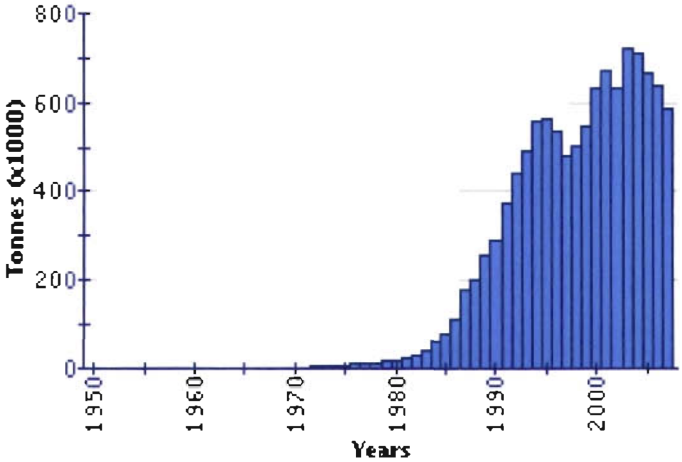
Contents

1.1 General introduction

Shrimp aquaculture is one of the most economically important commercial activities all over the world, including India. The recent world shrimp catch is about 3.4 million tonnes per year, with Asia as the most fertile area for shrimp fishing. World production of shrimp, both from captured and farmed, is about 6 million tonnes, of which about 60 percent enters the world market. Shrimp is the most important internationally traded fishery commodity in terms of value (FAO, 2008), accounting for 15 percent of the total value of internationally traded fishery products in 2008. The culture production of penaeids (shrimps) in 2008 was 73.3 percent of the total production. Cultured shrimp plays an important role in the market, but it experienced a decline in production in 2009 for the first time since it entered international trade in the 1980s. In 2009, shrimp trade was affected by the economic crisis. While export volumes remained stable, average shrimp prices declined substantially in the course of the year (FAO, 2010).

There are more than 50 species/ varieties of shrimps available in marine waters, with a very wide distribution in both tropical and temperate ecosystems. Most of them are very small and not suitable for farming or human consumption. However, the giant tiger shrimp (*Penaeus monodon*), which is internationally known as tiger shrimp, has been and continues to be the leading cultured species. This species accounts for more than half of the total world shrimp aquaculture output, and it is the largest and fastest growing of all shrimp species. Even at high stocking densities, *P. monodon* can reach marketable sizes of 20 cm and 35 g in three to six months, and therefore holds promise for the commercial aquaculturist for a more rapid cash flow than other cultured fish and shellfish (Muir and Roberts, 1982). *P. monodon* is tolerant to a wide range of salinities, but is highly susceptible to two of the most lethal shrimp viruses - yellowhead and whitespot.

The global shrimp farming industry had a rapid growth in the 1980s mainly due to technological breakthroughs both in hatchery production of seed and development of feed technology. High demand for shrimp resulted in higher price and high profit in shrimp farming, and public support. Farmed shrimp amounted to about 712,000 metric tons in 1995, which accounted for about 27% of total shrimp production from both wild-caught and farm-raised sources. Black tiger shrimp *P. monodon* is the prime species farmed, accounting for 57% of cultured shrimp production, followed by western white shrimp *Litopenaeus vannamei* (20%) (Rosenberry, 1995). The global aquaculture production of *P. monodon* is given in Fig1.1. The culture of *P. monodon* species spread throughout southeast and south Asia due to its faster growth rate compared to the other species and due to the high value and demand in the international markets.



(FAO Fishery Statistics)

Figure 1.1 Global aquaculture production of *Penaeus monodon*

Though shrimp farming had been practiced for more than a century for food and livelihood of coastal people in some Asian countries, commercial shrimp farming, the production of marine shrimp in impoundments, ponds and tanks, started only in early 1970's. Named for its huge size and banded tail, the giant tiger shrimp is the primary species farmed in Asia. Overstocking, pollution and outbreaks of disease have led to widespread mortalities of *P. monodon* in many Asian nations.

In Asia, shrimps had for centuries been traditionally grown in low-density monoculture, in polyculture with fish, or in rotation with rice in the *bheries* of West Bengal and *pokkalis* of Kerala in India (Shiva and Karir, 1997). The shrimp production in these systems was low-yielding and aimed for domestic markets. The shrimp culture received a boost in the mid-1970s, when fishermen and hatchery operators began supplying large quantities of penaeid shrimp post larvae to farmers. With improved technologies and the introduction of commercial formulated feeds, the industry boomed during the 1980s. Asia has always led the world production of cultivated shrimp with a market value of billions of US dollars per year.

1.1.1 Shrimp aquaculture in India

The giant tiger shrimp, *Penaeus monodon* Fabricius 1798, is widely distributed in the Indian Ocean and Western Pacific Ocean (Holthuis, 1980). India, by virtue of its 8118 km long coastline, 2.02 million sq. km of Exclusive Economic Zone (EEZ) and extensive geographical stretch with varied terrain and climate, supports a wide diversity of inland and coastal wetland habitats. There are 3.9 million ha of estuaries and 3.5 million ha of brackish water areas in the country. The potential area available in the coastal region of the country for shrimp farming is estimated to be between 1.2 million to 1.4 million hectares.

Shrimp industry is a key sector in India's economy owing to its significant contribution to export earnings and gainful employment. Tiger shrimp, *P. monodon* has been the mainstay of India's seafood exports and has immense potential as a foreign exchange earner. It also has substantial contribution towards socio-economic development in terms of income and employment. Compared to 2010, sea food exports recorded a growth of 19.85% in volume. Europe is the leading market for Indian seafood products followed by US, China and Japan. According to the provisional figures, Frozen shrimp continued to be the major export item accounting 46% of the total US$ earnings. Shrimp exports during the period increased by 13%, 35% and 41% in quantity, Rupee value and US$ value respectively (MPEDA, 2011).

The over exploitation of shrimp from natural sources and the ever increasing demand for shrimp and shrimp products in the world food market has resulted in a wide gap between the demand and supply of shrimp. This has necessitated the need for exploring new avenues for increasing shrimp production.

In India, commercial shrimp farming started gaining roots only during the mid-eighties. It was a relatively late start in India; by this time, shrimp farming had reached its peak in most of the neighbouring Asian countries, especially China and Taiwan; in some countries the disease and poor farm management practices had already taken a heavy toll. In India, at least 10 potential penaeid species are available for the coastal aquaculture. However, *P. monodon* is the only one species cultured and it constitutes about 95-99% of total farmed shrimp production of the country. There is no doubt about the suitability of *P. monodon* for farming as a candidate species with highest growth rate and high market value. The culture of shrimp received maximum importance due to its unique taste, high nutritive value and persistent demand in world market. In India, aquaculture industry is growing at an alarming rate surpassing some major hurdles (disease outbreak and pollution) during its development. The boom period of commercial-scale shrimp culture in India started in 1990 and the bust came in 1995-96, with the outbreak of viral disease. The higher stocking densities and poor water quality management might be the reasons for disease outbreak. So sustainable shrimp farming is need of the hour to overcome the above problems.

Presently, coastal aquaculture in India is synonymous with shrimp aquaculture and mainly carried out by small scale farmers. In India, shrimp farming has been traditionally practiced in the coastal states of West Bengal and Kerala. Apart from these, Andrapradesh, Orissa and Gujarat contribute to farmed shrimp. Kerala, situated on the south-western part of India has a coastline of 590 km and a continental shelf area of 40000 sq. km within 200 m depth. The utilization of all available area for shrimp farming can increase the output and yield from shrimp farming. The state has a sprawling brackish water area of nearly 65000 ha suitable for shrimp farming. Out of this about 14500 ha has been utilised for shrimp aquaculture. The introduction of scientific farming techniques will increase production and profitability from the shrimp culture.

1.1.2 Issues related to shrimp farming

High demand and high price of shrimp generated high profit in shrimp farming. This lured an increasing number of investors into shrimp farming in many countries (Shang et al., 1998). The whole process resembled a gold rush. However, the rapidly expanding shrimp industry started to face problems since 1988 (Csavas, 1995) as the negative impact of aquaculture became evident, which amounted to the environment and severe social and economic problems for the coastal communities.

Environmental impacts of shrimp aquaculture arise from the consumption of resources, such as land, water, seed and feed, their transformation into products valued by society, and the subsequent release of wastes into the environment from uneaten food, faecal and urinary products, chemotherapeutants, as well as microorganisms and parasites (Beveridge et al., 1997; Kautsky et al., 2000). Negative effects may be direct, through release of eutrophicating substances, toxic chemicals, the transfer of diseases and parasites to wild stock, and the introduction of exotic and genetic material into the environment, or indirect, through loss of habitat and niche space, and changes in food webs.

Most aquaculture systems are so-called throughput systems (Daly and Cobb, 1989). This means that resources, collected over large areas, are introduced and used in the aquaculture production site, and released back into the environment in concentrated forms as nutrients and pollutants, causing various environmental problems (Folke and Kautsky, 1992). Uneaten food, faecal and urinary wastes may lead to eutrophication and oxygen depletion, the magnitude of which is dependent on the type and size of operation, as well as the nature of the site, especially size, topography and water retention time (Kautsky et al., 2000).

In semi-intensive and intensive farms, artificial feeds provide most of the nitrogen, phosphorous and organic matter. Only 17% (by dry weight) of the total amount of feeds applied to the pond is converted into shrimp biomass (Primavera, 1993). The rest is leached or otherwise not consumed, egested as faeces, eliminated as metabolites, etc. Effluent water during regular flushing and at harvest can account for 45% of nitrogen and 22% of organic matter output in intensive ponds (Briggs and Funge-Smith, 1994). Pond sediment is also a major sink of, phosphorous and organic matter in intensive shrimp ponds (Briggs and Funge-Smith, 1994). As shrimp biomass and food inputs grow, the water quality in high-density ponds deteriorates over the cropping cycle. In intensive ponds total nitrogen , phosphorous, silicate, dissolved oxygen and biological oxygen demand increases and water visibility decreases in the grow-out period (Macintosh and Phillips, 1992). Quality of receiving waters may deteriorate if the assimilative capacity of the environment is exceeded. Levels of nitrates, nitrites, phosphorous, sulphide, turbidity and biological oxygen demand increases considerably and have great potential to cause pollution and subsequent collapses in shrimp production (through negative feedback). It is well established that the re-use of waste-laden pond water discharge, so-called self pollution, is a major triggering factor behind disease susceptibility for cultured shrimp.

Like any other agriculture / animal husbandry practice, shrimp culture has also been affected by health and disease problems. Initially, some bacterial diseases in localized shrimp farms with low mortality rates were noticed. However, viral diseases such as *Monodon Baculo virus* and *White Spot virus disease* syndrome were reported from shrimp farms in 1995 followed by a slump in Indian shrimp farming. Heavy stocking densities and poor farm management practices were attributed as the major reasons for such disease outbreaks in the country. Diseases have emerged as a major constraint to the sustainable growth of shrimp aquaculture. Many diseases are linked to environmental deterioration and stress associated with farm intensification. Disease prevention is more important than treatment. The solution to the problem must deal with site selection, design and sustainable farm management, especially the sediment and water quality.

Water is usually not included as a cost item in aquaculture production except for the pumping cost. However, the quantity and quality of water may deteriorate in the future and the cost of suitable water could become an important cost item for sustainable operation. Deteriorated water quality causes diseases and low survival rate for intensively managed farms. Better pond management is essential to improve water quality. Sediment management and polyculture are some of the alternatives to improve water quality if they are to be economically feasible.

Properties of the pond bottom soil (sediments) and processes occurring in the bottom soil and in the soil-water interface are very important regarding the well being and growth of fish or shrimp in ponds. Tiger shrimp and white shrimp require clean water for optimum growth and good health. Shrimps are primarily bottom dwellers and are easily stressed when exposed to poor pond conditions such as low pH, high organic matter concentration, and soft sediment containing reduced microbial metabolites. When shrimps are stressed they become susceptible to many diseases especially, viral diseases. Providing a good quality environment in ponds is the only means to prevent stress, minimize the risk of diseases, and to enhance survival rate and production.

One of the important issues related to the farming is the cost of feed. Feed is the most important cost item for a relatively intensive operation. Cost of feed per unit shrimp produced depends primarily on the conversion ratio of feed to shrimp and unit price of feed. Therefore, the cost of feed can be reduced by improving the conversion ratio or by lowering the wastage of feed, or by retaining the residual feed nitrogen by recycling using proper techniques. The conversion ratio in turn can be improved by reducing waste and improving feed formula. Wastage can be reduced by feeding the right amount of feed or by recycling the excess feed as nutrients. Overfeeding results in higher cost of feed per unit shrimp produced and also the pollution of sediment and water.

1.1.3 Development of anaerobic conditions in sediment and its consequences

An important issue related to the shrimp farming is the condition of bottom soil. A cultured aquatic animal assimilates 5%-40% nutrients from the feed. The average nutrient retention is evaluated as 13% for carbon, 29% for nitrogen and 79% for phosphorous. Most of the nitrogen and phosphorous not retained in the harvested biomass is found accumulated at the pond bottom. Nutrients and organic residues tend to accumulate at the bottom, and hence, to some extent, removed from the water phase. However, an excessive accumulation beyond what could be defined as the carrying capacity of the sediments may result in the deterioration of the pond system, as specified later. Such development seems to be of special importance for shrimp culture, since shrimps live in the soil-water transition zone. Reactions and fluxes within and across the water-soil interface are very significant in natural aquatic systems and even more in intensive aquaculture systems. The organic matter settles and accumulates on the pond bottom in extensive, semi-intensive and intensive ponds. Anaerobic conditions develop in the sediments of intensively stocked and fed shrimp ponds, the process being more pronounced with increase in intensification of culture. The development of anaerobic conditions constraints production and is a barrier to further intensification (Avnimelech and Ritvo, 2003).

Anaerobic conditions may affect aquaculture production both due to unfavorable conditions in the pond bottom and the diffusion of the reduced toxic compounds from the bottom soil upward to the water column. The development of anaerobic conditions in the pond bottom clearly coincides with the growth retardation of shrimp (Avnimelech and Zohar, 1986).

The important consideration that emerges from these budgets is that the character of the pond soil will have significant effect on water quality and production of shrimp ponds. Despite the importance of soil in pond aquaculture, there has been relatively little effort to correlate properties of pond bottom with production of fish or shrimp (Boyd, 1990). Most of the studies focus only on the pond water quality. Anoxic and anaerobic processes in sediment lead to the buildup of sulphides, reduced organic sulfur, reduced iron, manganous ions, ammonium, nitrite and other reduced species (Blackburn, 1987). Some of these metabolites, and especially ammonia, hydrogen sulphide, nitrite, manganous ions and certain organic compounds, are potentially toxic to shrimp.

1.1.4 Redox reactions in the pond bottom soil

Pond bottom conditions change with time affected to a large extent by the accumulation of organic matter residues, such as dead algae, faeces and feed residues, leading to high oxygen consumption and the development of reducing conditions (Boyd, 1995; Avnimelech and Ritvo, 2003). Pond sustainability is determined, to a large extent, by the capacity of the pond bottom to metabolize the organic loading before reaching a point of deterioration, when excessive accumulation of reduced components affects fish or shrimp growth. Accumulation of organic residues beyond the oxidising capacity of the sediment leads to the development of anaerobic conditions, formation of reduced species, high sediment oxygen demand, fish (or shrimp) growth inhibition and deterioration of the pond (Avnimelech and Ritvo, 2003). The redox system in the pond bottom is made of both organic components as well as reduced inorganic species (such as sulphides, Fe and Mn ions), yet, the driving force to the development of the high oxygen demand and anoxic conditions is the organic matter accumulated in the sediment.

The degradation of organic matter occurs in the pond bottom in aerobic conditions and oxygen serves as electron acceptors. When oxygen is depleted, other terminal electron acceptors are used to mediate the decomposition of organic matter. Subsequently, iron, manganese, sulphate and CO_2 serve as electron acceptors, and the anaerobic processes taking place in the pond bottom lead to the production of reduced and potentially toxic compounds such as organic acids, reduced manganese and reduced sulphur compounds (Avnimelech and Ritvo, 2003). Metabolites like ammonia and nitrite accumulate when nitrification is inhibited in the absence of oxygen.

1.1.5 Effect of toxic metabolites and nitrogenous waste products on the production of shrimp

Reduced inorganic species and nitrogenous waste products may affect biological activity. Sulphide oxidation appeared to represent the majority of the sediment oxygen demand (SOD) in shrimp ponds and it accounted for about 84% of the total SOD (Suplee and Cotner, 1996). Alongi et al. (1999) estimated that the contribution to carbon oxidation was 41-60% through O_2 respiration, 7-22% from Mn reduction, 5-25% from iron reduction and 13-26% from sulphate reduction in shrimp pond sediments. Burford and Longmore (2001) reported that 50-80% of the carbon degradation in shrimp pond soil was anaerobic, mostly coupled with sulfate reduction. The undissociated species H_2S is highly toxic to fish and shrimp. Hydrogen sulphide can inhibit aerobic respiration by binding to the heme of Cytochrome C oxidase in place of molecular oxygen (Smith et al., 1977). Hydrogen sulphide toxicity is inversely related to dissolved oxygen concentration. Consequently, sulphide increases the sensitivity to hypoxia (Adelman and Smith, 1970). Sulphide also inhibits nitrification (Joye and Hollibaugh, 1995) and will, thus, lead to an increase of ammonium ions in the pond. Reduced divalent manganese (Mn^{2+}) is toxic to fish. The release of hypolimnetic, anaerobic water from reservoirs to rivers led to fish kills due to the high Mn concentrations (Nix and Ingols, 1981).

Nitrogen plays a key role in aquaculture systems due to its twin functioning as a nutrient and toxicant. The excessive accumulation of toxic inorganic nitrogen in culture systems deteriorates the pond system by reducing the growth rate and survival rate of cultured organisms. Without effective control, farming operations usually increase the nutrient and organic matter load to the ecosystem well beyond the carrying capacity of the environment. This often results in self-pollution which leads to more frequent disease outbreaks followed by crop failure.

Ammonia is the principal end product of protein catabolism in crustaceans (Hartenstein, 1970; Kinne, 1976; Dall et al., 1990) and accounts for 40%-90% of nitrogenous excretions (Parry, 1960) and is also produced from the ammonification of organic matter in a culture system (Chen et al., 1990). The accumulation of ammonia can cause mortality of organisms (Chen et al., 1990; Spotte, 1979) and/or cessation of feeding in shrimp. The unionised form of ammonia is usually toxic; since it has high lipid solubility and is able to diffuse quite readily across cell membranes (Chen and Kou, 1993; Armstrong et al., 1978; Thurston et al., 1981). Ammonium ions (NH_4^+) are also toxic, especially at low pH levels (Allan et al., 1990). Ammonia and its intermediate product of oxidation, nitrite, are the most common toxicants in culture systems and are toxic to fish, mollusc and crustaceans (Colt and Armstrong, 1981). Therefore, the accumulation of ammonia and nitrite may have detrimental effects on shrimp rearing. Toxicities of ammonia and nitrite to *P. monodon* larvae have been reported (Chin and Chen, 1987; Chen and Chin, 1988). A safe level was calculated to be 4.26 mg/l NH_4^+-N, 0.08 mg/l NH_3-N and

10.6 mg/l NO_2^--N based on the incipient LC_{50} value and an application factor of 0.1 for *P. monodon* adolescent rearing (Sprague, 1969, 1971).

Pond bottom conditions are more critical for shrimp than for other aquaculture species because shrimp spend most of their time on the bottom, burrow into the soil and ingest pond-bottom soil (Boyd, 1989; Chien, 1989). The distribution of penaeid shrimp in the natural environment can be influenced by sediment characteristics (Williams, 1958; Hughes, 1968; Rulifson, 1981; Somers, 1987). Penaeid shrimp commonly burrow into the substrate to hide from predators (Fuss and Ogren, 1966; Boddeke, 1983). *Litopenaeus indicus* avoids regions in the pond with sediments containing sulphide although sulphide was undetected in the water column (Gopakumar and Kuttyamma, 1997). Feed acceptance by shrimp was significantly lower in pond areas with sulphide containing sediment. Mixing and resuspension of bottom soil can expose the organic particles to the oxygenated water and may induce aerobic processes. Pond bottom soil mixing can be done by aerators (Boyd, 1998), by towing chains above the bottom (Pullin, 1987) or by aquatic organisms (Bioturbation) (Blackburn et al, 1988; Scheffer, 1997; Riise and Roos, 1997; Avnimelech, 1999).

Mixing of the bottom soils can be assessed through the evaluation of several chemical species related to or are indicators of reducing conditions. Easily reduced organic matter, EOM, represents the active fraction of organic matter (Avnimelech et al., 2004). It is readily oxidized under aerobic conditions and accumulates when oxygen is restricted. Manganous ion (Mn^{2+}) is the reduced and toxic species of Mn. Besides, it is more soluble than the sparingly soluble manganese species (Mn^{4+}) and thus, high concentrations of soluble Mn (Mn^{2+}) in the soil are indicative of reducing conditions (below 200-300 mv: Reddy et al., 1986). Iron solubility is also affected in a similar way, since the ferrous (Fe^{2+}) species is more soluble than the oxidized ferric ion (Fe^{3+}). Ammonium (NH_4^+) is also indicative of reducing conditions, both due to restricted nitrification under anaerobic conditions as well as accelerated release of ammonium as a product of anaerobic respiration due to the low energy yield of this process. Determination of the concentration profiles of these species is beneficial in understanding whether the sediment system is aerobic or anaerobic.

1.1.6 Towards the sustainable growth

After an impressive growth phase, shrimp farming entered a lag phase and the sustainable development of the industry is presently haunted by many socio economic and environmental problems. Many shrimp farmers often seek to maximize their short-term gain at the expense of the environment. The 'rape and run' practice in shrimp farming, where ponds in mangrove areas are farmed intensively and quickly abandoned as observed in Thailand and the Philippines, is a good example. This type of farming violates the criterion of sustainability and therefore, is unsustainable. Shrimp farming is sustainable if it is in harmony with other economic activities in using natural resources. It should produce a reasonable and relatively stable net income benefit to producer's society on a long-term basis without degrading the environment. Its development has to be balanced among production, marketing and other supporting services such as hatchery, feed mill and legal measures. Therefore, a sustainable shrimp farming system has to be bio-technically feasible, environmentally sound and socio-economically viable (Shang and Tisdell, 1996).

At the farm level, net farm income is affected by the level of production, farm price and operating cost. Increase in farm productivity, reduction in production costs and increase in average farm price received are major measures to improve economic viability. Farm productivity depends mainly on (1) stocking rate, survival rate and growth rate of the stock, which are in turn affected by bio-technical factors such as rate of feeding, fertilization, mono or polyculture, stocking and harvesting strategies, and (2) environmental factors such as water quality, water temperature, dissolved oxygen, pH levels, chemical inputs to treat diseases and predators. The best combination of inputs and the most suitable culture intensity of the system are mainly determined by available resources and cost, and the farmer's management ability. Development of inexpensive or low cost technologies for soil and water quality management, reduction of toxic metabolites and removal or recycling of wasted feed as nutrients with minimum utilization of high protein feed coupled with improved growth and production must be a strategy for sustainable shrimp farming. Natural and biological techniques like bioturbation can be used in this regard as it is an inexpensive method to make the shrimp farming more sustainable by reducing toxic metabolites and improving nutrient recycling.

1.1.7 The soil bioturbation process

Bioturbation is the turnover or mixing of soil by animals. Some common definitions include, the biologically driven mixing of materials in the soil layer between the underlying geological formations and the overlaying atmosphere (Smallwood et al., 1998), and "the churning and stirring of sediment by organisms" (Bates and Jackson, 1984). Some authors (Eldridge and Rath, 2002; Whitford and Kay, 1999) also refer to soil disturbance by animals as bioperturbation.

Bioturbation occurs in different ways, depending on the species involved and the way they move the soil. Invertebrates such as earthworms, that live underground and move through the soil by pushing particles aside, or termites and ants, that excavate large quantities of soil and physically alter the soil characteristics. In addition, those animals that consume organic matter, like gophers that displace soil while burrowing to eat plant roots, affect the soil structure and biogeochemistry (Gabet et al., 2003).

According to Anderson (1988), invertebrates can affect the transport of organic and inorganic materials in soils. He demonstrated the ability of a small biomass of soil macro fauna to cause significant effects on fluxes of organic matter and dissolved materials in the superficial horizons of forest soils. Body size and feeding behaviour of fauna influence these processes.

The bioturbation refers to the displacement of sediment particles as a consequence of animal mobility and feeding. A faunal community, once established in a suitable area, will also change its geochemical environment by bioturbation. The redistribution of organic matter within the sediment by effective bioturbating species will influence depth, distribution and community structure as well (Dauwe et al., 1998). Bioturbation has been identified as classic example of ecosystem engineering (Levinton, 1995) and is likely to occur in soft sediments, which provide an "abiotic substrate amenable to biogeomorphic action" where many abiotic resources are integrated (Jones et al., 1997).

1.1.8 Bioturbation in aquatic environment

Bioturbation is the reworking and mixing of the sediment at sediment-water interface, accomplished, collectively by burrowing, feeding, irrigation, resuspension, secretion, excretion and transporting activities. The mixing of pore water solutes and sediment particles by the activities of benthic organisms collectively is also referred to as bioturbation (Richter, 1952). The process of bioturbation which involves both the dispersal of sediment particles and transport of interstitial pore water by benthic organisms is of global importance. Bioturbation, the diffusion like transport of solutes and solids in sediments caused by movements of fauna, is often a significant transport process (Aller, 1982; Aller and Yingst, 1985; Aller and Aller, 1992; Forster et al., 1995).

Sediment reworking by fish, helps to expose more organic particles to the oxygenated water, enhance aerobic metabolism of the organic residues and prevent the development of anaerobic condition. Under intensive culture densities, bioturbation can be a significant process, which can accelerate microbial activity, enhance aerobic decomposition of sediment, and increase the rate of suspension of nutrients into the water column. Through bioturbation, organic residues become frequently resuspended in the water column favoring aerobic decomposition over anaerobic decomposition. Through bioturbation, there is an increased and sustained nutrient influx to the water column (Yusoff et al., 2001). This is believed to be responsible for the more stable plankton bloom observed by aqua farmers.

1.1.9 Significance of fish bioturbation in shrimp aquaculture

Soil composition is the major factor in the pond aquaculture, and the condition of pond bottom greatly influences water quality and production. The composition of shrimp pond soil is altered constantly by residues from feeds and fertilizers, settling of dead plankton, and accumulation of sediments and salts (Hopkins et al., 1994; Boyd, 1995). Organic matter accumulation in pond soil from uneaten feed, dead plankton and shrimp faeces increases oxygen demand and facilitates reducing conditions and release of toxic metabolites such as ammonia, nitrite, hydrogen sulphide, ferrous iron, manganous manganese and methane (Boyd, 1995). Hence the process of bioturbation is of utmost importance in aquaculture. Aquatic sediments are continuously reworked due to the activity of local infauna, a process typically referred to as bioturbation (Richter, 1952). Bioturbation also forms the driving force for the dispersal of various solid particles, which can be both mineral (e.g. organic matter, metal oxides and contaminants) as well as biological (e.g. bacteria, viruses, cysts and resting stages of plankton) in nature. Bioturbation directly affects organic matter remineralisation and decomposition (Anderson and Kristensen, 1991; Aller, 1994), nutrient cycling (Furukawa et al., 2001), pollutant release (Gilbert et al., 1994), sediment resuspension (Rowden et al., 1998) and microbial activity (Aller and Yingst, 1985). As a consequence, bioturbation plays a crucial role in the geochemistry and ecology of aquatic sediments (Aller and Yingst, 1978; Aller, 1988; Meysman et al., 2006a). By increasing the depth of oxygen penetration in organically enriched sediments, bioturbation can supply the additional oxygen required during the processing of the additional organic material (Aller, 1994), thus reducing the impact of oxygen depletion on species sensitive to low oxygen levels whilst also stimulating the activity of aerobic microbial decomposers and accelerating carbon processing.

Bioturbation activity generated by fish has potential to improve bottom soil quality by increasing oxygen supply to greater depth in aquaculture pond bottoms (Ritvo et al., 2004). Improvement of the anaerobic condition at the pond bottom soil-water interface helps to decrease the concentration of reducing components (total dissolved sulphide, reduced manganese, easily oxidized matter and exchangeable ammonium). Another effect of bioturbation is the oxygenation of the sediment, which enhance mineralization process by which nutrients are released to the overlying water column (mainly phosphate and ammonia). Nutrient flow can be augmented by fish driven bioturbation and resuspension (Rahman and Verdegem, 2007). Detritivorous fish is a suitable organism to regenerate the nutrients from the sediments (Kang and Xian, 2007). By feeding on benthic organic detritus and then releasing a portion of the consumed nutrients as excreta, detritivorous fish may provide an important source of nutrients to stimulate phytoplankton growth (Schaus et al., 1997; Moore et al., 2004). This added release can increase the total nutrient contents of the water column and thus can be regarded as a new nutrient (Dugdale and Goering, 1967; Caraco et al., 1992). Accordingly bioturbation helps to recycle the nutrients into water column through resuspension process and makes it available for assimilation in the water column.

In aquatic ecosystems, ecological processes such as organic matter mineralization and nutrient cycling are regulated by microbial activities in sediments (Boulton et al., 1998; Sundback et al., 2004). Bioturbation potentially affects bacterial communities by modifying the supply of nutrients and oxygen to the microorganisms (Yingst and Rhoads, 1980). Microorganisms mineralise organic matter and return it into the trophic chain as nutrients for phytoplankton consumption (Lalli and Parsons, 1997; Rubcova, 2000). The recycling of nitrogenous waste products helps in the incorporation of these nutrients into the biomass of the culturing organism and the resultant improvement of growth and production. The bottom feeding fish resuspend bottom particles and nutrients; the latter enhances phytoplankton that becomes available to zooplankton and finally to the culturing organism.

Although the precise form and rate of bioturbation is generally species-specific (Forster and Graf, 1995; Shull and Yasuda, 2001) and may covary with respect to other environmental variables (Solan and Kennedy, 2002; Biles et al. 2003), the combined effect of infaunal activity on the properties of the sediment has important implications for many ecosystem processes. Understanding and quantifying the mechanisms of bioturbation is therefore of primary importance in disentangling organism-sediment interactions as they relate to the provisional and long-term sustainability of ecosystem function. Bioturbation is getting more attention presently in the removal of ammonium from water through its assimilation into the biomass of culturing organism through food web by the recycling process of nutrients. This approach offers a practical and inexpensive means to reduce the accumulation of inorganic nitrogen in the rearing water and sediment.

Thus a pioneer attempt was made at the brackish water shrimp culture unit of School of Industrial Fisheries, CUSAT in controlling the accumulation of toxic nitrogen species by resuspending and recycling the nutrients accumulated in the pond bottom and the reduction of the levels of toxic metabolites by the application of fin fish mediated bioturbation. It was hypothesized that bioturbation ultimately results in making the shrimp grow out system economically feasible by ensuring the reduced dietary protein levels with a steady and increased growth and production.

Thus, the specific objectives of the present study are:

a) Evaluation of the bioturbation efficiency of four fin fish species in the culture system of *Penaeus monodon*

b) Effectiveness of fish driven bioturbation in the resuspension of nutrients and its utilization in shrimp-fish aquaculture ponds

c) Bioturbating effect of various size groups of *Etroplus suratensis* in shrimp-fish aquaculture ponds

d) Optimisation of the stocking density of *Etroplus suratensis* in the shrimp-fish aquaculture ponds

e) Evaluation of the protein requirement of *Penaeus monodon* in shrimp-fish aquaculture ponds

1.2 Review of literature

Aquaculture pond bottom soils are recipients of large amounts of nitrogen, phosphorous and organic matter (Boyd, 1990, 1992; Boyd et al., 1994b; Munsiri et al., 1995), and these substances tend to accumulate in bottom soil. Pond bottom soils are enriched in organic matter (Maguire and Allen, 1986; Boyd, 1992; Boyd et al., 1994a; Smith, 1996; Martin et al., 1998), nitrogen and phosphorous (Chien and Tay, 1991; Smith, 1993, 1996; Boyd et al., 1994a; Hopkins et al., 1994; Martin et al., 1998; Ritvo et al., 2000).

Accumulation of carbon, nitrogen and phosphorous in the bottom of culture ponds have been reported by many authors (Eren et al., 1977; Avnimelech and Lacher, 1979). Avnimelech and Lacher (1979) reported that most of the nitrogen (75%) and phosphorous (80%) not retained by the harvested animal was recovered in the pond bottom soil. Organic carbon accumulation in the sediments was about 25% of the organic carbon added to the feed. Lin and Nash (1996) estimated that 26% of the nitrogen and 24% of the phosphorous applied as feed accumulated in the sediments of intensive shrimp ponds. Munsiri et al. (1996) reported a higher concentration of nutrients in bottom soil of older ponds compared to newer ones. Paez-Osuna et al. (1997) reported that in a semi-intensive shrimp operation the sediments accumulated 63.5% of the added Phosphorous. Ritvo et al. (1998) found that concentrations of several elements in the soil increased during one shrimp culture cycle. Funge-Smith and Briggs (1998) found that the sediments accumulated 24% of the feed nitrogen and 84% of the phosphorous. Martin et al. (1998) found that up to 38% of the total nitrogen input accumulated in the sediments, while Paez-Osuna et al. (1999) estimated that 47.2% of the phosphorous input was adsorbed by the sediments in shrimp ponds. Lemonnier and Brizard (2001) reported a correlation between sediment accumulation rate and final shrimp density in 13 earthen shrimp ponds in New Caledonia.

Aerobic mineralization of organic matter occurs only in a zone of few millimetres deep at the sediment surface (Jensen et al., 1994). Dissolved oxygen cannot enter rapidly in bottom soil because it must diffuse through the tiny, water-filled pore spaces among soil particles. At a depth of only a few millimetres below the soil surface, the demand for dissolved oxygen by microorganisms will exceed the rate that dissolved oxygen can move to that particular depth, and anaerobic conditions will develop.

One major ecological impact of marine aquaculture is the generation of vast quantities of particulate organic waste products leading to organic matter loading of underlying sediments, increasing mineralization rates and favouring of anoxic conditions (Hall et al., 1990; Holmer and Kristensen, 1992). Boyd (1992) reported that high concentrations of organic matter in bottom soil may result in anaerobic conditions in the surface layer of soil and at the soil-water interface. Hopkins et al. (1994), working in plastic lined ponds, suggested that sources of the accumulated sediments in a pond are uneaten food, faeces, decaying plankton, airborne debris, eroded soil and microorganisms. An unknown fraction of primary productivity, together with fish faeces and uneaten food, reaches the sediment as particulate organic matter, where it is available for microbial degradation (Blackburn et al., 1998). There is increasing evidence that the condition of pond bottoms and the exchange of substances between soil and water strongly influence water quality (Boyd, 1995). Due to the continuous input of organic matter to the bottom, microorganisms are continually decomposing both fresh, easily degradable (labile) organic matter and older, resistant (refractory) organic matter. Holmer et al. (2005) also reported the development of anoxic conditions in the pond bottom by the degradation of excess organic matter by the microorganisms.

Accumulated sediments in shrimp ponds are highly reduced (Guo, 1986). In anaerobic sediment, some microorganisms able to take up oxygen from nitrate, nitrite, iron, manganese oxides, sulphate and carbon dioxide to decompose organic matter, and releases toxic metabolites (Blackburn, 1987). Anaerobic metabolic pathways, such as methanogenesis, sulphate reduction and denitrification, are regarded as being responsible for at least 50% of the mineralisation of organic matter in sediments (Fry, 1987; Schroeder, 1987). Anaerobic soil does not effectively remove toxic reduced substances (nitrite and hydrogen sulphide in particular) produced by microbial activity in anaerobic soil layers and enters the pond water if the soil-water interface is anaerobic (Masuda and Boyd, 1994). These metabolites are potentially toxic to fish or shrimp.

Concurrence of growth retardation of fish/shrimp with development of anaerobic conditions in pond bottom was reported by Avnimelech et al. (1981). Similar conclusions related to fish culture was reported by Avnimelech and Zohar (1986) regarding the retardation of fish growth in intensive fish ponds. It is also reported that the conditions of pond bottom are exceptionally important for shrimps because they are animals that normally live on or near the bottom and are in proximity to the conditions on the pond bottom. Hopkins (1994) observed total mortality of shrimps in untreated ponds, whereas normal growth of shrimp occurred in these ponds with resuspended sediments. Avnimelech (1995) reported that reduced sediments negatively affected the shrimp growth, activity and health. The effect of accumulated sediment on shrimp feeding was studied by Allen et al. (1995) and found that shrimp feed intake was lesser from reduced sediments. Exposure of toxic materials endangered the well being of the cultured shrimp by reduced feeding and growth rate or by subjecting them highly sensitive to disease and mortality (Avnimelech and Ritvo, 2003). This demonstrated the potential effect of accumulated sediment on shrimp growth and survival.

The accumulated sediment is loosely consolidated and is readily relocated by small movements and can be affected by physical and biological factors such as resuspension and bioturbation. It is reported that the control of accumulated soil is possible in ponds since the organically enriched upper layer of soil being relatively light, is selectively resuspended compared to the denser mineral soil particles (Avnimelech and Ritvo, 2003).

The exposure of organic particles of bottom soil to the oxygenated water through mixing and resuspension may induce aerobic processes and it might enhance the recycling and utilization of feed residues (Hopkins et al., 1994). However, resuspension of reduced sediments is to be practiced with caution, since resuspension of reduced sediments into the water consumes oxygen and could expose shrimp to a flux of reduced toxic materials. Resuspension can be practiced only if it is done as a routine to prevent accumulation of reduced sediment (Avnimelech and Ritvo, 2003). Beveridge et al. (1994) reported that occasional resuspension of sediments by dragging a chain across fish pond bottom increases pond water turbidity levels and may also release reduced compounds into the water and should be practiced with extreme caution. Movements, foraging near the pond bottom or other activities of fish facilitate the continuous and routine resuspension of sediment in aquaculture ponds (Avnimelech et al., 1999). Hence fish bioturbation can act as an important mechanism for the continuous resuspension of sediment.

The bioturbation helps to maintain an oxidised layer of sediment in the soil-water interface by exposing more particles to the oxygenated water column by the particle reworking. The oxidized layer at the sediment surface is highly beneficial and should be maintained throughout the aquaculture crop. The oxidized layer at the sediment surface prevents diffusion of most toxic metabolites into pond water because they are oxidized to non-toxic forms by chemical and biological activity while passing through the aerobic surface layer. Nitrite will be oxidized to nitrate, ferrous iron converted to ferric iron, and hydrogen sulphide will be transformed to sulphate. Thus, it is extremely important to maintain the oxidized layer at the sediment surface in aquaculture ponds. Ponds should be managed to prevent large accumulations of fresh organic matter in the soil surface, or in the upper few millimetres of soil. Toxic metabolites entering well-oxygenated pond water will be quickly oxidized. However, if the rate of release of toxic metabolites into water exceeds the rate that metabolites are oxidized, equilibrium levels of metabolites in the water may be high enough to have detrimental effects on culture animals (Boyd et al., 2002).

Many studies have examined the importance of infaunal bioturbation in increasing the flux of dissolved nutrients from sediments to the overlying water (Aller, 1980; Kristensen and Blackburn, 1987). Reworking of soft sediments by benthic fauna has been recognized as an elemental process that modifies the key physical, chemical and biological features of the sediment (e.g. Rhoads, 1974; Aller, 1988). For instance, bioturbation accelerates chemical transformations and stimulates microbial processes related to organic matter degradation (Kristensen and Blackburn, 1987; Aller, 1988; Kristensen, 1988, 2000) and, hence, enhances the functioning of the entire benthic ecosystem (Rosenberg, 2001). Bioturbation by macro-invertebrates (Austen et al., 1998; Tita et al., 2000; Widdicombe et al., 2000) and physical disturbance (Schratzberger and Warwick, 1998, 1999) are important processes that influence the distribution, species composition, trophic structure and abundance of sediment-dwelling organisms ranging in size from microbes to macro fauna. The burrowing activity of benthic fauna, e.g. oligochaetes, is known to increase sediment flux rates by up to several folds (Chatarpaul et al., 1980; Fisher, 1982; Fry, 1982; Blackburn, 1987). Sediment reworking by benthic fauna results in redistribution of particles and destruction of sediment layers (Rhoads and Young, 1970; Rhoads, 1974), which enables introduction of reduced compounds to the oxidized surface environment and, on the other hand, burial of fresh organic matter beneath the surface (Kristensen, 2000). Such transport enhances the growth and activity of microbial communities and stimulates the degradation of redox-sensitive compounds, thereby accelerating the rate of organic matter turnover (Kristensen and Blackburn, 1987; Aller, 1988; Kristensen and Mikkelsen, 2003). Although less frequently studied, benthic and demersal nekton also influences conditions at the sediment-water interface (Hall, 1994). Metabolism by macrofauna directly contributes to the overall sediment metabolism and mineralization, while several indirect routes operate as well, biogenic constructions (e.g. burrows) serve as favourable microenvironments for microorganisms (Meyers et al., 1987; Marinelli et al., 2002), excretion provides nutrition for bacteria, and irrigation and ventilation of burrows enhance solute transport between pore water and the overlying water (Aller and Aller, 1998).

Bioturbation may increase the sediment phosphorous-binding ability by increasing the sediment O_2 content by resuspension, which increases the number of oxidized surfaces in particles suspended in water (Lehtoranta and Pitkanen, 2003). On the other hand, enhanced mineralization and burrow irrigation, resulting in increased transport of solutes from pore water to the overlying water, lead to increased regeneration and release of bioavailable phosphorous (Lehtoranta and Heiskanen, 2003). In the mineralization of organic nitrogen, the NH_4^+ released may be nitrified to NO_3^- under oxidized conditions and further reduced to molecular N_2 (denitrification), or may be directly converted to N_2 by NO_2^- (anammox). Both denitrification and anammox are strongly dependent on the supply of NO_3^- and NO_2^- and, hence, nitrogen removal through these processes occurs mainly at the interface of oxidized and reduced conditions (Hulth et al., 2005). Mchenga et al. (2007) reported that bioturbation activity of grapsid crab *Helice formosensis* significantly influences the physicochemical properties, fatty acid composition and organic matter profiles of the surrounding environment. Volkenborn et al. (2007) observed that bioturbation and bioirrigation activities of lugworms, *Arenicola marina* led to altered sediment and pore water characteristics and helped to maintain habitat properties suitable for population persistence. Their movement also prevented clogging of sediment interstices by fine particles and organic compounds. Cuny et al. (2007) also reported that bioturbation by the polycheate *Nereis diversicolor* favoured the development of bacteria which played an active role in natural bioremediation process of the oil polluted environments. On the other hand, the study of Cardoso et al. (2008) demonstrated that the bioturbation by *Hediste diversicolor* did not influence the remobilisation of mercury from the sediment to the water column, as it is a tedious process. Bioturbation potential of benthic macro invertebrates like chironomid larvae were reported by Biswas et al. (2009) and concluded that chironomids induced mobilisation of bound phosphates of subsurface sediments to the overlying water, additionally reduced the build-up of toxic metabolites in the sediment and thus promotes positive interspecific interactions. Thus the internal nutrient load in the aquaculture system was managed by decreasing allochthonous input of fertilisers. The study of bioturbation using species *Corophium volutator* revealed that density of species is an important parameter in particle transport and hence organic matter processing (Backer et al., 2011).

Very few studies, however, have considered the possible effects of fish bioturbation, although Tenore et al. (1982) suggested that fish bioturbation might explain the discrepancy between the rate of metabolite production and the flux of these metabolites from the system. Krom et al. (1985) showed that the amount of sediment resuspended into the water column was proportional to the size of the fish in the pond. Ten Winkel and Davids (1985) found that disturbance by a fish increased predatory mite feeding rates on chironomid larvae, and thereby indirectly influenced chironomid abundance, suggesting that trophic cascades may be altered by bioturbation. Spot (*Leiostomus xanthurus*) turns over about 1% of the upper 2 mm of the sediment surface per day while feeding (Billheimer and Coull, 1988), and epibenthic fish can influence benthic invertebrate abundance more by impacts associated with bioturbation than by predation (Flecker, 1992). Thus, ecological processes may differ greatly with and without bioturbation in ways that may influence infaunal assemblages. *Gobiosoma bosc* increased physical disruption of the sediment-water interface which may hinder diatom mat formation. Accumulation of organic matter in pond sediments is highly dependent on the rate of mineralisation and the activity of fish and benthic fauna.

The influence of perturbation activities of fish bioturbation on the pond ecosystem was reported by many authers (Scheffer, 1997; Riise and Roos, 1997; Avnimelech et al., 1999; Saha and Jana, 2003; Ritvo et al., 2004). Ritvo et al. (2004) demonstrated that the major effect of fish bioturbation in aquaculture system is the improvement of aerobic conditions at the pond bottom soil-water interface and it helps to decrease the concentration of total dissolved sulphide, soluble manganese, easily oxidized matter, and exchangeable ammonium at the sediment-water interface. The bioturbation activities of fish would also improve the vertical distribution of temperature and dissolved oxygen in the water column, especially in the lower water layers of ponds, which lead to better treatment efficiencies of pollutants. This fact significantly demonstrates the double benefit of integrating fish culture with wastewater stabilization, where there is an increase in the production of fish biomass coupled with the enhancement of waste treatment efficiency. Ritvo et al. (2004) and Rasmussen et al. (2005) reported that the presence of fish or any active moving objects in the water can generate mixing of the water column, thereby enhancing the dispersion/diffusion of dissolved substances throughout its environment. There is evidence that fish bioturbation can impact water layers as deep as the water-soil interface at the superficial layer of pond bottom. Fish bioturbation enables oxidation of sedimenting organic matter by resuspending it into aerobic upper water layers (Scheffer, 1997), enhances the release of phosphorous (Jana and Das, 1992; Jana and Sahu, 1993; Saha and Jana, 2003), and changes the structure of soil particles. Fish bioturbation not only enhances the aerobic conditions inside the whole water column but also in the pond bottom soil (Ritvo et al., 2004; Rasmussen et al., 2005). The bioturbatory activities of tilapia can override the vertical distribution of water temperature in stabilization ponds to a depth of 1 m during the dry season. At the same time it can bring some dissolved oxygen downwards to the lower layers of the water column, improving aerobic conditions on the pond bottom (Phan-Van et al., 2008).

Nutrients are predominantly supplied as fertilizer and/or feed; yet, in ponds that receive protein pellets, less than approximately 35% of the supplied nitrogen and phosphorous are retained in fish biomass (Avnimelech and Lacher, 1979; Krom et al., 1985; Foy and Rosell, 1991). The sediments are very significant sources of nitrogen to the overlying water, and it must be inferred that most organic nitrogen mineralization occurs in the sediments, rather than in the overlying water (Blackburn et al., 1988). Although a slow process, diffusion is the main mechanism by which nutrients become available from sediment to the water column.

Benthivorous fish influence the nutrient fluxes between water and sediment in several ways as they feed on benthic fauna or detritus (Schroeder, 1978; Zur, 1979, 1980). Positive effects of resuspending sediments for enhancing the release of nutrients have been found by stocking benthivorous fish and were reported by Jana and Sahu (1993) and Cline et al. (1994). Selection of a suitable benthivorous fish can increase the nutrient flux, which greatly influences the abiotic and biotic properties of the overlying water column (Northcote, 1988; Rahman, 2006). The foraging behaviour of different fishes is a critical factor affecting sediment disturbance and nutrient resuspension (Avnimelech et al., 1999; Rahman, 2006). Nutrient flow can be increased by fish driven resuspension, which is especially important in semi-intensively managed ponds, where fish production depends nearly entirely on natural food availability (Rahman and Verdegem, 2007).

Resuspension of bottom soil exposes organic particles to oxygenated water and induces aerobic decomposition and mineralization of organic matter (Graneli, 1979; Avnimelech et al., 1999; Hargreaves, 1998). Mineralization of organic matter occurs faster under aerobic than anaerobic conditions (Beristain, 2005). Due to the continuous resuspension and redeposition of particles and stirring of the surface sediment by fish and other organisms, organic matter becomes rather uniformly mixed in the upper layer of sediment. Nevertheless, usually there is a layer of fresh organic matter at the sediment surface that has not been completely mixed into the sediment. Organic matter concentration usually is greatest near the sediment surface (Munsiri et al., 1995).

Semi-intensive production is characterised by the dependency on natural feeds produced in the ponds (Edwards, 1993). Pond production is enhanced by inputs of manures and/or commercial fertilisers supporting a high primary and secondary production in the pond for the benefit of fish. Semi-intensive ponds are usually stagnant and the pond bottom is a potential trap for the sedimented decaying algae and allochthonic inputs. Recycling of the organic detritus deposited in the pond sediment is commonly believed to enhance pond productivity (Schroeder, 1987; Ayyappan et al., 1990; Edwards, 1993). Resuspension of bottom soil by benthivorous fish not only affected the water quality but also the nitrogen and phosphorous accumulations in fish, plankton, benthic macro invertebrates, and water and bottom soil. Greater resuspension may have resulted in a higher mineralization rate, creating highly eutrophic water with greater NO_3^--N, TAN, total nitrogen, PO_4^{3-}.P and total phosphorous concentrations in common carp tanks. Higher nitrogen and phosphorous availability in the water stimulated higher photosynthesis rates (Hepher et al., 1989; Poxton, 1991; Milstein and Svirsky, 1996), which resulted in greater phytoplankton, zooplankton and fish biomass in culture tanks with a bottom stirring fish. In fresh water aquaculture, Costa-Pierce and Pullin (1989) reported that common carp is a vigorous benthic feeder that has more potential to stir bottom soil due to their foraging behavior. The authors also observed 2.5 times higher yield in tilapia stirred ponds compared to unstirred one.

Measuring sediment fluxes enables elucidation of the major pathways in the mineralisation process and identification of the relative importance of the sediment in the overall recycling of nutrients in a fish pond. Aerobic mineralisation, measured as the rate of oxygen uptake, is regarded as responsible for approximately half of the organic carbon mineralisation in the sediment under eutrophic conditions in fertilised ponds (Fry, 1987). The rate of oxygen uptake is important in fish ponds as it affects the general health conditions of the fish (Schroeder, 1975).

Sediment-reworking fauna may stimulate coupled nitrification-denitrification by enhancing nitrogen mineralization, by excreting NH_4^+-N and by increasing the surface area of the oxic-anoxic interfaces (Henriksen and Kemp, 1988; Seitzinger, 1988; Tuominen et al., 1999). For example, denitrification forms a quantitatively important pathway for nitrogen removal in the northern Baltic Sea; accounting for 20-30% of external nitrogen input (Stockenberg and Johnstone, 1997; Tuominen et al., 1998). In the Gulf of Finland, the bulk nitrogen formation occurs via coupled nitrification- denitrification and is correlated with the abundance of sediment-dwelling fauna (Tuominen et al., 1998).

In coastal and estuarine ecosystems, benthic regeneration of bioavailable nutrients is a key factor that determines the overall productivity of the system, supplying dissolved inorganic nitrogen (DIN) (Blackburn and Henriksen, 1983; Blackburn et al., 1988; Stockenberg and Johnstone, 1997; Tuominen et al. 1997; Lehtoranta and Heiskanen, 2003) for primary producers. In ecosystems with progressed eutrophication, efficient internal nutrient cycling may form a strong feedback, a vicious circle that inhibits ecosystem recovery (Vahtera et al., 2007), organic matter decay and benthic nutrient exchange dynamics.

It is most likely that the current wastewater treatment technologies conventionally based on highly optimized physical, chemical and microbial processes (Brix, 1999) are not affordable for farmers to tackle the water pollution. Within such a context, the ecological technology, especially fish culture-integrated waste stabilization pond, has been demonstrated as an appropriate alternative for its cost effectiveness and nutrient recycling into fish biomass (Bartone and Khouri, 1990; DePauw and Salomoni, 1991; Edwards, 1992; Oswald, 1995; Liang et al., 1998; Saha and Jana, 2003; Phan-Van and De Pauw, 2005).

Jana et al. (2007) reported that polyculture with carps and prawn exerted considerable influence on the growth and activities of some biogeochemical cycling bacteria that regulate the nutrient cycle of the culture system. Jana et al. (2007) also reported greater utilisation of feed remnants and less accumulation of organic load in the bottom in carp-prawn polyculture. As a consequence, microbial communities, especially the biogeochemical cycling bacteria have been suitably modified to promote stability and create favourable food chain in the system. Phan-Van et al. (2008) reported that fish bioturbation enhances aerobic conditions inside the whole water column and pond bottom soil and also affects water quality reclamation process, nutrient cycling and variations of food web structure.

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Chapter 2

EVALUATION OF THE BIOTURBATION EFFICIENCY OF FOUR FIN FISH SPECIES IN THE CULTURE SYSTEM OF *PENAEUS MONODON*, FABRICIUS

Contents

2.1 Introduction

Penaeus monodon (tiger prawn) is a prime candidate species among brackish water farmers in Asian countries due to its high domestic and international market demand and consumers preference. However, farming of the species faces serious challenges due to the formation of toxic metabolites in the pond bottom, release of farm effluents having high inorganic nitrogen species and the consequential environmental issues related to its farming. Fish and shrimp grown in ponds are fed with a generous supply of feed. However, only a fraction of the feed nitrogen, phosphorous, and organic carbon are retained in the biomass of fish and shrimp (Avnimelech and Lacher, 1979; Foy and Rosell, 1991; Krom et al., 1985; Alongi et al., 2000; Briggs and Funge-Smith, 1994; Casillas-Hernandez et al., 2006, Jackson et al., 2003; Paez-Osuna et al., 1997). On the basis of more than 10 research publications, Avnimelech and Ritvo (2003) reported that the average amount of nutrients assimilated by shrimp and fish are 13, 29 and 16% of the C, N and P applied with the feed, respectively. A large fraction of the unused C, N and P accumulate in the sediment which can store 100-1000 times more nutrients than water (Biro, 1995).

Sediments are enriched with nutrients and organic matter by the sedimentation of organic matter from the overlying water column to the pond bottom. This sedimentation process as well as a slow downward movement of oxygen develops anaerobic conditions in the bottom soil of intensively stocked and fed fish ponds (Avnimelech and Ritvo, 2003). As a result, anaerobic conditions constrain production and restrict future intensification. Anaerobic conditions may adversely affect the aquaculture production due to unfavourable conditions in the pond bottom and by the diffusion of toxic compounds from the bottom soil to the upper water column. The development of anaerobic conditions in the pond bottom clearly coincides with fish growth retardation (Avnimelech and Zohar, 1986). Furthermore, shrimp that normally live on or near the bottom may be critically affected by adverse conditions in the pond bottom. Exposure to toxic materials accumulating in anaerobic sediments endangers the well being of the cultured shrimp. Reduced feeding, slower growth, mortality and possibly higher sensitivity to disease have been reported (Avnimelech and Ritvo, 2003). For example, TAN and NO_2^--N liberated in anaerobic or poorly oxygenated environments have led to mortality and provoked feeding changes in post larvae and juvenile shrimp (Chin and Chen, 1987; Chen and Chin, 1989; Chen et al., 1990; Frias-Espericueta et al., 1999, 2000).

Some microorganisms decompose organic matter in anoxic sediments by fermentation reactions that produce alcohols, ketones, aldehydes, and other organic compounds as metabolites. Other anoxic and anaerobic processes lead to the build-up of sulphides, reduced organic sulphur, ammonium, manganese ions and other reduced species (Blackburn, 1987).

Some of the metabolites, such as ammonia, hydrogen sulphide, nitrite, manganous ions and certain organic compounds, are potentially toxic to fish and shrimp (Avnimelech and Ritvo, 2003).

One of the major and critical parameters that affect processes and conditions in the pond bottom soil-water interface is the dissolved oxygen concentration. Pond bottom soils are found to have low dissolved oxygen concentration due to insufficient and slow diffusion of oxygen to the deeper layers. Oxygen does not penetrate deeper than 1 mm in stagnant, intensive and semi-intensive pond bottom soil cores (Meijer and Avnimelech, 1999). Pond bottom soil mixing can be done by aerators (Boyd et al., 1998), by towing chains above the bottom (Pullin, 1987) or by aquatic organisms (bioturbation) (Avnimelech et al., 1999; Blackburn et al., 1988; Riise and Roos, 1997; Scheffer, 1997). The process of bioturbation which involves both the dispersal of sediment particles and transport of interstitial pore water by benthic organisms is of global importance. Through various activities (mainly feeding, burrowing, locomotion and ventilation), benthic fauna modifies the physical, chemical and biological properties of the sediment (Aller, 1982; Rhoads, 1974). Bioturbation helps to expose more organic particles to the oxygenated water, enhance aerobic metabolism of the organic residues and prevent the development of anaerobic conditions (Ritvo et al., 2004).

Incorporation of a benthivorous fish into shrimp culture systems can increase bioturbation activity and reduce toxic materials, thus improving the growth and production of shrimp. In fresh water, several species of finfish have been used to induce bioturbation, the most well-known being the common carp (*Cyprinus carpio*) (Costa-Pierce and Pullin, 1989). In brackish water aquaculture, information on fish species with bioturbation activity is lacking. The selection of a suitable benthivorous fish can increase nutrient flux, which greatly influences the abiotic and biotic properties of the overlying water column (Northcote, 1988; Rahman et al., 2006).

The objective of this study was to assess the potential of four important brackish water finfish species to improve bottom soil conditions by bioturbation and thereby increase growth and production of shrimp. Fish tested in this study were selected according to their known or assumed sediment mixing activity. As an added advantage, all selected finfish species were edible.

2.2 Materials and methods

2.2.1 Finfish selected:

a) Pearl spot, *Pearlspot suratensis* (Bloch, 1790): Pearl spot is the native Indian representative of Cichlidae family, and has promising cultural potential. This species has wide market acceptability and is considered a delicacy in certain regions (Rattan and Parulekar, 1998). *E. suratensis* is an omnivore and bottom feeder, feeding mostly on algae growing on substrates and the associated bacterial and zooplanktonic biomass (Keshava et al., 1988).

b) Mullet, *Mugil cephalus* (Linnaeus, 1758): Member of Mugillidae family, with wide distribution in the Indian subcontinent. Grey mullet feed on detritus, diatoms, algae, microscopic invertebrates, desmids, annelids, crustaceans, bivalves and fish parts. Feed is filtered from mud and sand through their mouth and gills (Soyinka, 2008).

c) Milkfish, *Chanos chanos* (Forsskal, 1775): Milkfish is one of the most important food fish species in the world (FAO, 2000) and a member of family Chanidae. Milkfish is an inhabitant of brackish-water lagoons and are mostly benthic feeders, obtaining their food from the cyanobacterial mats and plant debris found in the littoral zone of the lagoon. Since approximately 90% of its natural diet consists of plants or detritus, the cultured fish is fed as an herbivore (Hiatt, 1944). The success of milkfish as a cultured food fish species may be attributed to its ability to tolerate extremes of environmental conditions.

d) Tilapia, *Oreochromis mossambicus* (Peters, 1852): Like most tilapias, *O. mossambicus* is thought to be primarily herbivorous or a mixed herbivore and detritivore (Bruton and Boltt, 1975; De Silva et al., 1984; Whitfield and Blaber, 1978) and belongs to the family Cichlidae. Tilapia disturbs bottom sediments to a greater degree than shrimp, both in foraging and nest building activities.

2.2.2 Experimental design

Bioturbation activity among four different brackish finfish species in combination with shrimp was studied in tank experiments. Five treatments were executed in triplicates (4 experimental and one control) in a completely randomized design. Treatment T1 was a combination of shrimp and Pearlspot (S+E), T2, a combination of shrimp and milkfish (S+Mi). Shrimp and mullet (S+Mu) combination was used in Treatment T3, and Treatment T4 was shrimp and tilapia (S+T) combination. Shrimp alone (SA) was used as a control in treatment T5.

2.2.3 Experimental setup

The experiment was carried out in 6 m^3 concrete tanks. All of the tanks were provided with a uniform 7 cm deep loamy sediment layer taken from an extensive shrimp culture pond. The soil consists of 20.7% clay, 29.8% silt and 49.5% sand. Lime was added at a rate of 3 kg per tank prior to stocking. Culture tanks were filled with 22 ppt saline water from the Cochin estuary, India. Water level in the culture tanks was maintained at 1 m throughout the culture period. Overflow pipes facilitated a runoff of rainwater with minimum tank water mixing. To stimulate phytoplankton development, culture tanks were fertilized with urea and super phosphate at the respective rates of 4 and 1 g/m^2/week during the first six weeks of culture. Cattle dung was also added to the tanks at a rate of 0.5 kg/m^2 before stocking. Twenty day old post larvae (PL 20) of *P. monodon* were purchased from a commercial hatchery and stocked in the tanks at a density of 6 PL/m^2,which is within the common density range for semi-intensive production systems (FAO, 2011). Tilapia, Pearlspot, mullet and milkfish fingerlings, 3 cm long, were purchased from Puduvypin fisheries research station of Kerala Agricultural University, India and stocked at a density of 3 $fingerlings/m^2$. Commercial shrimp feeds containing 30% crude protein were used in the experiment (Higashimaru Feeds India Limited, Kuthyathodu, S.India). Daily feeding rates of the shrimp were 15% body weight at the start of the experiment, reduced gradually to 3% at the end of the culture period. Fish were not fed separately. The feed was distributed evenly over the tank's surface, twice daily at 08:00 and 18:00 hrs. Shrimp and fish were harvested by completely draining the tanks at 105 days after stocking. Individual length, weight and survival of *P. monondon* and fish in each tank were recorded.

2.2.4 Incorporation of Luminophore (fluorescent tracer) to the sediment column

In order to evaluate sediment mixing by fish, the change in location of surface applied sand particles stained with fluorescent dyes was followed (Ritvo et al., 2004). Washed sand particles of 180-250 µm diameters were dyed according to Yasso (1966), with orange, green and violet daylight fluorescent spray paint (Kobe-Acrylic lacquer-Thailand). Orange stained particles were added to the soil in the tanks 3 days prior to fish stocking, green-dyed particles were added 45 days after fish stocking and violet-dyed particles were added 90 days after fish stocking. Using three different colours and application dates may enable to detect the effect of time and fish behaviour on sediment mixing. One hundred g of stained particles were mixed with 1 kg of dry pond soil and manually distributed over the top of each tank (Ritvo et al., 2004).

2.2.5 Sediment sampling and Fluorescent particle counting

Sediment samples were collected from six locations in each tank using a 2 cm diameter glass corer with a minimal disturbance of the soil stratification. Core sediment samples were collected 3 days prior to fish and shrimp stocking and 105 days after stocking. Sediment sampling was carried out in triplicate from each tank. Each core was sectioned into 0-1, 1-2, 2-3, and 3-7 cm deep slices. The 3 slices representing the same depth in a single tank were pooled together to obtain a composite sample of each depth layer in each tank. Fluorescent particles obtained from the sediment samples were illuminated under ultraviolet light. The stained fluorescent sand particles were counted manually using an optical microscope.

2.2.6 Laboratory analysis of sediment toxic metabolites and organic carbon

Organic carbon in the sediment was determined following EL Wakeel and Riley (1957). EOM was measured according to Avnimelech et al. (2004). Exchangeable total ammonium nitrogen, TAN, was determined following Grasshoff et al. (1983). Bottom soil pore water samples were extracted by centrifuging for 3 min at 6000 rpm. The supernatant was analyzed using the methylene blue method for dissolved sulphide (Cline, 1969) and inductively coupled plasma system for Fe and Mn (Ritvo et al., 2004).

2.2.7 Growth parameters

Shrimp and fish growth parameters such as average weight, yield (g/tank) and percentage survival were calculated. Average weight = Final weight – Initial weight

2.2.8 Statistical analysis

Chemical and fluorescent tracer particle data were analyzed by two-way ANOVA with treatment and tank depth as factors. Shrimp and fish indices were analyzed by one-way ANOVA. Post-hoc comparisons were made using Tukey's HSD test (Hayter, 1984). All descriptive statistics and model testing were calculated using SPSS version 18.

2.3 Results

Mixing of the sediment was evaluated using tracer sand particles. The percentages of the given sets of tracers as compared to all relevant particles along the sediment profile are given in Table 2.1, 2.2 and 2.3.

All (100%) of the stained sand particles in the control (shrimp alone) were found only in the topmost 0-1 cm layer, and none in the deeper layers, regardless of tracer sand application time. Different results were obtained for other treatments in which fish were co-stocked with shrimp, where tracer particles were found in deeper layers. This would happen due to the mixing of soil and indicated the bioturbation activities of fishes.

The orange dyed tracer particles, placed prior to stocking, were mixed with the topmost 2 cm in all cases except control. These particles were found down to the 3-7 cm layer in Pearlspot treatment, and found extensively in the 3-7 cm layer in tilapia treatment (Table 2.1).

Table 2.1 Orange dyed fluorescent tracer particles (Placed before stocking) along the bottom soil profile (percentage of total number in the soil profile)*

Treatments	**0-1 cm**	**1-2 cm**	**2-3 cm**	**3-7 cm**
Shrimp+Pearlspot	$44^{b} \pm 1$	$44^{c} \pm 1$	11^{b}	1^{a}
Shrimp+Milkfish	$36^{a} \pm 2$	$64^{d} \pm 2$	0^{a}	0^{a}
Shrimp+Mullet	$57^{c} \pm 1$	$43^{c} \pm 1$	0^{a}	0^{a}
Shrimp+Tilapia	$31^{b} \pm 6$	$25^{b} \pm 6.50$	$31.3^{c} \pm 1.15$	$12.7^{b} \pm 1.54$
Shrimp alone	100^{d}	0^{a}	0^{a}	0^{a}

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

The green stained sand particles, applied 45 days following stocking, were similarly mixed along the sediment profile. In mullet and milkfish treatment, mixing resulted in penetration of green fluorescent particles down to the 1-2 cm layer, while tanks co-stocked with Pearlspot showed mixing at depths 3-7 cm. More extensive mixing at that layer (3-7 cm) was found in tilapia treatment (Table 2.2).

Table 2.2 Green dyed fluorescent tracer particles, placed 45 days after stocking along the bottom soil profile (Percentage of total number in the soil profile) *

Treatments	0-1 cm	1-2 cm	2-3 cm	3-7 cm
Shrimp+Pearlspot	$53^{b} \pm 3$	$2^{b} \pm 3$	$11.67^{b} \pm .58$	$6.33^{b} \pm .58$
Shrimp+Milkfish	$60^{c} \pm 1$	$40^{c} \pm 1$	0^{a}	0^{a}
Shrimp+Mullet	$53^{b} \pm 4$	$47^{d} \pm 4$	0^{a}	0^{a}
Shrimp+Tilapia	$29^{a} \pm 1$	$27^{b} \pm 1$	$29.67^{c} \pm .58$	$14.33^{c} \pm .58$
Shrimp alone	$100^{d} \pm 0$	0^{a}	0^{a}	0^{a}

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

The violet stained sand particles, applied 90 days after stocking and just 15 days prior to harvest and sediment sampling, were found only in the topmost 0-1 cm layer for Pearlspot, mullet, and milkfish. The tilapia co-stocked tanks showed most of the tracer particles (71%) in the 1-2 cm layer with some (11%) in the 2-3 cm layer. In control, (shrimp alone) also particles were recovered in the upper layer of the sediment. It can be concluded that the shrimp (reaching an average size of more than 5 g at harvest), located on or near the bottom, did not supply enough shear force to significantly re-suspend and mix the sediment (Table 2.3).

Table 2.3 Violet dyed fluorescent tracer particles, placed 90 days after stocking along the bottom soil profile (Percentage of total number in the soil profile) *

Treatments	0-1 cm	1-2 cm	2-3 cm	3-7 cm
Shrimp+Pearlspot	$100^{b} \pm 0$	0^{a}	0^{a}	0^{a}
Shrimp+Milkfish	$100^{b} \pm 0$	0^{a}	0^{a}	0^{a}
Shrimp+Mullet	$100^{b} \pm 0$	0^{a}	0^{a}	0^{a}
Shrimp+Tilapia	$18^{a} \pm 3$	$71^{b} \pm 3$	11^{b}	0
Shrimp alone	$100^{b} \pm 0$	0	0	0

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

Organic carbon concentrations along the sediment profile are given in Fig. 2.1. Organic carbon concentrations were high in the topmost layer in all treatments except the tanks co-stocked with Pearlspot and tilapia (Table 2.4). Although treatment with Pearlspot showed the lowest concentration of organic matter and varied significantly compared to all other samples, where the concentration of organic matter in the top most 0-1 cm layer were significantly ($p<0.05$) lower as compared to all other treatments. The levels of EOM along the sediment profile in the different treatments are given in Fig. 2.2. The EOM levels were high all along the sediment profile in the shrimp alone tanks (control). The other extreme was found in tanks co-stocked with Pearlspot, where EOM was approximately one third of the level found in the control, all along the tested soil profile. In tanks co-stocked with other fish, the EOM levels in the topmost layers (0-1 and 1-2 cm) were significantly ($p<0.05$) high, on the other hand EOM was low in the bottom layers, (2-3cm and 3-7 cm).

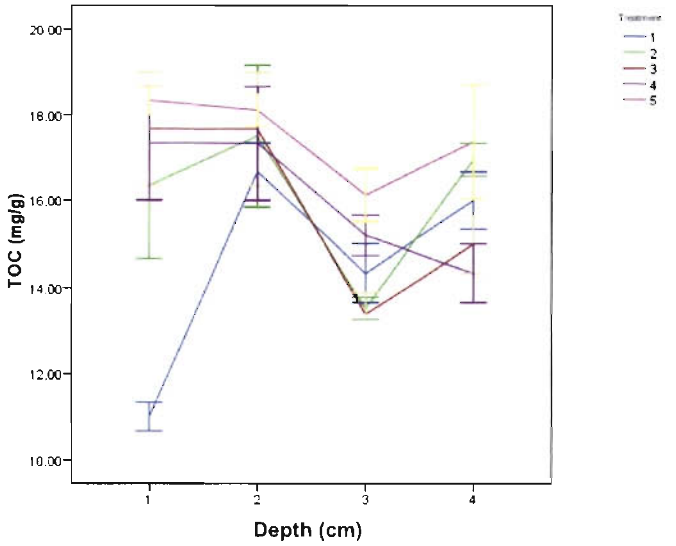


Figure 2.1 Concentrations of total organic carbon (TOC) in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

Table 2.4 Concentrations of parameters in the tank bottom soil, 105 days after stocking *

Depth	Treatment	Ammonium (mg/g)	EOM (mgC/g)	Soluble Iron (mg/l)	Soluble Mn (mg/l)	Total C (mg/g)	Sulphide (ppm)
0-1 cm	**Etroplus**	$0.03^{a} \pm 0$	$0.14^{a} \pm 0.02$	$0.38^{a} \pm 0.03$	$0.08^{a} + 0$	$11.0^{a} \pm 0.1$	$0.10^{a} + 0$
	Milkfish	$0.12^{b} \pm 0$	$0.35^{b} \pm 0.02$	$0.40^{a} + 0$	$0.09^{b} \pm 0$	$18.3^{b} \pm 0.1$	$0.70^{b} \pm 0$
	Mullet	$0.13^{c} \pm 0$	$0.34^{b} \pm 0.01$	$0.40^{a} + 0$	$0.13^{c} \pm 0$	$17.3^{b,d} \pm 0.38$	$0.72^{b} \pm 0.01$
	Tilapia	$0.17^{d} \pm 0$	$0.51^{c} \pm 0.01$	$0.53^{b} + 0$	$0.12^{d} \pm 0$	$13.4^{c} \pm 0.15$	$1.09^{c} \pm 0$
	Control	$0.17^{c} \pm 0$	$0.43^{d} \pm 0.01$	$0.59^{b} \pm 0$	$0.14^{c} \pm 0$	$16.9^{d} \pm 0.11$	$1.25^{d} \pm 0.01$
1-2 cm	**Etroplus**	$0.50^{a} \pm 0$	$0.18^{a} \pm 0$	$0.42^{a} \pm 0.01$	$0.06^{a} \pm 0$	$16.3^{ac} \pm 0.48$	$0.25^{a} \pm 0.01$
	Milkfish	$0.16^{c} \pm 0$	$0.33^{b} \pm 0.01$	$0.52^{b} \pm 0$	$0.08^{b} \pm 0$	$16.7^{a} \pm 0.19$	$1.15^{b} \pm 0.01$
	Mullet	$0.13^{b} \pm 0$	$0.33^{b} + 0$	$0.53^{b} \pm 0$	$0.09^{b} \pm 0$	$18.1^{b} \pm 0.11$	$2.00^{c} \pm 0.06$
	Tilapia	$0.17^{d} \pm 0$	$0.41^{c} + 0$	$0.71^{c} + 0.01$	$0.13^{c} \pm 0$	$15.2^{cd} \pm 0.13$	$1.86^{c} \pm 0.04$
	Control	$0.18^{c} \pm 0$	$0.39^{c} + 0.01$	$0.68^{d} \pm 0.01$	$0.13^{c} \pm 0$	$15.0^{d} \pm 0.19$	$2.31^{d} \pm 0.01$
2-3 cm	**Etroplus**	$0.06^{a} \pm 0$	$0.13^{a} + 0$	$0.29^{a} \pm 0.03$	$0.06^{a} \pm 0$	$17.7^{a} \pm 0.38$	$0.11^{a} \pm 0.01$
	Milkfish	$0.16^{d} \pm 0$	$0.17^{b} \pm 0$	$0.22^{a} \pm 0.01$	$0.08^{b} \pm 0$	$17.5^{a} \pm 0.48$	$1.50^{b} \pm 0.12$
	Mullet	$0.15^{c} \pm 0$	$0.13^{a} \pm 0$	$0.22^{a} \pm 0$	$0.09^{b} \pm 0$	$14.3^{b} \pm 0.19$	$1.67^{bd} \pm 0.01$
	Tilapia	$0.11^{b} \pm 0$	$0.18^{b} \pm 0$	$0.78^{b} \pm 0.01$	$0.13^{c} \pm 0.01$	$16.1^{a} \pm 0.18$	$1.16^{c} \pm 0.01$
	Control	$0.18^{c} \pm 0$	$0.35^{c} \pm 0$	$0.83^{b} \pm 0.02$	$0.13^{c} \pm 0$	$14.3^{b} \pm 0.19$	$1.86^{d} \pm 0.03$
3-7 cm	**Etroplus**	$0.13^{a} \pm 0$	$0.15^{a} \pm 0.01$	$0.47^{ad} \pm 0.01$	$0.05^{a} \pm 0$	$17.3^{ac} \pm 0.38$	$0.05^{a} \pm 0$
	Milkfish	$0.19^{b} \pm 0$	$0.21^{b} \pm 0.01$	$0.33^{b} \pm 0.02$	$0.17^{b} \pm 0$	$17.7^{a} \pm 0.38$	$0.33^{b} \pm 0.01$
	Mullet	$0.20^{c} \pm 0$	$0.18^{c} \pm 0$	$0.41^{a} \pm 0.02$	$0.15^{b} \pm 0.01$	$13.5^{b} \pm 0.07$	$0.27^{c} \pm 0$
	Tilapia	$0.20^{c} \pm 0$	$0.28^{d} \pm 0.01$	$0.58^{c} \pm 0.01$	$0.19^{b} \pm 0$	$16.0^{c} \pm 0.19$	$0.90^{d} \pm 0.01$
	Control	$0.22^{d} \pm 0$	$0.38^{c} \pm 0$	$0.52^{cd} \pm 0.01$	$0.17^{b} \pm 0.02$	$17.4^{ac} \pm 0.39$	$0.90^{d} \pm 0.01$
Effects							
Treatment		***	***	***	***	***	***
Depth		***	***	***	***	***	***
Treatment*Depth		***	***	***	***	***	***

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

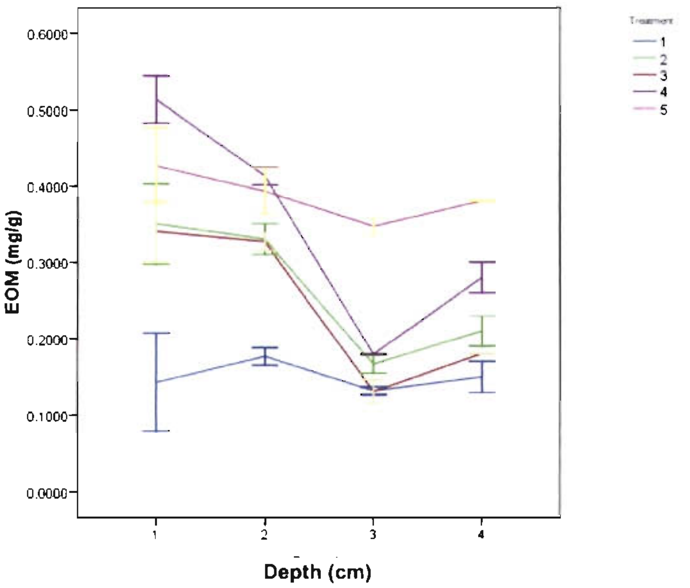


Figure 2.2 Concentrations of easily oxidized matter (EOM) in the tank bottom soil, 105 days after stocking. Error bars represent standard

Concentrations of iron and manganese in the sediment pore water along the bottom soil profile in the different tanks are shown in Figs. 2.3 and 2.4, respectively. Soluble iron in the control, (shrimp only) and in tanks co-stocked with tilapia, was higher, in most cases significantly ($p<0.05$) higher, than in the other treatments, indicative of minimal reducing conditions. Soluble manganese concentrations in the control tanks were the highest, about 1.3 mg/l in the 1-2 cm layers and 1.9 mg/l in the 3-7 cm layer. Soluble manganese in the Pearlspot co-stocked tanks was very low all along the soil profile, significantly lower in the control, averaging 0.06±0.01 mg/l. The other treatments were in an intermediate position between the two extremes viz. the control and the Pearlspot treatments. The tilapia co-stocked tanks had high manganese concentrations in the two deeper soil layers (2-3 and 3-7 cm), similar to the equivalent concentrations in the control, yet soluble manganese in the top 2 layers was low (0.1 mg/l).

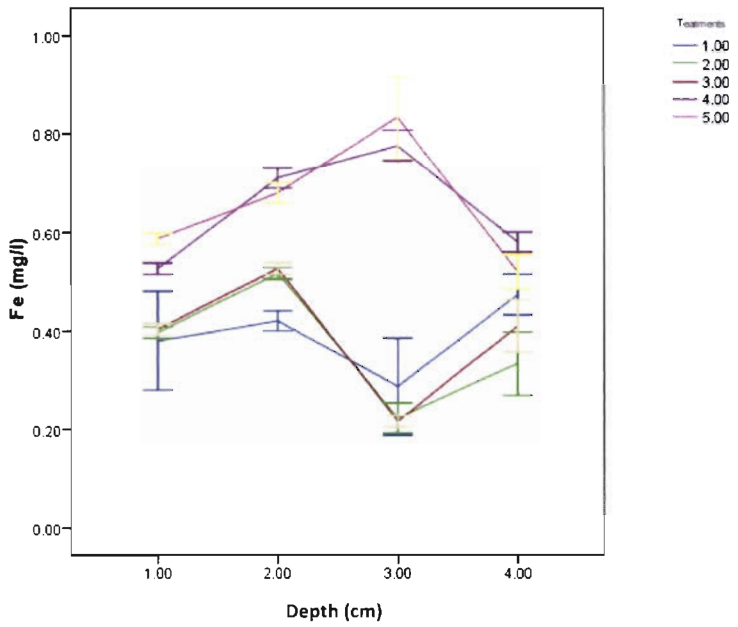


Figure 2.3 Concentrations of soluble iron in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

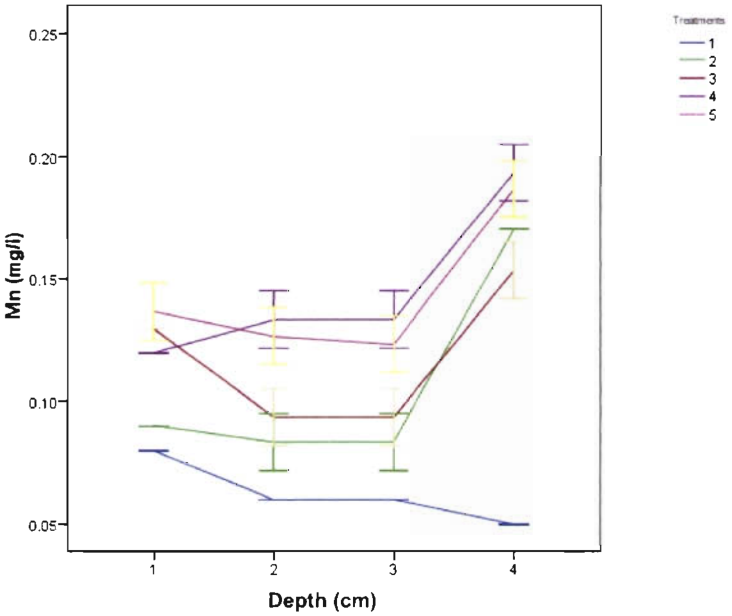


Figure 2.4 Concentrations of soluble manganese in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

Soluble manganese in the mullet and milkfish co-stocked tanks followed the same pattern of low concentrations in the topmost and bottom soil layers, with rather high levels in the intermediate layers (1-2 and 2-3 cm). This may be an indication of high redox potential during the initial stages of the experiment and subsequent accumulation of organic matter resulting in the depletion of oxygen in the intermediate soil layers.

Sulphide concentration in the pore water of the bottom soil profiles is shown in Fig. 2.5. Sulphide pore water concentrations in the control tanks (T5; shrimp alone) were significantly ($p<0.05$) high along the whole profile. The highest concentrations, in the range of about 1.86-2.31 mg/l, were found in the intermediate layers (1-2 and 2–3 cm), characterised by apparently accumulated oxygen consuming organic matter and consequently had a limited supply of oxygen. The topmost layer of sediment that was in contact with the aerated water had a lower concentration (1.25 mg/l), and the deepest layer (3-7 cm) a level of 0.8 mg/l, probably due to a lower concentration of oxygen consuming organic matter. Completely different results were obtained in tanks co stocked with Pearlspot, where sulphide pore water concentrations were significantly ($p<0.05$) low all along the soil profile. The average concentration of sulphide in the pore water of this treatment was only $0.10^{a}\pm0$, $0.25^{a}\pm 0.01$, $0.11^{a}\pm0.01$, $0.05^{a}\pm0$ mg/l for 0-1, 1-2, 2-3 and 3-7 cm layers respectively. The sulphide concentrations of the tanks co-stocked with other finfish were lower than those of the control, yet followed a similar pattern. Maximum sulphide concentrations were found in the intermediate soil layers (range of 1-2mg/l), while concentrations in the topmost layer were slightly lower than those in the control.

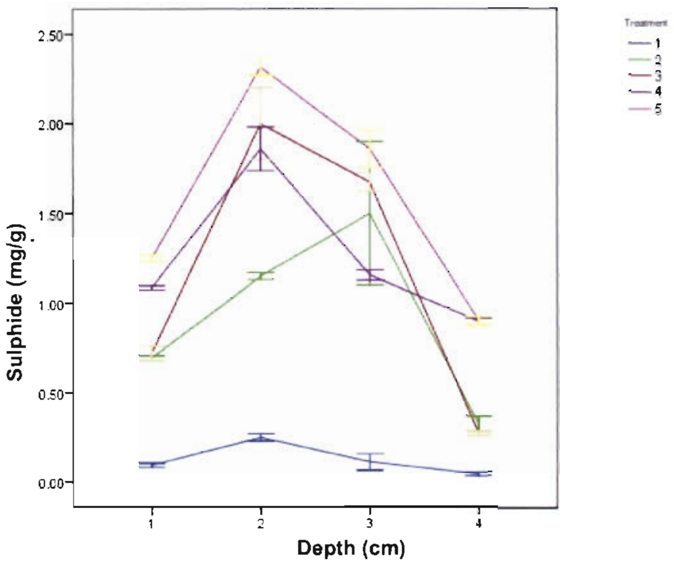


Figure 2.5 Concentrations of sulphide in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

Another potential indicator of redox conditions in pond bottom sediments is TAN. TAN concentrations along the sediment profile are shown in Fig. 2.6. TAN concentrations greater than 0.15 mg/g were found in the sediment of the control following 105 culture days. TAN concentrations were a bit lower along the sediment profile for all co-stocked fish treatments, especially for Pearlspot. TAN concentrations in all layers, especially down to 3 cm, in the tanks co-stocked with Pearlspot were about one third of those found in other treatments ($p<0.05$).

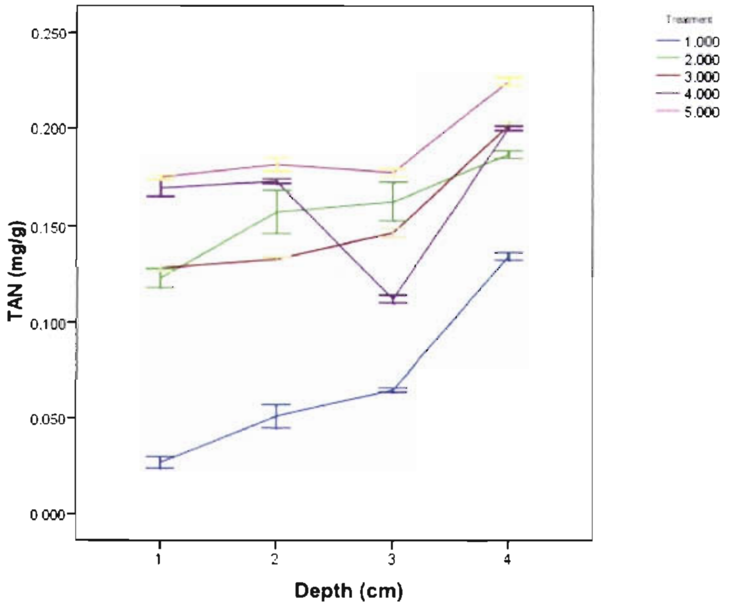


Figure 2.6 Concentrations of total ammonium nitrogen (TAN) in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

Shrimp growth parameters in the different treatments are given in Table 2.5. The lowest average weight of shrimp at harvest, 6.3^{a}±2.5 g, was found in the control ($p<0.05$). Average shrimp weights in the tanks co-stocked with tilapia, milkfish and mullet were 6.7^{a}±2, 8.5^{a}± 0.5 and 9.0^{a}±0.3 g, respectively (Fig. 2.7). The average weight of shrimp harvested in the tanks co-stocked with Pearlspot was 13.3^{b}±0.5 g, twice as high as the control and about 50% higher than in tanks co stocked with the other tested fish. Survival was low in the tanks co-stocked with tilapia (58±14%) and highest ($p<0.05$) in tanks co-stocked with Pearlspot (97±4%; Fig. 2.8). Survival in other treatments was in the range of 74-89%. Yield of shrimp was also found to be higher in Pearlspot co-stocked treatment (Fig. 2.9). Fish growth parameters are also given in Table 2.6. Fish production per tank was 609, 1012, 296 and 667 g for Pearlspot, milkfish, mullet and tilapia, respectively. Interestingly, in T4 treatment, prolific breeding of tilapia led to a doubling of the original population during the tenure of the experiment. During the experimental period, *O.mossambicus* attained sexual maturity and produced many offspring. Competition between fingerlings for shrimp feed might be a reason for the observed low shrimp yield in the tilapia co-stocked tanks.

Table 2.5 Shrimp Growth Parameters

Treatment	Average weight (g)	Survival (%)	yield (g/tank)
T1 (Shrimp+Pearlspot)	$13.3^{b} \pm 0.47$	$97^{d} \pm 4$	$463.7^{c} \pm 26.36$
T2 (shrimp+Milk fish)	$8.5^{a} \pm 0.50$	$89^{c} \pm 15$b	$251.0^{b} \pm 36.25$
T3 (Shrimp+Mullet)	$9.0^{a} \pm 0.27$	$74^{b} \pm 9$a	$239.8^{b} \pm 28.75$
T4 (Shrimp+Tilapia)	$6.7^{a} \pm 1.97$	$58^{a} \pm 14$	$145.2^{a} \pm 72.18$
T5 (Shrimp alone)	$6.3^{a} \pm 2.45$	$79^{abc} \pm 10$	$169.6^{ab} \pm 45.13$

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

Table 2.6 Fish Growth Parameters

Fish	Average weight (g)	yield (g/tank)	Survival (%)
Pearlspot	33.9±15.4	609±277	100±0
Milkfish	61.6±8.2	1012±242	91±11
Mullet	18.8±11.5	296±165	89±10
Tilapia	37.0±6.1 (*)	667±110	~300(*)

(*) Number of fish found at harvest was about three times number of fish stocked. Average fish weight is given for mature fish only, not including off springs

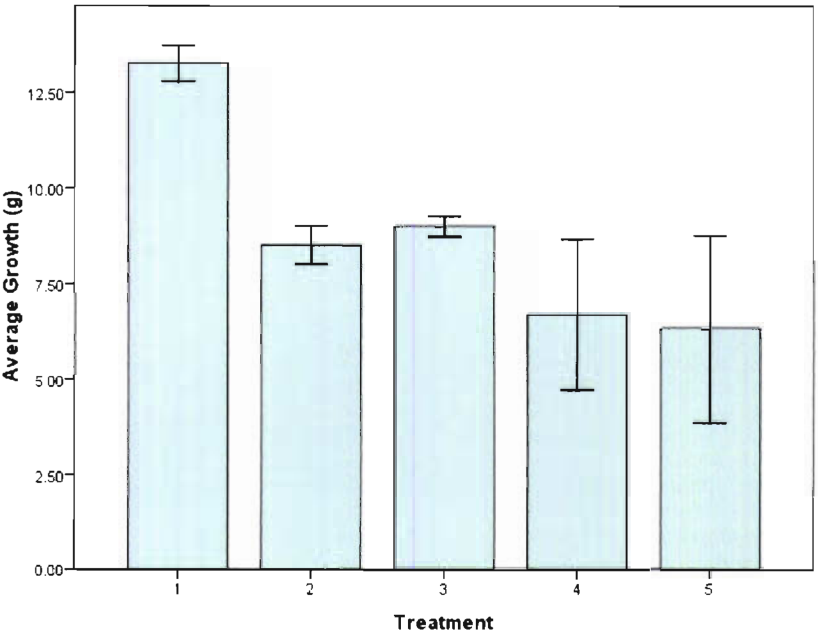


Figure 2.7 Average growth of shrimp. Error bars represent standard deviation

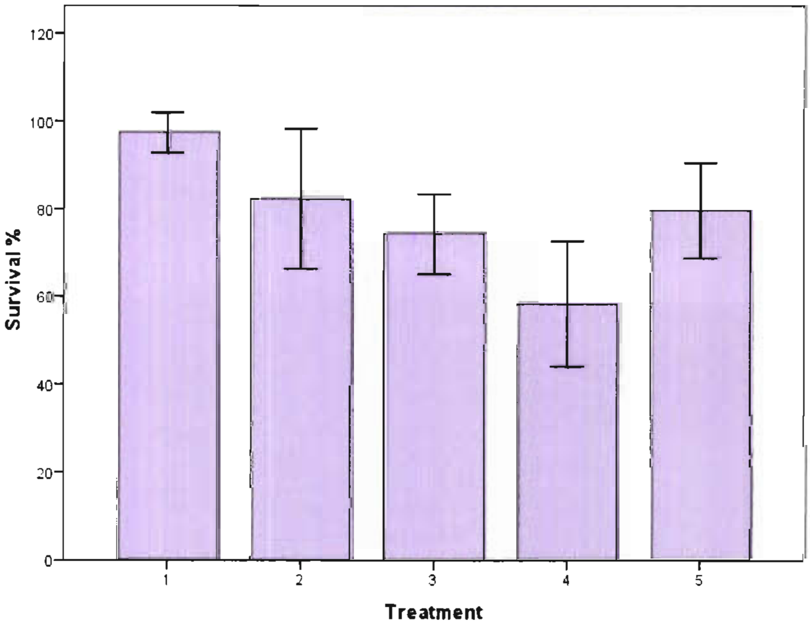


Figure 2.8 Survival % of shrimp. Error bars represent standard deviation

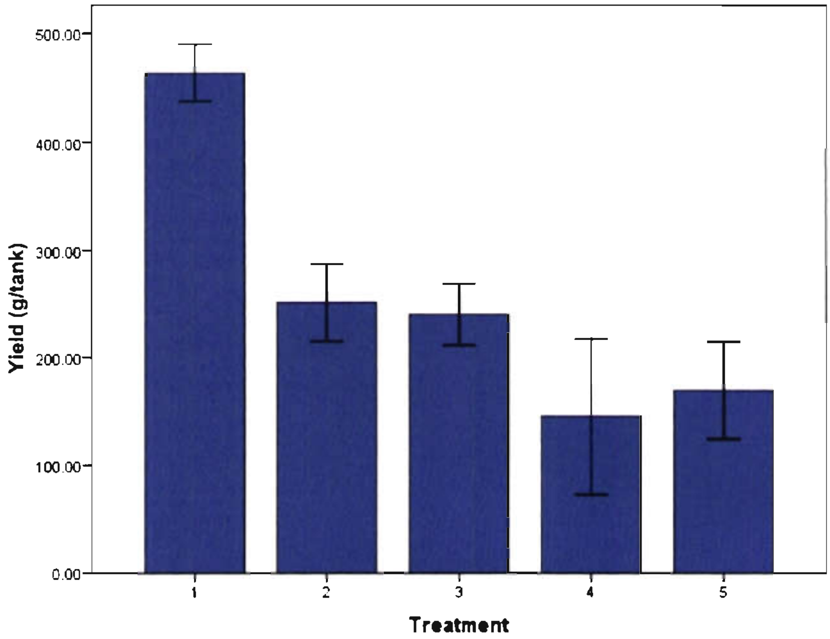


Figure 2.9 Yield of shrimp. Error bars represent standard deviation

2.4 Discussion

Vertical displacement of sand particles stained by fluorescent pigments is a direct method to evaluate physical mixing processes in the sediment, a method proposed and developed by Ritvo et al. (2004).

All tested finfish induced penetration of stained sand particles through the disturbance and mixing of the sediment. Most of the vertical displacement, in all fish species except tilapia and Pearlspot, was limited to the depth of 2 cm. In addition, the sand particles applied 15 days prior to harvest were not found below application site for all finfish, except tilapia. This may be due to the short time interval, change in the sediment mechanical properties (e.g. higher viscosity) or changes in fish behaviour. Sediment mixing was clearly higher in the case of tilapia. This may be attributed to the foraging and nest building activities of tilapia, which led to the deep vertical penetration of the stained sand particles and was intensive enough to induce a clear effect at just 15 days prior to harvest.

Resuspension of sediments may affect the chemical and biological characteristics of the sediment. Undisturbed sediments are typified by a steep oxygen gradient from the overlying oxidized water to a poorly oxidized water-sediment interface and anaerobic sediment just a few mm below this interface (Meijer and Avnimelech, 1999). Resuspension of the sediment raises particles and organic matter up toward the oxidized water, where it can undergo chemical oxidation processes and relatively fast aerobic microbial degradation processes. Several chemical components of the sediment, including: OC, EOM, soluble iron and manganese, soluble sulphite, and TAN that were likely to be affected by the resuspension process were determined along the sediment profiles in the different treatments.

Organic carbon concentrations were high in the superficial layer of all treatments except that of Pearlspot co-stocking. Normally, the level of organic matter in fish pond sediments is highest in the topmost layer, where organic particles accumulate (Munsiri et al., 1996). The results followed this trend in all cases, except for Pearlspot. The reduced levels of organic carbon in Pearlspot treatment is an indication of vigorous mixing and resuspension of this shallow sediment layer through the activity of the Pearlspot.

The pond sediment organic matter contains a large fraction of stable, slowly degradable, organic carbon. Thus, it is not a sensitive indicator to environmental conditions in the pond soil (Avnimelech et al., 2004). A fraction of the soil organic matter, EOM, is oxidised relatively faster and was proposed as a better indicator of pond bottom soil properties and changes (Avnimelech et al., 2004). It seems that vigorous resuspension by the Pearlspot leads to an effective microbial degradation of the EOM. On contrary, with the other fish, resuspension of pond sediments was not vigorous enough to compensate for the sedimentation of organic residues (feed, feces, algae etc.) from the water onto the topmost bottom soil layers.

The concentrations of soluble iron and manganese are very sensitive indicators of the redox conditions. The oxidized species, Fe^{3+} ferric ion and Mn^{4+} manganese ions form highly insoluble salts, oxides and hydroxides in the pond bottom soil. The concentrations of either iron or manganese in oxidized systems are very low. However, the reduced species, the divalent, Fe^{2+} (ferrous) or Mn^{2+} (manganous ions) form soluble species in the pond bottom soils (Ritvo et al., 2004). In addition to the use of manganese as a sensitive indicator of redox conditions, it should be mentioned that high concentrations in solution may be toxic to fish (Nix and Ingols, 1981). In the present study high concentrations of iron and manganese were found in control and significantly lower values in Pearlspot co-stocking treatment. The results clearly indicated that very vigorous resuspension of the sediment by Pearlspot and ensuing soil oxidation due to this activity minimised the production of low redox conditions / toxic metabolites.

Sulphides are present in sediments as a result of the reduction of sulphates or other oxidized sulphur species under very low redox conditions of ca ~100 mv (Reddy et al., 1986). Sulphide is highly toxic and affects shrimp at a very low concentration range of below 1 ppm (Avnimelech and Ritvo, 2003). The control of sulphides in ponds is an essential means to provide good environmental conditions and high potential shrimp production. Concentrations of sulphide were low in Pearlspot co-stocked treatments than control. It was clearly seen, in accordance with the results obtained for the other redox indicators studied here, that the Pearlspot seems to vigorously mix the soil down to the bottom layer, aerating the soil and dramatically lowering the sulphide concentrations.

Higher concentrations of TAN were observed in control. High TAN concentrations are expected in chemically reduced sediments due to the effect of low oxygen on both organic nitrogen mineralization and on nitrification. Anaerobic metabolism of organic matter has a low energy yield as compared to aerobic processes (Reddy et al., 1986). Thus, for a given energy yield, much more organic substrates are metabolized and more TAN is released. Under aerobic conditions TAN is nitrified, yet, is not oxidized and thus accumulates when oxygen is absent or low. The effects of anoxic conditions on TAN accumulation may have a negative impact on shrimp growth. For example, the accumulation of ammonia may lead to fish mortality. Spotte (1979), Chen et al. (1990) and Colt and Armstrong (1981) observed that ammonia and its intermediate oxidation product, nitrite, are toxic to fish, molluscs and crustaceans. The toxicity of *P. monodon* larvae to ammonia and nitrite has been reported by Chin and Chen (1987) and Chen and Chin (1988). The interaction between ammonia and shrimp production is thus an important consideration for aquaculturists (Frias-Espericueta et al., 1999). The 0-1 cm layer of Pearlspot added treatment showed lower concentration of $0.03^{a}\pm0$ mg/g, while it was $0.17^{c}\pm0$ mg/g in control. The low concentrations of TAN along the soil profiles of Pearlspot co-stocked treatments indicated the aerobic conditions induced by Pearlspot stirring activity.

Toxic reduced components such as sulphides, ammonia and manganese ions are produced in anaerobic condition and may diffuse up to the water column, affecting the shrimp. An aerobic sediment layer may serve as a barrier, within which the diffusing anaerobic species are oxidized and detoxified. In order to achieve this aerobic layer, the use of suitable finfish has been suggested as a means to efficiently resuspend, mix and oxidize the soil surface (e.g. Ritvo et al., 2004). In the present study finfish incorporated treatments produced enhanced growth for shrimp. The highest growth and survival were recorded especially in Pearlspot co-stocked treatment due to more favourable conditions. In the absence of finfish, high concentrations of organic matter, EOM and low redox indicators such as ammonium, soluble iron, soluble manganese and soluble sulphide were found to accumulate in superficial layers following a period of 105 days. However, in the presence of finfish, top layer sediment concentrations of EOM and of the different low redox indicators were clearly decreased, particularly in the presence of Pearlspot. The lowest average weight of shrimp was seen in the control. Low redox metabolites such as sulphide, manganese and ammonium are toxic; their presence in the upper layers of the sediment may negatively affect shrimp growth and survival. In support of this, it was found that co-stocking the aquaculture pond with fish that re-suspend and aerate the sediment raises both shrimp survival growth and production, would be further enhanced even greater extent when Pearlspot co-stocking was carried out.

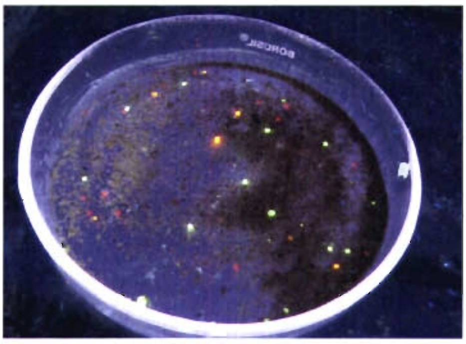
In addition to recording the effect of finfish presence on bottom soil chemistry, mixing of the sediment was evaluated using tracer stained sand particles. Interestingly, maximum mixing with the deeper layers was observed in the case of co-stocking with tilapia, even though tilapia had the lowest beneficial effect on bottom soil oxidation. This apparent controversy seems to be due to two different modes of bioturbation. In one mode, the tilapia digs site-specific holes and mixes the various bottom soil layers only during nest building activities. In the second mode, a continuous resuspension of bottom material occurs. Soil particles, specifically the lighter organic rich particles, are raised up to the aerated water column where they are aerobically metabolized and thus dominance of anaerobic conditions is prevented. The results of this study indicate that Pearlspot is very effective in this second mode of bioturbation. Nevertheless, the biological and physical aspects controlling the various bioturbation modes are not yet fully understood.

2.5 Conclusion

The objective of this work was to test the hypothesis of selecting suitable finfish and investigating potential methods to evaluate the efficiency of the induced bioturbation. Shrimp, (*P. monodon*) were stocked at a relatively low density (6 PL/m^2, final biomass of 30-75 g/m^2) in systems without exchanging water. The present study directs attention towards the potential advantage of co-stocking with fish in shrimp culture. Finfish may act as an efficient bioturbating agent, effectively improving shrimp production and in addition, enhance the economic growth from shrimp production at no additional cost. The present study highlights the potential advantage of co stocking *P. monodon* with Pearlspot, both as a bioturbating agent and fish as an additional income to the farmers. Co-stocking with Pearlspot was found to be highly advantageous, a highly marketable fish, may add significantly to shrimp production economy. Further studies implementing the principles demonstrated in this study, investigating the optimal ratio of finfish to shrimp, developing efficient and less expensive feed regimes and studying the implications under field conditions are needed. Clear and practical indicators to characterize the effect of bioturbation on bottom soil properties, as demonstrated in this work, may help and ease further studies in this direction.

Preparation of fluorescent tracer particles

6m^3 concrete tanks prepared for the experiment



Fluorescent tracer illuminated under UV light

Collecting sediment core samples from experimental tanks

Digging of soil by nest building of Tilapia

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Chapter 3

EFFECTIVENESS OF FISH DRIVEN BIOTURBATION IN THE RESUSPENSION OF NUTRIENTS, EFFECTS ON WATER QUALITY AND UTILIZATION OF RESUSPENDED NUTRIENTS IN SHRIMP-FISH AQUACULTURE TANKS

Contents

3.1 Introduction

The species dominating marine shrimp culture in South East Asia are penaeid shrimp, especially *Penaeus monodon*, commonly known as black tiger shrimp or giant tiger shrimp. In the shrimp culture system, the actual amount of nutrients assimilated in to shrimp biomass is a small fraction of the total applied as feed. Unconsumed feed waste and excreta potentially contaminate both inside and outside of the culture environment. The main environmental concerns in the shrimp farming sector are the increased levels of nutrients including nitrogen and phosphorous, the excess quantities of suspended solids and particulate organic matter in the waste water released from the farms. The sediment of aquatic ecosystem is a reservoir of nutrients, organic compounds, and a large variety of solutes (Boyd, 1995; Reddy et al., 1995; Woodruff et al., 1999). Organic matter and nitrogenous waste from uneaten feed, dead plankton, mineral soils, airborne debris, shrimp faeces and pathogenic microorganisms form organic sludge (Bergheim and Asgard, 1996) and accumulate in pond bottom through sedimentation process. Nitrogenous waste accumulation occurs at an increased rate as a result of the fact that, in most aquaculture systems shrimp retain only 20-30% of nitrogen applied as feed nitrogen (Avnimelech and Ritvo, 2003; Lin and Nash, 1996; Funge-Smith and Briggs, 1998). Thus, nitrogen not retained by shrimps accumulates in sediment column (Avnimelech and Lacher, 1979). Reduced penetration of oxygen to the deeper layers of the sediment column exert more adverse effects and water quality deterioration is enhanced by diffusion of toxic materials and nitrogenous waste to the overlying water column (Avnimelech and Ritvo, 2003). High concentration of ammonia and other nitrogenous products cause growth retardation and ultimately lead to the death of shrimp at extreme levels (Chin and Chen, 1987; Chen and Chin, 1989; Chen et al., 1990; Frias-Espericueta et al., 1999, 2000).

Diffusion is an important mechanism by which nutrients become available from sediment to the water column, yet it is a slow process. Nutrient flow to the water column can be augmented by fish driven bioturbation and resuspension (Rahman and Verdegem, 2007). Bioturbation is the reworking and mixing of sediment at the sediment-water interface, accomplished collectively by burrowing, feeding, irrigation, resuspension, secretion, excretion and transporting activities of benthic organisms, which alter the structure and properties of the sediment and thereby influence the diffusive or advective transport of both solutes and particulate organic matter (Koretsky et al., 2002; De Haas et al., 2005; Janssen et al., 2005; Meysman et al., 2006b). Bioturbation plays an important role in the nutrient flux and resuspension in a co-ordinated manner (Mulsow et al., 1998; D'Andrea et al., 2002; Risnoveanu et al., 2002, 2004) in aquaculture systems. The effect of bioturbation is oxygenation of the sediment, which prevent toxic material formation and enhances mineralization process, by which nutrients are released to the overlying water column (Fukuhara and Sakamoto, 1987; Hansen et al., 1998) as resuspension being the physical aspect of bioturbation. Bioturbation mediated resuspension is hence an important mechanism in nutrient resuspension and nutrient transfer between sediment and water (Avnimelech et al., 1999).

Resuspension is a possible solution to enhance nutrient flux in shrimp ponds (Hopkins et al., 1994) and it might enhance the recycling and utilization of the feed residues (Avnimelech and Ritvo, 2003). Resuspension may affect the rate at which pore water nutrients are released from the sediment into the overlying water column (Tarvainen et al., 2005). The hypothesis that resuspension might have a significant influence on the redistribution of sediments in shrimp ponds is supported by Avnimelech and Ritvo (2003). Many works on waste stabilization ponds integrated with fish culture have been carried out, as reviewed by Edwards (1992, 2005). There is a concept that fish integration can augment the resuspension of organic matter and nutrients, clogged in sediment and thus increase nutrient flux and its conversion to the biomass of the culture organisms (Andersson et al., 1988; Bartone and Khouri, 1990; De Pauw and salomoni, 1991; Edwards, 1992; Deegan, 1993; Oswald, 1995; Liang et al., 1998; Saha and Jana, 2003; Phan-van and De Pauw, 2005). The effect of bottom material resuspension through fish activities were observed and evaluated in ponds by Havens (1991); Avnimelech and Ritvo (2003); Matsuzaki et al. (2007). The effect of bioturbation brought about by mullet, milkfish, tilapia and Pearlspot on minimizing the levels of toxic metabolites like reduced iron, reduced manganese and sulphides in pond bottom especially by *Etroplus suratensis* co-stocking in shrimp culture system was reported by Joyni et al. (2011). Even though an integration of the different brackish water fin fishes with shrimp culture system, especially on resuspension of nutrients and water quality management and in turn their effect on growth and production of shrimp utilizing the resuspended nutrients are poorly known. The present study was designed to evaluate the effects of resuspension of sediments using four brackish water fin fishes- mullet, milkfish, tilapia and Pearlspot on water quality and nutrient retention for the enhancement of production of *P. monodon* considering their known bioturbation activities.

3.2 Materials and methods

3.2.1 Experimental design

Bioturbation mediated resuspension or nutrient flux and its effects among four different combinations of different fin fishes adapted to saline water integrated with shrimp was studied using tank experiment. Five treatments were executed in triplicates (4 experimental and one control) in a completely randomized design. Treatment T1 was a combination of Shrimp and Pearlspot abbreviated as S+E, T2, a combination of Shrimp and milkfish (*Chanos chanos*) as S+Mi. Shrimp and Mullet (*Mugil cephalus*) (S+Mu) combination was used in Treatment T3 and Treatment T4 was Shrimp and Tilapia (*Oreochromis mossambicus*) (S+T) combination. Shrimp (*Penaeus monodon*) alone (SA) was used as a control in treatment T5.

3.2.2 Experimental arrangement

Experimental arrangement is same as described in chapter 2- section 2.2.3

3.2.3 Sediment analysis

Total ammonia nitrogen (TAN), Nitrite-N (NO_2^--N) and Nitrate-N (NO_3^--N) in the sediment were measured according to Mudroch et al., 1996.

The phosphate in sediment was measured by ascorbic acid method (Grasshoff et al., 1983). The organic carbon in the sediment was determined following El Wakeel and Riley (1957). Total heterotrophic bacteria (THB) count in the sediment was estimated following the standard procedures (APHA, 1995) and expressed as colony forming units (CFU). pH (pH-Scan-Eutech instruments, Singapore) were measured daily in situ at 09.00 a.m.

3.2.4 Water sampling and analysis

Temperature, salinity, pH and dissolved oxygen were measured by collecting water samples on biweekly basis between 09.00 and 10.00 hours. Water samples were collected using a horizontal water sampler from three locations of each tank and pooled together before analysis. Composite water column samples were filtered through a GF/C Whatman glass fiber filter and the filtrate was analyzed for total ammonia nitrogen (TAN) (phenol hypochlorite method), NO_2^--N (Grasshoff et al., 1983) and NO_3^--N (cadmium reduction). The phosphate in water was measured by ascorbic acid method (Grasshoff et al., 1983). Monthly analysis of chl-*a* in non-filtered water column samples was carried out following standard methods (APHA, 1995). Monthly observation of total heterotrophic bacteria (THB) count in the water was estimated following the standard procedures (APHA, 1995) and expressed as colony forming units (CFU).

3.2.5 Growth parameters

Growth related parameters are measured same as described in chapter 2- section 2.2.7.

2.6 Statistical analysis

Water and sediment quality data were analyzed by two way ANOVA with treatment and biweek as factors. Shrimp and fish indices were analyzed by One way ANOVA. Post hoc comparisons were made using Tukey's HSD test (Hayter, 1984). All the descriptive statistics and model testing were calculated using SPSS version 18.

3.3 Results

3.3.1 Sediment characteristics

Table 3.1 shows the characteristics of sediment column during the culture period. The pH varied significantly ($p<0.05$) in all experimental treatments (Fig. 3.1) with lowest values of $5.50^{a}\pm0.90$ in the control and the highest in Pearlspot ($6.9^{d}\pm0.19$) and milkfish ($6.67^{c}\pm0.42$) treatment. The incorporation of fishes in the shrimp culture system significantly ($p<0.05$) reduced the levels of TAN and NO_2^--N in the experimental tanks (Fig.3.2 & 3.3) compared to the control ($0.221^{d}\pm0.16$ mg/g and $0.748^{d}\pm0.41$ mg/g), especially in Pearlspot combination tanks ($0.055^{a}\pm0.02$ mg/g TAN and $0.424^{a}\pm0.23$ mg/g NO_2^--N).

Table 3.1 Concentrations of parameters in bottom soil of different treatments*

Parameters	T1(S+E)	T2(S+Mi)	T3(S+Mu)	T4(S+T)	T5(SA)
TAN (mg/g)	$0.055^{a}\pm0.02$	$0.081^{b}\pm0.03$	$0.094^{c}\pm0.06$	$0.271^{e}\pm0.22$	$0.221^{d}\pm0.16$
NO_2^--N (mg/g)	$0.424^{a}\pm0.23$	$0.518^{b}\pm0.26$	$0.698^{c}\pm0.30$	$0.751^{d}\pm0.38$	$0.748^{d}\pm0.41$
NO_3^--N (mg/g)	$8.726^{a}\pm3.58$	$8.922^{a}\pm3.58$	$9.424^{b}\pm3.33$	$10.464^{c}\pm4.48$	$11.001^{d}\pm4.41$
TOC (mg/g)	$6.811^{a}\pm4.48$	$7.429^{b}\pm4.58$	$7.449^{b}\pm4.45$	$7.983^{c}\pm4.99$	$9.042^{d}\pm5.53$
THB (x10^{-3} CFU)	$52.00^{d}\pm17.02$	$47.10^{c}\pm10.1$	$46.71^{c}\pm8.20$	$45.05^{b}\pm9.11$	$36.67^{a}\pm0.57$
pH	$6.9^{d}\pm0.19$	$6.67^{c}\pm0.42$	$6.17^{b}\pm0.48$	$6.26^{b}\pm0.52$	$5.50^{a}\pm0.90$

(*) Means within a row not sharing a letter are significantly different ($p<0.05$; Tukey's HSD test)

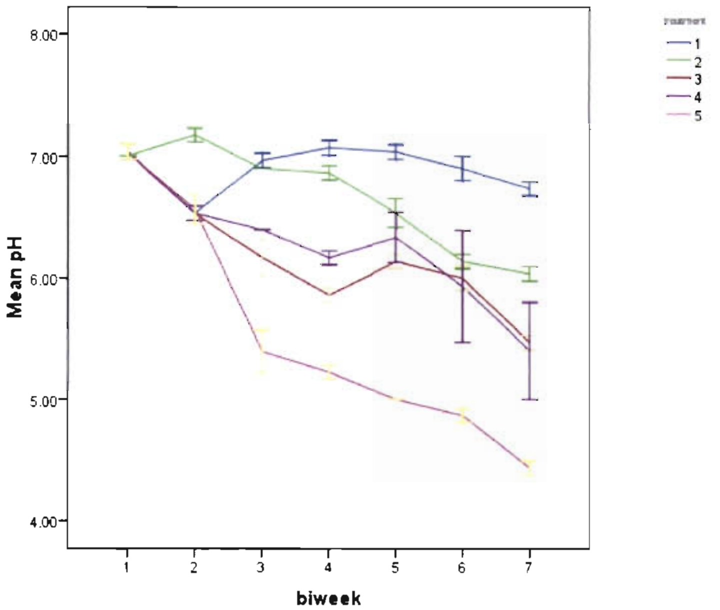


Figure 3.1 pH in the tank bottom soil at different biweeks. Error bars represents standard deviation

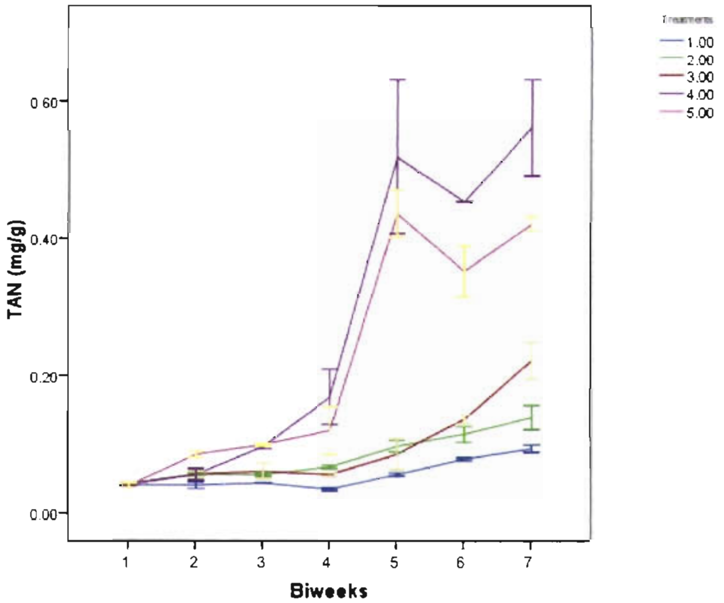


Figure 3.2 Concentrations of Total Ammonia-Nitrogen in the tank bottom soil at different biweeks. Error bars represents standard deviation

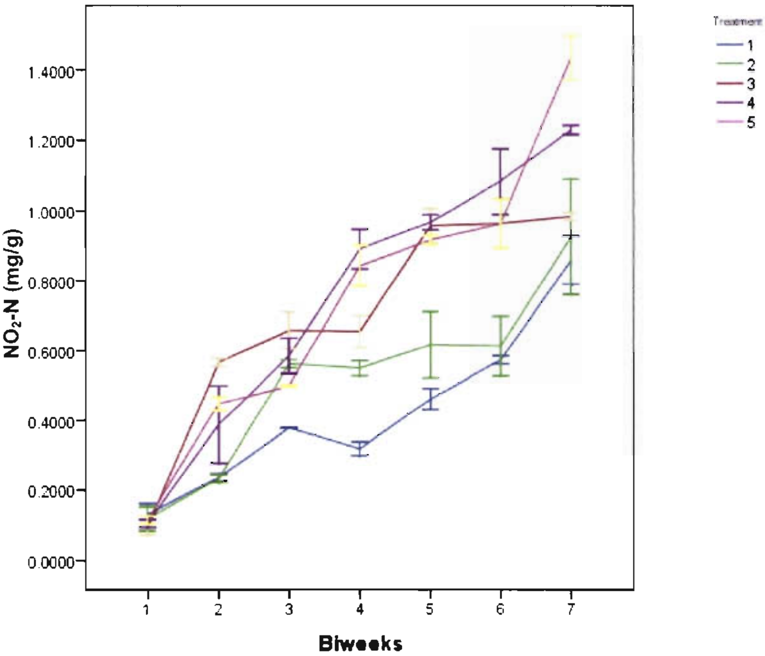


Figure 3.3 **Concentrations of Nitrite-Nitrogen in the tank bottom soil at different biweeks. Error bars represents standard deviation**

NO_3^--N concentrations were also varied significantly (Fig. 3.4) among experimental treatments and control ($p<0.05$). NO_3^--N concentrations were found decreased in both T1 (S+E) and T2 (S+Mi) (8.726^a±3.58 mg/g and 8.922^a±3.58 mg/g).The phosphate levels in the tanks were also reduced significantly ($p<0.05$) by the incorporation of fishes. The lowest and highest values of phosphate were in Pearlspot (0.610^a±0.17 mg/g) and control (0.761^d±0.43 mg/g) treatments respectively and the difference was statistically significant ($p<0.05$; Fig. 3.5).

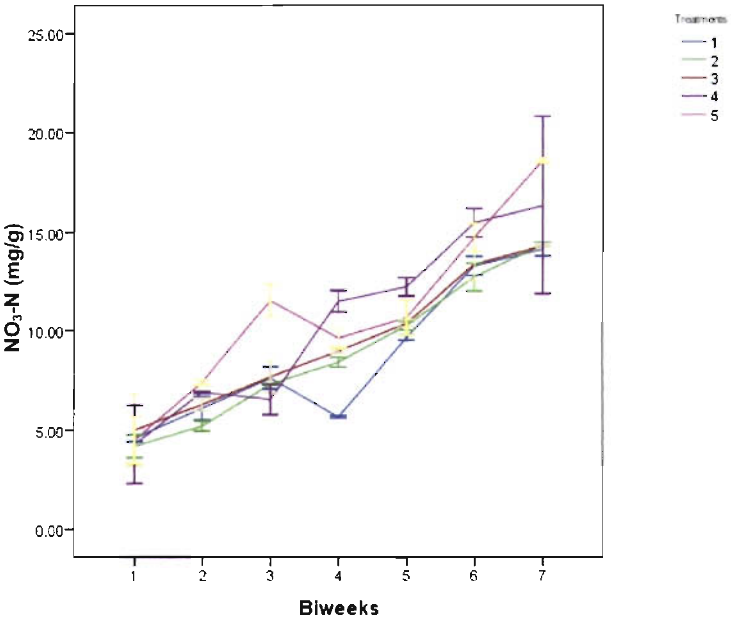


Figure 3.4 Concentrations of Nitrate-Nitrogen in the tank bottom soil at different biweeks. Error bars represents standard deviation

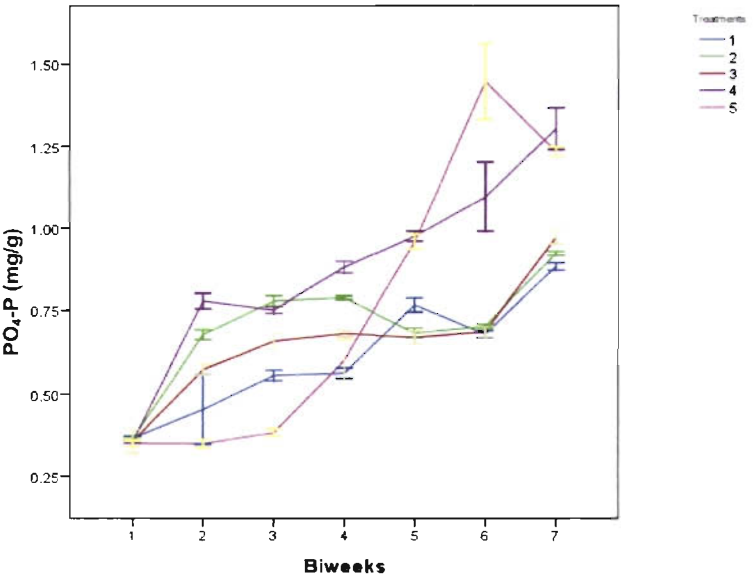


Figure 3.5 Concentrations of Phosphate-Phosphorous in the tank bottom soil at different biweeks. Error bars represents standard deviation

An increase in proportion of organic carbon in the control was observed compared to the other experimental treatments (Fig. 3.6). The lower concentrations in fish co-stocked treatments especially that of Pearlspot are indicative of sediment resuspension and increased degradation of organic matter in these treatments due to fish induced bioturbation. Maximum concentrations were observed in control ($9.042^d \pm 5.53$ mg/g). THB count were found to be significantly higher ($p<0.05$) in Pearlspot incorporated treatment ($52^d \pm 17$ mg/g) compared to control ($36.67^a \pm 4.3$ mg/g) and other fish incorporation treatment (Fig. 3.7).

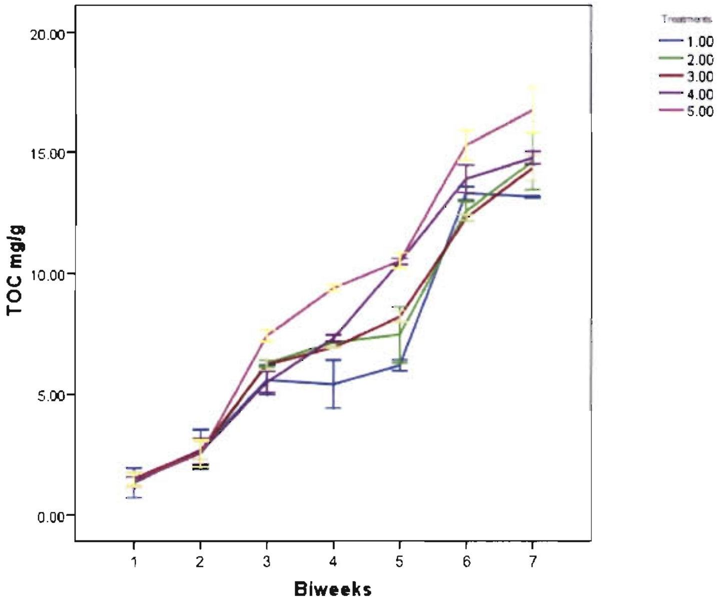


Figure 3.6 Concentrations of Total Organic carbon in the tank bottom soil at different biweeks. Error bars represents standard deviation

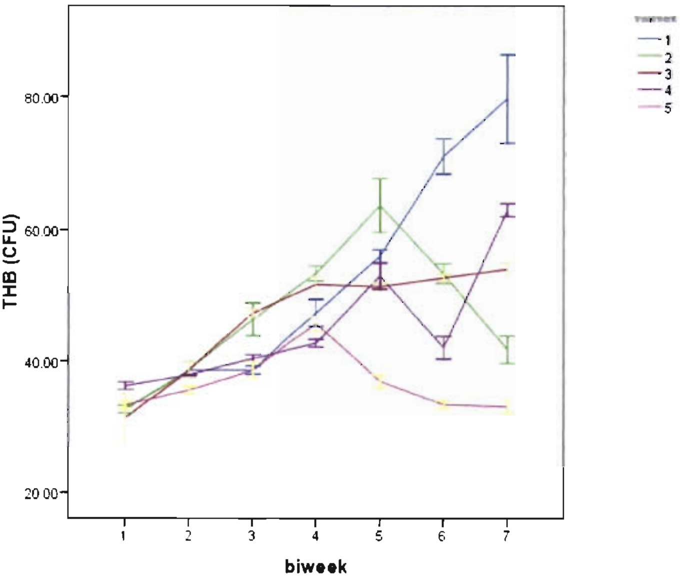


Figure 3.7 **Total Heterotrophic Bacterial count in the tank bottom soil at different biweeks. Error bars represents standard deviation**

3.3.2 Water column parameters

The water quality parameters (Table 3.2) such as temperature, salinity and dissolved oxygen concentration in surface water were not affected by fin fish integration ($p>0.05$). But the dissolved oxygen concentration of the bottom layer varied among treatments (Fig. 3.8). The lowest dissolved oxygen in the bottom layer is observed in the shrimp alone control ($3.76^a \pm 2.6$ mg/l). Mullet and tilapia incorporated treatments showed a relatively higher dissolved oxygen concentration of $6.34^b \pm 0.66$ mg/l and $6.39^b \pm 0.82$ mg/l respectively. Compared to the control, other two combinations, milk fish and Pearlspot incorporated treatments were showed highest concentrations ($6.82^c \pm 0.35$ mg/l and $7.14^d \pm 0.28$ mg/l) and were statistically significant ($p<0.05$).

Table 3.2 Concentrations of parameters in the water column of different treatments*

Parameters	T1(S+E)	T2(S+Mi)	T3(S+Mu)	T4(S+T)	T5(SA)
Temperature	29.06^{a}±0.30	29.06^{a}±0.30	29.07^{a}±0.30	29.08^{a}±0.27	29.07^{a}±0.30
Salinity	24.52^{a}±0.51	24.29^{a}±0.46	23.57^{a}±43.4	24.14^{a}±0.57	23.95^{a}±0.60
pH	7.58^{d}±0.42	7.38^{c}±0.27	6.47^{a}±0.43	6.50^{a}±0.53	6.74^{b}±0.54
Surface DO (mg/l)	8.10^{a}±0.28	8.10^{a}±0.28	7.73^{a}±0.30	7.22^{a}±2.36	6.74^{b}±0.54
Bottom DO (mg/l)	7.14^{d}±0.29	6.82^{c}±0.35	6.34^{b}±0.67	6.39^{b}±0.82	3.76^{a}±2.62
TAN (mg/l)	0.057^{a}±0.01	0.064^{b}±0.02	0.066^{b}±0.02	0.075^{c}±0.02	0.073^{c}±0.02
NO_2^--N (mg/l)	0.168^{a}±0.08	0.229^{c}±0.09	0.200^{b}±0.09	0.186^{b}±0.08	0.248^{d}±0.16
NO_3^--N (mg/l)	5.436^{a}±2.08	5.512^{a}±2.49	5.954^{b}±2.62	6.396^{c}±2.89	6.679^{c}±2.61
PO_4^{3-}-P (mg/l)	0.312^{c}±0.34	0.152^{a}±0.13	0.144^{a}±0.13	0.247^{d}±0.27	0.127^{a}±0.13
Chl-*a* (mg/l)	0.698^{c}±0.17	0.403^{a}±0.03	0.391^{a}±0.07	0.474^{b}±0.18	1.349^{d}±1.05
THB (x10^{-3}CFU)	1.801^{c}±1.08	1.53^{b}±0.61	1.154^{a}±0.36	1.928^{c}±0.85	1.047^{a}±0.17

(*) Means within a row not sharing a letter are significantly different ($p<0.05$; Tukey's HSD test)

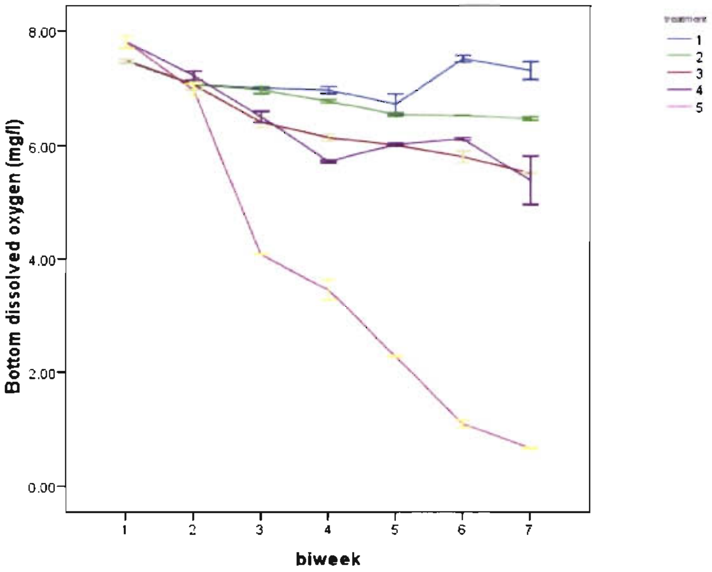


Figure 3.8 Concentrations of bottom dissolved oxygen in water column at different biweeks. Error bars represents standard deviation

Water column pH had significant ($p<0.05$) variation between treatments. The lower pH values were observed in Mullet ($6.47^{a}\pm0.43$) and Tilapia ($6.50^{a}\pm0.53$) co-stocked treatment. pH was observed to be higher in milkfish ($7.38^{c}\pm0.27$) and Pearlspot ($7.58^{d}\pm0.42$) incorporated treatments with statistically significant ($p<0.05$) concentrations.

The ANOVA results (Table 3.2) showed that fin fish addition to shrimp culture system had a significant effect ($p<0.05$) on the water TAN, NO_2^--N and NO_3^--N concentrations. All the treatments with bioturbation (T1, T2, T3, T4) showed a significant ($p<0.05$) reduction in the mean concentrations of TAN during the experimental period compared to the non bioturbated control. The overall mean concentration of TAN exhibited distinct treatment variability ($p<0.05$) and exhibited the following order $T4 \geq T5 > T3 \geq T2 > T1$. TAN concentrations were least ($p<0.05$) in T1 ($0.057^{a}\pm0.01$ mg/l) and highest in T4 and T5 ($0.075^{c}\pm0.02$ mg/l and $0.073^{c}\pm0.02$ mg/l). Significant variations were not observed between T2 & T3 and T4 &T5. TAN in the water column of different treatments varies with time. In treatment 1, during the initial phase on the 30th day sharp TAN peaks were observed, followed by a declining phase in 45-60 days and a gradual increasing phase in 75-90 days. In final phase, T3 and T5 showed highest TAN value of 0.095 mg/l and 0.092 mg/l respectively, while it was very low in T1 (0.067 mg/l), while T2 and T4 resulted a peak of TAN in 75th day and 60th day of the culture respectively (Fig. 3.9).

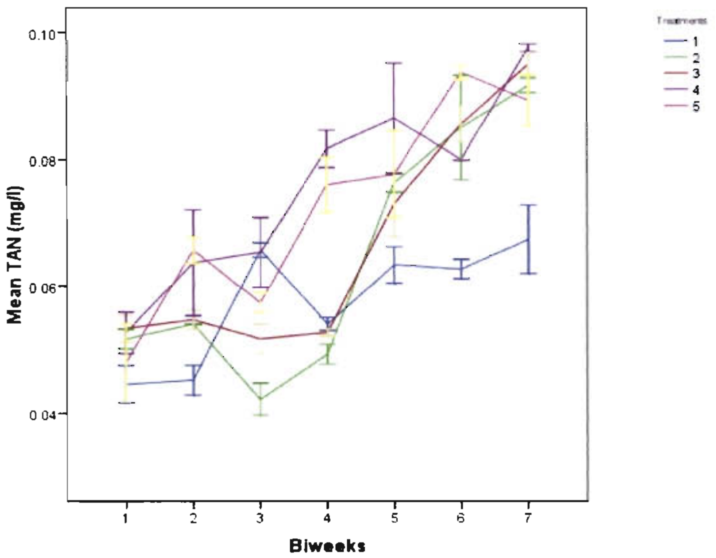


Figure 3.9 **Concentrations of Total Ammonia-Nitrogen in water column at different biweeks. Error bars represents standard deviation**

The variability of mean NO_2^--N concentration was 32% and 25% less than that of control in T1 and T4 respectively. Introduction of Pearlspot and tilapia into the experimental units resulted a significant ($p<0.05$) decrease with a concentration of 0.168^a ±0.08 mg/l and 0.186^b±0.09 mg/l of NO_2^--N respectively, when compared to the non bioturbated counterpart (0.248^d±0.16). Decrease in NO_2^--N attributable to the effect of mullet and milkfish were 7% and 16% compared to the control. This showed that fish incorporated culture systems especially Pearlspot exerted greater effect in maintaining water quality throughout the culture period. Temporal variations in NO_2^--N have been illustrated with the figure (Fig. 3.10). Peak concentration was observed in 45th day of the culture in T1, while in T5 (control), peak concentration was observed in 90th day of culture. In T1 concentrations were in the range of 0.0127 mg/l to 0.252 mg/l and in T5 it was 0.0170 mg/l to 0.4733 mg/l with a gradual increase in concentration.

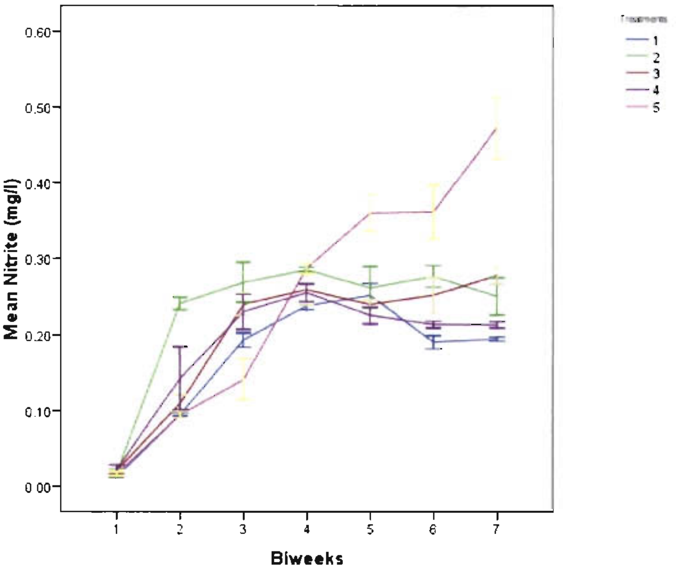


Figure 3.10 Concentrations of Nitrite- Nitrogen in water column at different biweeks. Error bars represents standard deviation

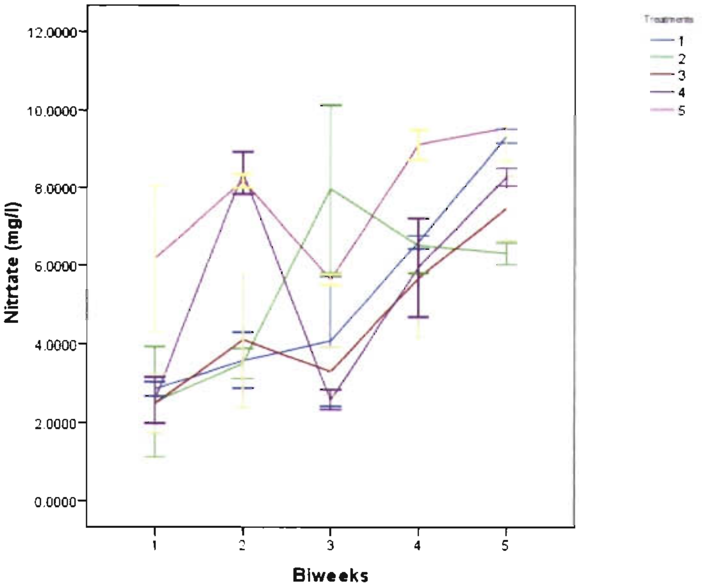


Figure 3.11 Concentrations of Nitrate-Nitrogen in water column at different biweeks. Error bars represents standard deviation

Differences in NO_3^--N concentrations were significant ($p<0.05$) between treatments, the mean concentrations of NO_3^--N in the water samples showed variability with the highest mean value in T5 (6. 679^{c}± 2.61 mg/l) and lowest in T1 (5.436^{a}±2.08 mg/l). In T2, the concentration observed was 5.512^{a}±2.49 mg/l (Fig. 3.11). T3 and T4 showed a moderate concentration of 5.954^{b}±2.62 mg/l and 6.396^{c}±2.89 mg/l. The reduced stirring effect of mullet and milkfish compared to Pearlspot leads to less decomposition of lower percentage of accumulated organic matter, which in turn liberated less phosphate and TAN into the water column.

As a result of the bioturbation and nutrient flux, the quantity of the mean phosphate in water tended to increase in all treatments and maintained its concentration higher than that of control ($p<0.05$). The higher mean values observed in T4 were 0.247^{d}±0.27 mg/l followed by T1 (0.312^{c} ±0.34 mg/l). T2, T3 and T5 were not statistically significant ($p>0.05$; Fig. 3.12).

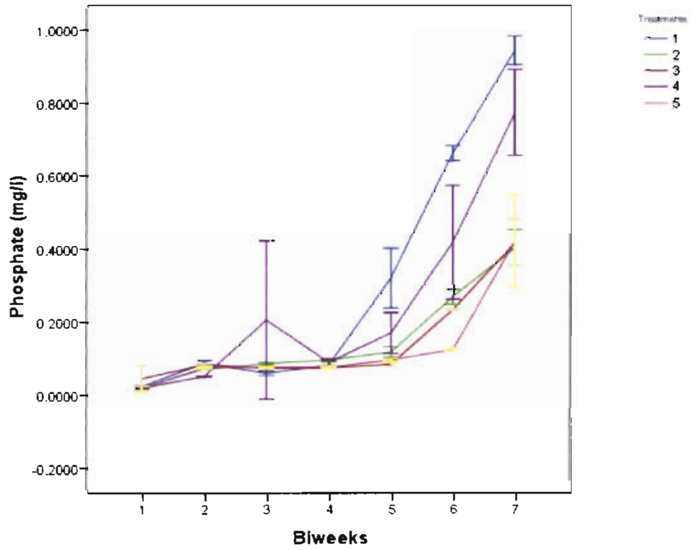


Figure 3.12 Concentrations of Phosphate-Phosphorous in water column at different biweeks. Error bars represents standard deviation

The counts of THB (Fig. 3.13) were 41% ($1.801^c \pm 1.08$) higher in T1 and 45% ($1.928^c \pm 0.85$) higher in T4 in mean values of bacterial count compared to the fish free treatment ($1.047^a \pm 0.17$). The effect of bioturbation was most prominent in Pearlspot and tilapia culture tanks in sustaining higher THB population in comparison to the non bioturbated control. But modest increases in THB population were observed in T2 and T3 and but it was in T4. This indicates that fishes especially Pearlspot and tilapia increased nutrient dynamicity of the system mediated through heterotrophic metabolism with accelerated heterotrophic bacterial populations in the culture system.

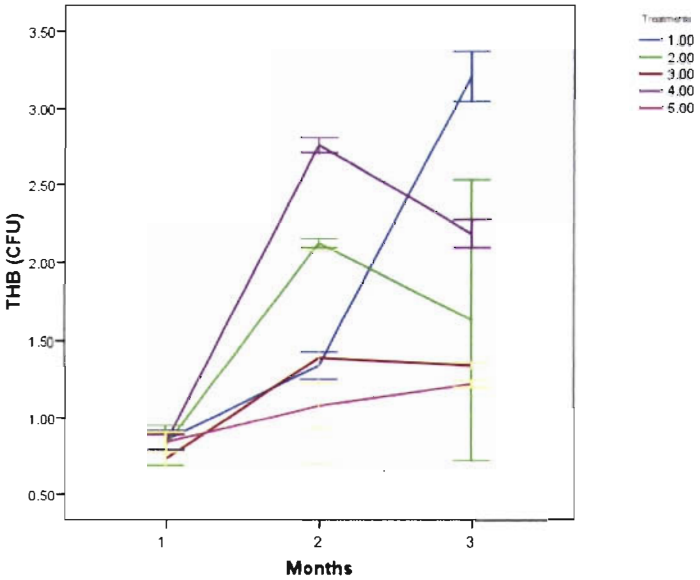


Figure 3.13 Total heterotrophic bacterial count in water column at different Months. Error bars represents standard deviation

Chl-*a* concentrations (Fig. 3.14) were low in T2 ($0.403^a \pm 0.03$ mg/l) and T3 ($0.391^a \pm 0.07$ mg/l) and was, not significantly different ($p > 0.05$). But other three treatments (T1, T4, T5) were significantly different ($p < 0.05$). Chl-*a* concentration was found to be higher in T5 ($1.34^d \pm 1.05$ mg/l) followed by T1 (0.698^{c}±0.17 mg/l), while in T4, the concentration was 0.474^{b}±0.18 mg/l.

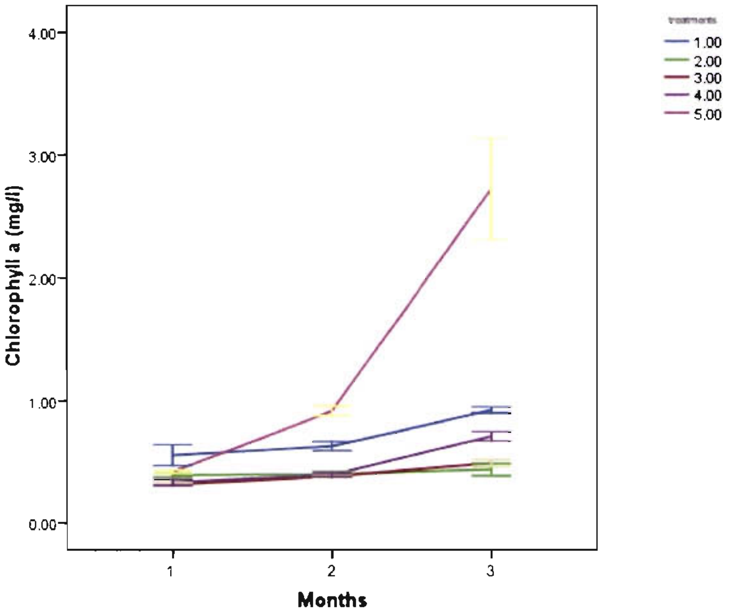


Figure 3.14 Concentrations of chlorophyll-*a* in water column at different months. Error bars represents standard deviation

3.3.3 Growth parameters

The growth parameters of shrimp are represented in table 3.3. The highest growth and production of shrimp was recorded in Pearlspot co-stocked treatment with significantly higher ($p<0.05$) weight gain (Fig. 3.15; 17.65^{c}±0.58 g), survival (Fig. 3.16; 96^{b}±1.73%) and yield (Fig. 3.17; 611.6^{d}±14.77 g). In S+Mi the average growth rate was 15.01^{b}±0.68 g and the corresponding survival was 88.67^{b}±11.93%. The average growth rate recorded in S+Mu and S+T treatments were 13^{a}±0.89 g and 13.34^{ab}±1.12 g respectively. Survival was lowest in S+T (68.33^{a}±4.04%) and SA (63.00^{a}±1.73%) and was not statistically significant ($p>0.05$). The average weight was also significantly low in SA treatment (12.06^{a}±.38g). Growth parameters of fishes are represented in table 3.4.

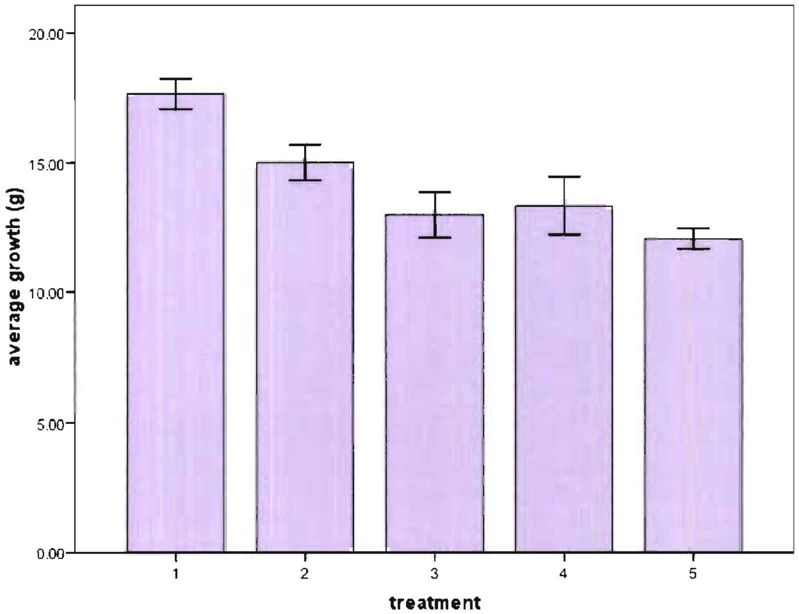


Figure 3.15 Average growth of shrimp. Error bars represents standard deviation

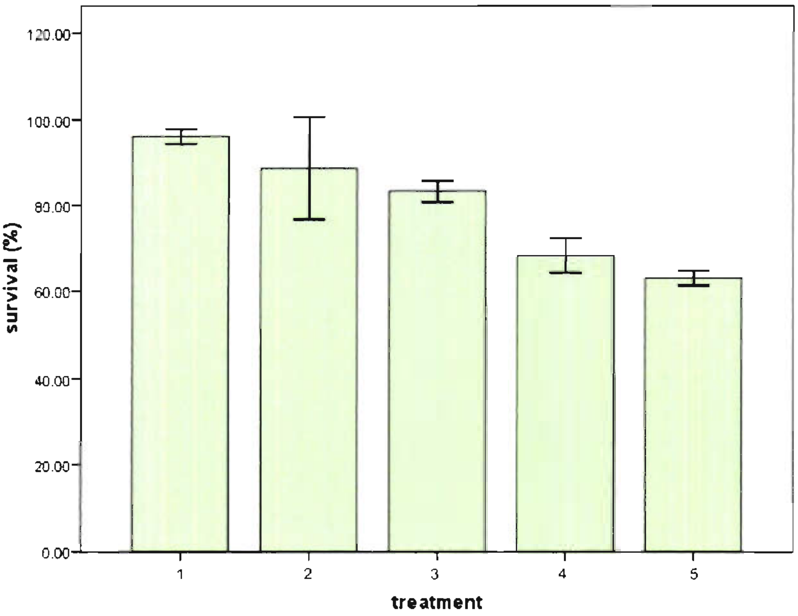


Figure 3.16 Survival % of shrimp. Error bars represents standard deviation

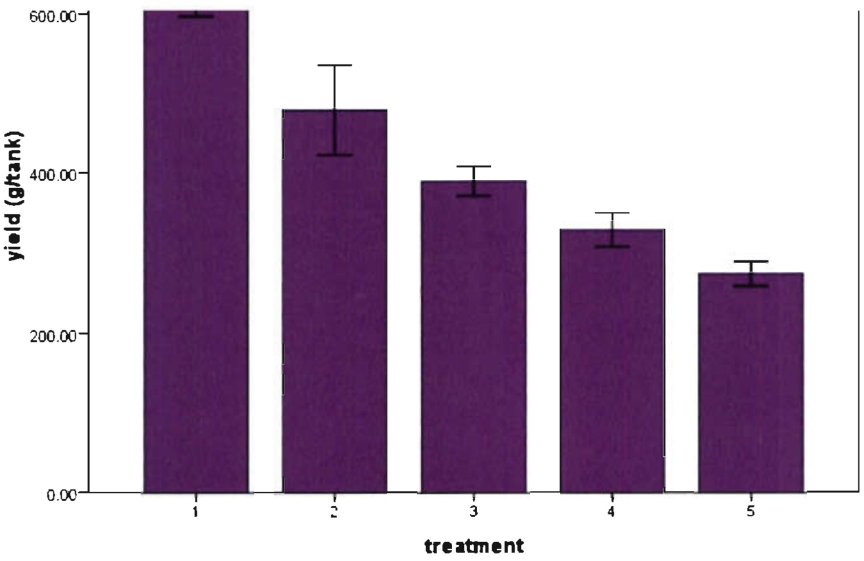


Figure 3.17 Yield of shrimp. Error bars represents standard deviation

Table 3.3 Growth parameters of shrimp in different treatments *

Treatments	Average weight (g)	Survival (%)	Yield/tank (g)
T1 (Shrimp+Pearlspot)	17.65^{c} ±0.58	96^{b} ±1.73	611.6^{d}±14.77
T2 (shrimp+Milk fish)	15.01^{b} ±0.68	88.67^{b}±11.93	479.22^{c}±56.65
T3 (Shrimp+Mullet)	13^{a} ±0.89	83.33^{b}c±2.51	389.46^{b}±18.42
T4 (Shrimp+Tilapia)	13.34^{ab} ±1.12	68.33^{a}±4.04	328.47^{ab}±20.89
T5 (Shrimp alone)	12.06^{a} ±.38	63.00^{a}±1.73	273.55^{a}±14.86

(*) Means within a column not sharing a letter are significantly different ($p<0.05$; Tukey HSD)

Table 3.4 Growth parameters of fish in different treatments *

Treatments	Yield/tank (g)	Survival (%)	Average weight (g)
Pearlspot	$521.67^{b}\pm8.39$	$90.67^{b}\pm2.89$	$33.97^{b}\pm0.95$
Milkfish	$846^{d}\pm35.04$	$87^{b}\pm3.46$	$54^{d}\pm1.0$
Mullet	$220.67^{a}\pm26.76$	$72.3^{a}\pm5.51$	$17^{a}\pm0.81$
Tilapia	$624^{c}\pm45.30$	$96.33^{b}\pm6.35$	$36^{c}\pm1.0$

(*) Means within a column not sharing a letter are significantly different ($p<0.05$; Tukey HSD)

3.4 Discussion

3.4.1 Nutrients in sediment column

The resuspension of fishes have a significant influence on the redistribution of sediments in shrimp ponds. The activity of hydrogen ions (pH) is a master geochemical parameter reflecting the thermodynamic state of acid-base processes within natural environments (Sorensen and Palitzsch, 1910; Takahashi et al., 1970; Dickson, 1993). In the present study soil pH varied greatly among treatments and the lowest was observed in treatment 5. The pH usually declines in water logged soils as a result of microbial activity in the absence of molecular oxygen (Boyd, 1995). Stagnant conditions may have promoted the formation of concentration gradients in the water and surficial sediment layers (Hulth et al., 2002). The highest concentrations were observed in fin fish added treatments especially in Pearlspot added one.

In the experiments with resuspension, the pH conditions in the soil were found to be improved by the stirring activity of fishes. The relatively large discrepancy between treatments may be caused by the disparity in stirring activity between treatments. A pH minimum at the subsurface oxic/anoxic boundary, related in large part to reoxidation of reduced compounds was reported by Revsbech et al. (1980).

In the present study, all the sediment parameters representing nutrients were found to be decreased significantly ($p<0.05$) in comparison to the shrimp alone system. TAN, NO_2^--N and PO_4^{3-}-P recorded specifically lower levels in Pearlspot co-stocking, whereas NO_3^--N concentrations decreased significantly ($p<0.05$) in both Pearlspot and milk fish co-stocked treatments. This may be due to the release of nutrients by their bioturbation activities (Joyni et al., 2011). Nitrogenous compounds in aquaculture system have dual role, as they act as both the toxicant and nutrient in the same system at different concentrations. When its concentration reaches above particular level, it is harmful to the shrimp growth and survival, and at extremely high concentrations, is lethal. On other hand it can act as source of nutrients, if present at optimum levels with better recycling. This is possible with frequent resuspension of accumulated and clogged nitrogenous waste nutrients with fish activities (bioturbation) and can lead to nutrient retention and utilization. Tarvainen et al. (2005) reported that the Ruffe fish can also release nutrients from the sediment through resuspension while searching for food. TOC concentrations were greatly decreased in the Pearlspot integrated treatments. Organic matter decomposition in aquaculture pond bottoms can lead to anoxic conditions, and resuspension would favour the aerobic decomposition of organic matter. Resuspension would also increase the exchange rate of materials from sediment to water.

Sediment disturbance and particle erosion through burrowing, feeding and movement enhance both the direct release of nutrients sequestered in pore water and nutrient cycling, through oxygenation of the sediment (Henriksen et al., 1980) and increasing the surface area available for microbial activity (Fry, 1982). Blackburn et al. (1988) attributed 30% of the solute flux from the sediment of a marine fish pond to disturbance by fish. In fish (tilapia and mrigal) exclosure experiments, Riise and Roos (1997) measured greater sediment oxygen uptake, ammonia flux and denitrification associated with oligochaete burrowing activities inside exclosures. Grazing by benthivorous fish on benthic invertebrates limits the loss of nitrogen through denitrification and promotes benthic-pelagic coupling and the internal recycling of nitrogen through sediment resuspension (Breukelaar et al., 1994; Cline et al., 1994; Riise and Roos, 1997). In ecosystems like pond having low interstitial flow rates, bioturbation is likely to increase the supply of resources and stimulate aerobic microbial respiration and thus bioturbators are very important in enhancing the biogeochemical processes (Mermillod-Blondin and Rosenberg, 2006). Diffusion of oxygen from the water into the soil was found to be limited by a very thin, 2 mm thick, diffusion barrier. It is anticipated that this diffusion barrier is very fragile and can be easily disturbed by bioturbation and resuspension activities of organisms. Any movement of the upper sediment layers may change the profile of oxygen and redox potential and thus change transfer reactions dramatically. Revsbech et al. (1980) found that the periodic introduction of oxygen into the sediment caused by bioturbation kept a 3 cm sediment layer oxidized compared to a 2 mm oxidized layer in non-disturbed sediments. Minimum daily resuspension rate in carp-tilapia polyculture ponds was estimated to be about 3 mm (Kochva and Avnimelech, 1995). In fishponds, the sediment surface is disturbed by resuspension induced by fish activity and the resuspension of organic matter accounted for 60–90% of the total solids flux (Avnimelech et al., 1999).

3.4.2 Nutrients in water column

Dissolved oxygen concentration is one of the critical factors affecting processes and conditions at the bottom soil-water interface (Avnimelech and Ritvo, 2003). Dissolved oxygen concentration at the surface in the present study was not statistically significant ($p<0.05$). On the other hand, concentrations of DO of water immediately above the sediment were low, especially in control. This is due to the slow and insufficient diffusion of oxygen to the deeper layers of water column. Mixing of water at the soil-water interface across the flocculent layer is very limited (Avnimelech and Ritvo, 2003). Whereas in finfish integrated treatments the concentrations of dissolved oxygen were found to be high. A possible benefit from stirring was observed in tilapia ponds where high concentrations of dissolved oxygen were obtained for stirred ponds as compared with unstirred ponds (Phan Van et al., 2008). The results of the present study corroborate with that of Phan Van et al. (2008), which indicates that the bioturbation activity of fish enhanced the diffusion of DO throughout the water column of the ponds. Rasmussen et al. (2005) also opined that the presence of fish or any active moving objects in the water can generate mixing of the water column, thereby enhancing the dispersion/diffusion of dissolved substances throughout the culture environment.

In the present study, the pH level was also found decreased in control and increased in fin fish added treatment. The lower pH level due to lack of stirring or aeration in overlying water was reported by Hulth et al. (2002). However, the stirring activity of fishes especially Pearlspot helped to maintain the higher pH level in aquaculture ponds.

All the treatments with fin fish integration especially Pearlspot have shown better results in both water quality and growth than shrimp monoculture. The effects of Ruffe on water quality via nutrient translocation from the bottom into the water column through bioturbation and nutrient release have been reported by Tarvainen et al. (2005). Bioturbation mediated resuspension in shrimp farm sediments are used for boosting decomposition of organic matter, recovery of nutrients as well as natural remediation of water quality. Fish move actively while searching for food, causing water turbulence (Havens, 1991; Tatrai et al., 1997) and positively affecting water quality. Associated with this resuspension process there is nutrient exchange between the water column and the sediments (Mortimer, 1941; Lam and Taquet, 1976; Ryding and Forsberg, 1977; Bates and Neafus, 1980).

The results of the present study revealed that the bioturbation of fin fishes had significant role in the reduction of toxic inorganic nitrogen levels in shrimp culture system, as lower TAN, NO_2^--N and NO_3^--N levels were recorded in the treatments, with bioturbation. The overall mean concentrations of TAN were low in Pearlspot incorporated treatment. While examining the entire culture period, two prominent peaks of inorganic nitrogen were observed in treatment 1 (S+E). The first peak was observed in the initial stage on the 30th day of culture with higher concentration within optimum range while a second peak with lowered inorganic nitrogen concentration was observed on the 90th day. This is comparable with the study of Hari et al. (2006) in which peaks of TAN concentrations were observed in 31st day of culture with enhanced nitrification in response to the carbohydrate addition in shrimp culture tanks. On the contrary, Thakur and Lin (2003) observed nitrification on the

56th day of the culture of *Penaeus monodon* in concrete culture tanks. The shorter time period for the establishment of nitrification in the present study can be attributed to the resuspension activity of Pearlspot which plays a part in biogeochemical processing of both soil and water. This clearly indicates the process of recycling of nutrients effectively with enhanced nitrification at an early stage (30th day) of culture. Nitrification in the water column is restricted by the availability of surfaces and possibly by light inhibition (Hargreaves, 1998). In the present study in bioturbated tanks, resuspended particles act as active sites of mineralization and nitrification and reduce the levels of both ammonia and nitrite in water column. Nitrifiers are lithotrophic, requiring organic or mineral surfaces for attachment. It is reported that water column nitrification is an important mechanism of ammonia transformation in high-intensity pond systems in which particles suspended by mechanical aeration are sites of active mineralization and nitrification (Hargreaves, 1998). The suspension of sediment particles by aeration may have promoted rapid desorption of exchangeable ammonium and stimulated nitrification in the water column (Sanares et al., 1986). Due to the accumulation of toxic substances and sulphides the soil seems to be acidic in non bioturbated tanks, whereas in treatments with bioturbation it was found that the sediment was somewhat alkaline in condition and this alkaline environment along with increased penetration of oxygen helped to maintain high nitrification.

Decrease in ammonia levels in water column is a coupled process, it happens either by nitrification or by uptake of phytoplankton. In the water column, nitrification becomes low when ammonia is present at substrate-limiting concentrations due to rapid uptake by large and actively-growing phytoplankton populations (Hargreaves, 1998). Contrary to this, nitrification is stimulated by increase in ammonia resulting from reduced uptake from phytoplankton (Hargreaves, 1998). The levels of inorganic nitrogen species in the water column were lower due to increased uptake by phytoplankton and by stimulated nitrification. Nitrifying bacteria require slightly alkaline pH (7-8.5) for optimal growth. Increased nitrification at alkaline pH suggests that NH_3 may be act as the substrate for nitrification (Hargreaves, 1998).

The increased release of nutrients at initial stage is an indicative of bioturbation generated nutrient flux. An increased and sustained nutrient flux into the water column through the bioturbation events (Yusoff et al., 2001) especially in initial stages were reported by Feuillet-Girard et al. (1997). The release of nutrients from sediment to overlying water mediated through physical disturbances of the sediment core that altered the status of sediment pool by soil-water exchange mechanism by the bioturbation activities of chironomid larvae was reported by Biswas et al. (2009). Bioturbation brought about by burrowing, bioirrigation and other basic biological activities influences a suite of physical, chemical and microbiological process in the sediment (Lohrer et al., 2004; Maire et al., 2008; Rhoads, 1974; Aller, 1994; Rosenberg, 2001; Gilbert et al., 2003; Stief et al., 2004). It increases the sediment water interface area for increased sediment oxygen uptake (Svensson and Leonardson, 1996; Hansen et al., 1997; Heilskov and Holmer, 2001; Lewandowski and Hupfer, 2005; Nogaro et al., 2008), creates more hospitable ecological niche for heterotrophic bacterial population (Harper et al., 1999; Cuny et al., 2007). It enhances microbial mineralization activities which result in more efficient regeneration of nutrients (Heilskov and Holmer, 2001; Banta and Andersen, 2003). Microorganisms enhance organic matter mineralization through increased oxygen penetration (Graneli, 1979; Fukuhara, 1987; Hansen et al., 1998) or increase of resource flows (O_2) (Mermillod-Blondin and Rosenberg, 2006) due to bioturbation and releases nutrients to water column, as microbial processes at water-sediment interfaces are mediated by hydrological exchanges between surface and pore water. In aquatic ecosystem, ecological processes such as organic matter mineralization and nutrient recycling are regulated by microbial activities in sediments. Benthic fauna interact with microbes and thus modifies biogeochemical transformations. The release of nutrients from sediment to the overlying water column (Fukuhara and Sakamoto, 1987; Hansen et al., 1998) is carried out by a combination of diffusion (Berg et al., 2001; Furukawa et al., 2001; Koretsky et al., 2002) and advection (Huettel and Webster, 2001; Janssen et al., 2005; Meysman et al., 2006b). Even though ammonia and nitrite were observed to be higher in initial stage, it was found to decline as the experiment progressed. Therefore the limit of ammonia and nitrite never reached up to lethal concentrations due to the continuous conversion of these nutrients to shrimp and fish biomass and also by the incorporation into food chain in the culture system. The safe levels reported for ammonia and nitrite was 4.6 mg/l and 10.7 mg/l respectively for *P. monodon* (Chen and Chin, 1988).

In the present investigation TAN and NO_2^--N concentrations showed a gradual declining trend towards the end of the culture in fish stocking experiments. Recently, Rasmussen et al. (2005), using Rhodamine tracer, demonstrated that irrespective of flow rate, the presence of fish enhanced the mixing process. The corresponding reduction of inorganic nitrogenous compounds is explained by the following process in water column. Due to bioturbation mediated resuspension and nutrient flux, the nutrients become available in the water column. Thus, utilization of available nitrogenous compounds by the bacterial population and phytoplankton for their growth and production resulted in the reduction of these compounds in the water column. In the shrimp culture system, the toxic nitrogenous wastes were effectively removed by the phytoplankton and microbial activities (Shilo and Rimon, 1982; Diab and Shilo, 1988). However, the mean TAN concentrations in treatment SA was higher when compared to treatment S+E. Low TAN concentrations were recorded in S+E treatment due to the addition of bioturbating agent, on the other hand, higher THB counts were obtained from the water column of this treatments. In the present study, bacteria utilized the resuspended organic matter as a substratum for their growth and synthesized microbial protein through the subsequent uptake of nitrogen from the system (Avnimelech et al., 1994). The control of inorganic nitrogen was made possible by the utilization of the inorganic-N to synthesize bacterial protein and new cells (Avnimelech, 1999). Subsequent reduction of inorganic nitrogen and enhancement of THB in resuspended treatment strongly agrees with the above statement.

NO_3^--N may follow several biochemical pathways following production by nitrification. Phytoplankton and microbes may reduce nitrate to ammonia for incorporation into cellular amino acids (assimilatory nitrate reduction). Nitrate may function as a terminal electron acceptor during the oxidation of organic matter and thereby supply energy for microbial growth. Nitrate respiration results in the reduction of nitrate to dinitrogen (denitrification) or ammonia (dissimilatory nitrate reduction to ammonia). Cline et al. (1994) reported that sediment resuspension by common carp increased nitrite and nitrate concentration in the overlying water.

Fish-mediated phosphate release from the sediment may have a stronger impact on water quality than previously believed (Sondergaard et al., 2001). In the present study, increased release of phosphate-phosphorous compared to control also support the resuspension mediated nutrient flux. The role of bioturbation in enhancement of phosphorous release from sediment to water column was also upheld by many authors (Biswas et al., 2009; Jana and Das, 1992; Jana and Sahu, 1993; Saha and Jana, 2003). Biswas et al. (2009) have also reported the increased number of phosphate solubilizing bacterial consortia due to bioturbation mediated activities. The higher phosphate levels are beneficial in water column for the growth of phytoplankton, the dearth of which may act as a limiting factor and prevent phytoplankton development in the culture system. In various other studies, resuspension has decreased, increased or had no effect on the phosphorous concentrations of water (Fitzgerald, 1970; Peters and Cattaneo, 1984; Sondergaard et al., 1992; Schallenberg and Burns, 2004). Thus in aquaculture systems benthivorous fish have significant implications, because they may substantially increase internal loading via the translocation of nutrients from the bottom into the water column through bioturbation and nutrient release (Breukelaar et al., 1994).

3.4.3 Microorganisms in sediment and water column

Microbial analysis of all the treatments has shown that *E. suratensis* co-stocked treatment has the highest count of total heterotrophic bacteria. Burford et al. (1998) observed that there was an increase in THB count with increased nutrient concentrations. The higher THB count was found in S+E treatments especially in the early stage of culture. This might be due to the fluxes produced by bioturbation at the water-sediment interface that might have strongly influenced microbial process in the sediment

(Mermillod-Blondin and Rosenberg, 2006; Boulton et al., 1998; Sundback et al., 2004). Oxygen penetration by bioturbation increased the oxygenated area of the sediment-water interface and in turn, stimulated the microbial growth rate. Studies on the fine sediment reworkers, such as bivalves, have shown that their bioturbation activities increased the oxygen consumption by the microbial communities at the water-sediment interface. *E. suratensis* produced higher sediment-water nutrient fluxes, causing greater stimulation of aerobic microbial activities and nitrogen fluxes like denitrification, whereas other fishes like milkfish, mullet and tilapia had lesser impact on these activities. The comparison of microbial growth of both *E. suratensis* and other fin fish co-cultured tanks demonstrated that the given bioturbation activities of different species had different impact on resuspension or nutrient flux and microbial activities in benthic system. In *P. monodon* alone control system, the THB count was found to be decreased due to the lesser penetration of oxygen in the absence of bioturbation.

Resuspension of organic particles to the overlying water may accelerate organic matter mineralization and nutrient regeneration, and is therefore an important factor controlling oxygen and nutrient dynamics and microbial ecology of the shallow aquaculture ponds. Some studies have proved that organic matter get resuspended back to water column due to bioturbation and these organic particles act as a substratum for the proliferation of heterotrophic bacteria which might contribute to the increase in count of THB in water column. The increased number of heterotrophic bacteria in water column leads to enhanced levels of nitrification due to their metabolic activities and converts the toxic forms of nitrogen like ammonia and nitrite to less toxic nitrate, substantiating for the reduced levels of ammonia and nitrite and increased levels of nitrate in water column. Highest THB were observed in Pearlspot co-stocked treatment, mullet, milk fish and tilapia showed intermediate count in THB due to their moderate action. The lowest THB was recorded by shrimp alone control. Bioturbation also influences the vertical distribution and the composition of sedimentary bacterial communities (Dobbs and Guckert, 1988; Findlay et al., 1990; Grossmann and Reichardt, 1991; Goni-Urriza et al., 1999; Wilde and Plante, 2002; Papaspyrou et al., 2004; Plante and Wilde, 2004; Grossi et al., 2006; Papaspyrou et al., 2006).

3.4.4 Chlorophyll-*a*

The total phytoplanktonic biomass was determined by the measurement of chl-*a* concentrations. Nutrient replenishment events are usually followed by a chlorophyll increase which has generally been attributed to an active growth of phytoplankton in the water column (Walker and O'Donner, 1981; Gabrielson and Lukatelich, 1985). Increased release of sedimentary nutrients was assumed to lead to higher planktonic production up to 40% (Tenore, 1976) and concomitant higher chl-*a* concentrations were reported by other scientists like Walker and O'Donner (1981), Gabrielson and Lukatelich (1985). Chl-*a* concentration in *P. monodon* tanks were very high during the final phase (90 days) due to excess amount of nutrients accumulated at the final stage of culture. Nutrient release did not occur at the appropriate time in these tanks and the accumulated ammonia and nitrite due to anaerobic reaction slowly diffused into the water column only at the last phase of culture which promoted enhanced phytoplankton growth and finally lead to the bloom of plankton in the system by eutrophication.

Pearlspot added treatment showed highest values of Chl-*a* and invariably made maximum shrimp production. It highlights the importance of optimum levels of nutrients in the culture tanks. Fish integration noticeably promotes a stable bloom of green algae, and it was found that the growth of luminous bacteria in shrimp ponds was effectively curtailed. The proliferation of undesirable plankton species, which negatively affected water quality and growth of cultured shrimp was also restricted (Cruz et al., 2008). The greater availability of nutrients to phytoplankton can also be explained by the enhanced diffusion across the sediment water interface by the stirring activities of *E.suratensis*. Hohener and Gachter (1994) reported that carp stirring activity enhanced the diffusion of nutrients across sediment-water interface. Bioturbation also increases the aerobic decomposition by aerating anaerobic sediments (Graneli, 1979; Beristain, 2005). The diffusion of nutrients across sediment-water interface and the aerobic decomposition enhances the TAN and phosphorous flux from the sediments to the water column (Hargreaves, 1998). Phosphorous is absorbed by phytoplankton, TAN by nitrifiers and phytoplankton that compete for it and both nutrients affect phytoplankton, which in turn determines zooplankton abundance. Grazing of phytoplankton by the herbivorous fish induces reproduction of phytoplankton, hence photosynthesis, and the resultant improvement in oxygen conditions favours the nitrification and organisms on the pond bottom (Milestein et al., 2009).

3.4.5 Growth and production of shrimp

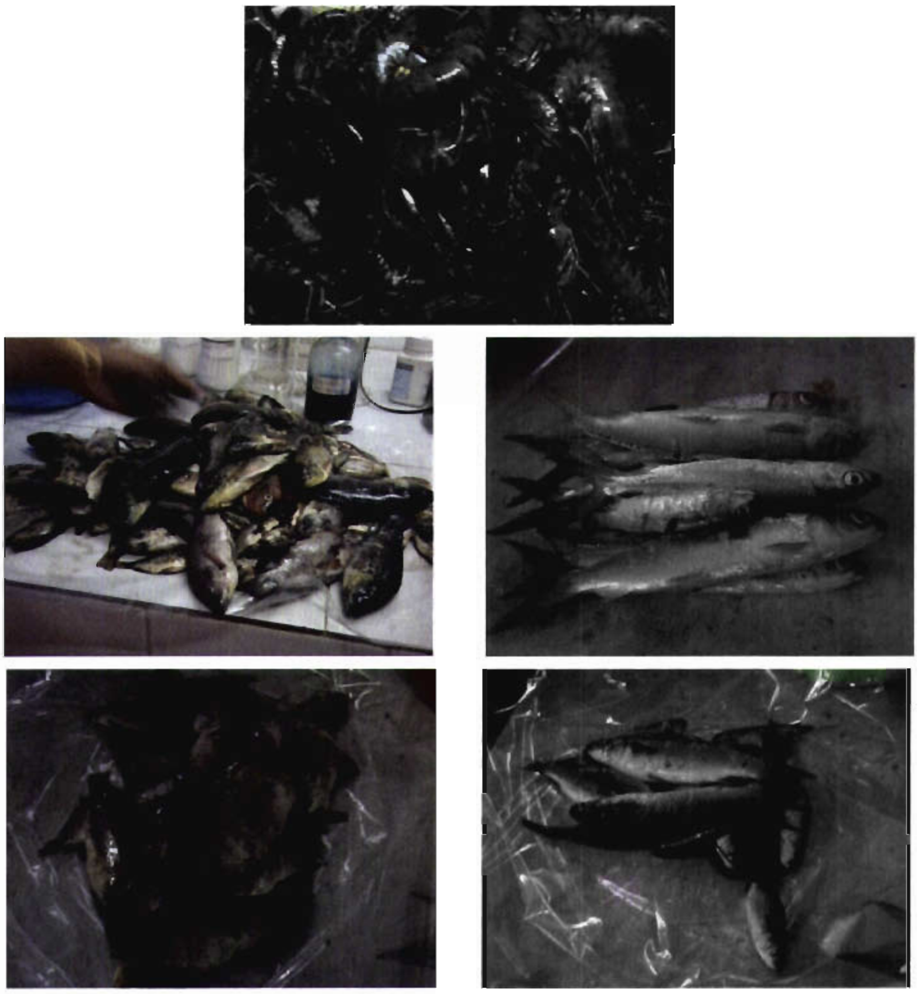
Growth and production was found to be high in fin fish incorporated treatments compared to control, especially in Pearlspot-shrimp combination. This is a strong evidence for the fact that resuspension plays an important role in fish growth and production by the recycling of wasted nutrients into the shrimp biomass. In finfish incorporated experiments, the toxic inorganic nitrogen species such as TAN, NO_2^--N and NO_3^--N in sediment and water column were reduced significantly ($p<0.05$). The higher growth rate and shrimp yield can be attributed to low concentrations of inorganic nitrogen species (Wahab et al., 2003). Furthermore, lower TAN levels in the sediment positively influenced shrimps by enhancing the food intake and health (Avnimelech and Ritvo, 2003). Benthivorous fish plays an important role in internal nutrient loading and enhances the growth of shrimp. Recycling of organic detritus deposited in the pond sediment is commonly believed to enhance the pond productivity (Schroder, 1987; Ayyappan et al., 1990; Edwards, 1993). The degree of variation in resuspension activities may be due to the different mode of behaviour of different fishes. The increased bioturbation activity of Pearlspot by continuous resuspension of bottom materials, specifically the lighter organic rich particles to the aerated water column was reported by Joyni et al. (2011). The bottom feeding behavior of Pearlspot was reported by Keshava et al. (1988) and Menon and Chacko (1956). Besides other studies highlighting the ability of Pearlspot in stirring up loose bottom materials by the vigorous and rapid beats of pectoral fin (Bindu, 2006) and fanning in bottom (Keenleyside, 1991; Zoran and Ward, 1983) were also reported. Bioturbation mediated resuspension and nutrient flux increased the availability of nutrients in the water column. Thus the toxic nitrogenous wastes were effectively used by the phytoplankton and microbial activities (Shilo and Rimon, 1982; Diab and Shilo, 1988) as nutrient.

The increased phytoplankton levels stimulated the food web in this treatment and increased the growth, production and survival of shrimp. Bioturbated tanks with resuspended nutrients also had good survival especially in Pearlspot co-stocking (96^c ±1.73%). Contrary to this, the lowest survival of 63.00^a±1.73% was recorded in control. In fish integrated treatments, increased bottom dissolved oxygen availability lead to better mineralization and stimulation of natural food web due to the fish driven movements and resuspension, whereas in shrimp alone ponds the growth was found to be constrained both due to the lesser availability of nutrients and diffusion of toxic materials from the sediment and due to the oversupply of nutrients beyond the optimum during the last phase. Moderate growth was observed in T2, T3 and T4 due to poorer bioturbation activities compared to T1, but these treatments were superior to the shrimp alone system. The higher yield in the pond with fin fishes as compared to the control revealed that *P. monodon* is capable of utilizing the nutrients recycled by the activities of fishes.

The increased levels of bacteria also play an important role in the production of microbial protein using the resuspended organic matter in water column. The utilization of microbial protein depends on the ability of target animal to harvest bacteria and its ability to digest and utilize the microbial protein (Avnimelech, 1999). Hari et al. (2004) reported the ability of shrimp in the utilization of microbial protein. In the present study, the increased growth and production in finfish integrated treatment revealed that both the recycled nutrients and resuspension mediated increase in the bacterial protein, served as an incentive for growth of shrimp. The juvenile and adult shrimp eat a wide variety of micro-invertebrates and phyto-zooplankton material (Dall, 1992; Smith et al., 1992). The cultured shrimp utilizes the organic matter, bacteria, phytoplankton, and zooplankton which results in high shrimp yield (Bombeo-Tuburan et al., 1993; Allan et al., 1995). The results of the present study reveals that resuspension process increased the level of nutrients available for shrimp, phytoplankton and organic matter and hence enhanced the overall performance of shrimp in finfish added treatments with distinctively good results in Pearlspot co-stocked treatment.

3.5 Conclusion

In the present study, the cultured fin fishes exhibited different degrees of effect on nutrient release. There were variations among the activity and regeneration of nutrients. Mullet and milkfish have shown a reduced activity compared to tilapia and Pearlspot but all treatments were invariably better than shrimp alone control. This might be due to the difference in the mode of bioturbation activities. Pearlspot was found to resuspend the fine soft sediment having high organic matter (Joyni et al., 2011), whereas tilapia pushed the soil aside by digging for its nest building activities (Phan-Van et al., 2008). The control of sludge distribution in ponds is possible since the organically enriched sludge particles are relatively light as compared with mineral soil particles and thus selectively resuspended as compared with denser mineral particles (Avnimelech and Ritvo, 2003). Anaerobic conditions developed inside the shrimp alone system, which affected the aquaculture production due to the diffusion of reduced compounds from the sediment upward to the water column. Sulphide formation inhibits nitrification and will thus lead to an increase of ammonium in the pond (Avnimelech and Ritvo, 2003) which ultimately leads to its increase beyond the safe levels of ammonia and nitrite during the culture. The addition of fin fishes particularly Pearlspot kept both the sediment and water quality parameters at an optimum level, than in control, with high growth rate, survival and production of shrimp, as a result of effective utilization of resuspended nutrients. Thus the result of the present study promises, in addition to the ability of these fishes in the minimization of toxic metabolites like ammonia, reduced iron, reduced manganese and sulphide by the way of bioturbation, they are also capable for the resuspension of excess feed and nitrogenous excretory products for reutilization of shrimps, especially in the Pearlspot co-stocked culture system. The higher growth rate, survival, yield of shrimp, good sediment and water quality and are indicative of effective utilization of resuspended nutrients and its incorporation into the biomass of target culture organism (*P. monodon*) besides having an additional benefit of rearing a highly marketable fish, *Etroplus suratensis*, which will definitely increase the profit from the aqua farming.



Shrimps and fishes after 105 days of culture

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Chapter **4**

BIOTURBATING EFFECT OF VARIOUS SIZE GROUPS OF *ETROPLUS SURATENSIS* IN SHRIMP-FISH AQUACULTURE TANKS

Contents

4.1 Introduction

Increased global demand for shrimp, which cannot be met by capture fisheries alone, continues to produce a strong economic incentive for shrimp farming. However the adverse environmental impacts resulting from the uncontrolled expansion of shrimp farming in many coastal regions in tropics and sub tropics have promoted widespread criticism and renewed global efforts for sustainable farming techniques.

One of the key environmental concerns about shrimp farming is the formation of toxic metabolites and nitrogenous waste material in the culture pond, its release into the adjacent waterways and the consequent degradation of the environment. In the sediment column, an accumulated sediment layer is formed mainly from the buildup of uneaten feed, faecal matter, phytoplankton debris and other fish wastes. The accumulated sediment is a sink for 91-94% of organic matter in the system. Problems associated with the pond bottom and accumulated sediment occur, when excessive organic material builds up and the subsequent development of anoxic condition, which causes the release of ammonia, organic sulphur compounds, hydrogen sulphides and other reduced compounds such as reduced iron and manganese. Water-sediment interface in aquatic ecosystem is a bridge connecting the bottom sediments with overlying water column. Through the water-sediment interface, by the process of diffusion these toxic and nitrogenous substances leaks into the water column. Diffusion is a major mechanism controlling the fluxes of dissolved material across the sediment-water interface.

An effective solution for the prevention of excess accumulation of nitrogenous wastes and the diffusion of toxic metabolite into the water column are the continuous recycling of the nutrients accumulating at the pond bottom, through oxygenation of sediment. Bioturbation and resuspension are the effective means as it is a biological and inexpensive method to reduce the risk of anoxic conditions at the pond bottom. The accumulated organic matter layer of the sediment develops into a dynamic flocculent layer at the sediment water interface and this poorly consolidated layer is sensitive to resuspension activities. Bioturbation and its effects on sediment chemistry and stratigraphy is a natural consequence of adaptation by organisms to live and forage in sediments. Resuspension can represent a major mechanism of material transfer (especially nutrients) between sediment and water by the activities of living organisms. Biogeochemical cycling and mineralization process in coastal marine soft sediment are controlled by the interplay of physical variables and the activities of bottom dwelling organisms (Malcom and Sivyer, 1997). The supply of oxygen to the sediment regulates the distribution of microbial communities (Fenchel and Finlay, 1995) and in turn organic matter degradation and nutrient recycling (Mackin and Swider, 1989). Detritivorous fish is an important organism to regenerate nutrients from the sediment (Kang and Xian, 2007). Fish driven bioturbation and resuspension has encompassed considerable attention in improvement of aquaculture systems. In shrimp aquaculture it is reported that Pearlspot has the ability to carry out the bioturbation activities and prevents toxic metabolite formation and its diffusion into the water column (Joyni et al., 2011) along with nutrient recycling by means of resuspension.

As fishes are known to have ontogenetic patterns of feeding habits, different size classes of a species are considered as different ecological units in its habitat (Stoner and Livingston, 1984) may reflect in the bioturbation activities. Pearlspot shows different feeding preferences as its size changes (Malcom and Sivyer, 1997). Studies have demonstrated that the feeding behavior and the foraging of the fishes have perceptive effect in bioturbation. Fish species, size, density and foraging behavior are critical factors affecting sediment disturbances. The effect of different size groups of Pearlspot in bioturbation is much relevant in sustainability of shrimp aquaculture systems. The aim of this study was to analyze the effect of varying size groups of Pearlspot on bioturbation and its effect in *P. monodon* culture system.

4.2 Materials and methods

4.2.1 Experimental design

Bioturbation activity among four different size groups of Pearlspot in combination with shrimp was studied in tank experiments. Five treatments were executed in triplicates (4 experimental and one control) in a completely randomized design. In all experimental treatments, a combination of shrimp and Pearlspot with varying size groups was used except in control (shrimp alone). In treatment T1, Pearlspot of size group 1-4 cm, in T2, 5-8 cm, T3, 8-12 cm, and T4, 13-16 cm. were used. Shrimp alone was used as a control in treatment T5.

4.2.2 Experimental setup

Experimental setup is same as described in chapter 2- section 2.2.3

Pearlspot of 1-4 cm, 5-8 cm, 9-12 cm and 13-16 cm sizes were purchased from Puduvypin fisheries research station of Kerala Agricultural University, India and stocked.

4.2.3 Incorporation of Luminophore (fluorescent tracer) to the sediment column

The procedure for incorporation of luminophore is same as described in chapter 2 section 2.2.4

4.2.4 Sediment sampling and Fluorescent particle counting

See chapter 2 section 2.2.5

4.2.5 Analysis of sediment toxic metabolites and organic carbon

The analysis is carried out as described in chapter 2 section 2.2.6

4.2.6 Water sampling and analysis

Refer chapter 3 section 3.2.4

4.2.7 Growth parameters and statistical analysis

Growth parameters are measured and statistical analysis is carried out as seen in chapter 2 sections 2.2.7 & 2.2.8

4.3 Results

4.3.1 Sediment reworking analysis by luminophores

The present study revealed that Pearlspot reworked the sediment particles in accordance with varying size (Table 4.1). The percentage of luminophores that penetrated into the sediment was higher for T1 and T2, whereas more intensive penetration was observed in T2. In control, most of the luminophores deposited in sediment surface at the beginning of the experiment were recovered in the upper sediment layer (0-1cm) at the time of harvesting (99.6^e±0.58%). In contrast, 5-8 cm sized Pearlspot added treatment (T2) displaced the luminophores vertically down to 7 cm at a more intensive rate (5.67^b±0.68%) compared to T1 (0.67^b±5.68%) with 1-4 cm sized Pearlspot. While for the treatment 5 (control), no significant ($p>0.05$) vertical displacement of luminophores was measured in 2-3 and 3-7 cm depth layers, whereas a small fraction of fluorescent particles were recovered in 1-2 cm layer of soil (0.33^a±0.58%). In T3 and T4 Pearlspot displaced tracer particles down to 2-3 cm and 1-2 cm, respectively.

Table 4.1 Fluorescent tracer particles (Placed before stocking) along the bottom soil profile (percentage of total number in the soil profile)*

Treatments	0-1 cm	1-2 cm	2-3 cm	3-7 cm
T1- Size 1-4 cm	57.33^b±1.53	27.33^c±2.08	14.67^b±3.51	0.67^a±0.68
T2- Size 5-8 cm	46.67^a±3.21	27.00^c±1.73	20.67^c±2.51	5.67^b±5.68
T3- Size 9-12 cm	67.67^c±2.08	28.67^c±1.53	3.67^a±0.58	0^a±0
T4- Size 13-16cm	87.33^d±0.58	12.67^b±0.58	0^a±0	0^a±0
T5- Shrimp alone	99.6^e±0.58	0.33^a±0.58	0^a±0	0^a±0

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

4.3.2 Sediment quality parameters

Concentrations of different indicators of toxic metabolites were quantified to examine the effect of size variation of Pearlspot on bioturbation and its effect in shrimp aquaculture system (Table 4.2). Treatments with varying size of Pearlspot showed a decrease in concentrations of TAN compared to control. Decrease in concentration of TAN was highly influenced by the size of Pearlspot. In 0-1 cm, sediment TAN concentration was lower in T2 ($0.033^a \pm 0.01$ mg/g) and higher in T5 ($0.220^c \pm 0.05$ mg/g). The sediment TAN content was low at 1-2 and 2-3 cm depths in all treatment except T5 (Fig. 4.1). In 3-7 cm layer of sediment, TAN concentration was higher in both T4 and T5 and were not statistically significant ($p>0.05$). Significantly ($p<0.05$) lesser TAN concentration ($0.87^b \pm 0.01$ mg/g) in 3-7 cm layer were observed in T2.

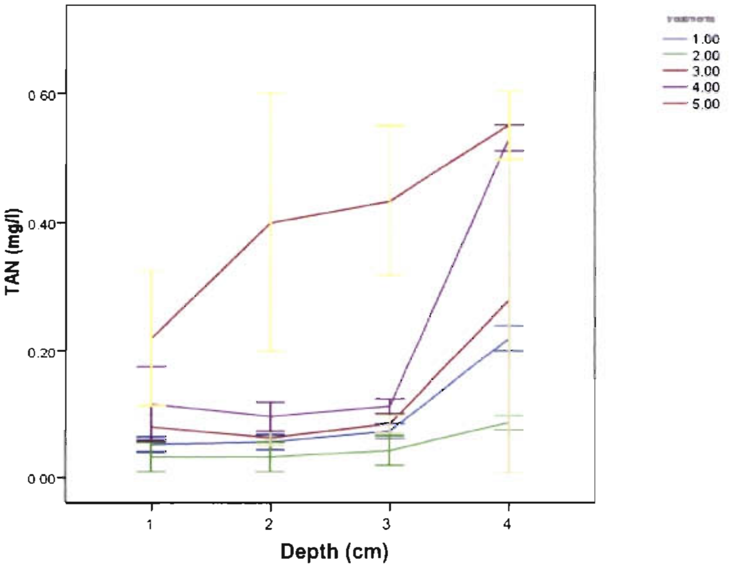

Figure 4.1 Concentrations of Total Ammonia-Nitrogen (TAN) in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

In all the soil layers (0-1, 1-2, 2-3 and 3-7cm) significantly ($p<0.05$) lower concentrations of reduced iron (Fe^{2+}) were recorded from T2 at the respective values of $0.220^{a}\pm0.01$, $0.237^{a}\pm0.01$, $0.277^{a}\pm0.02$ and $0.310^{a}\pm0.01$ (mg/l). Significantly ($p<0.05$) higher concentration was observed in T4 and T5 in the first three layers, while in the 3-7 cm layer of soil highest values were seen in T5 ($0.450^{c}\pm0.01$). Intermediate results were observed in all depth layers of T1 and T3 (Fig. 4.2). Analysis of the 3-7 cm of T4 also revealed the presence of higher concentration of reduced iron ($0.447^{b}\pm0.04$ mg/l).

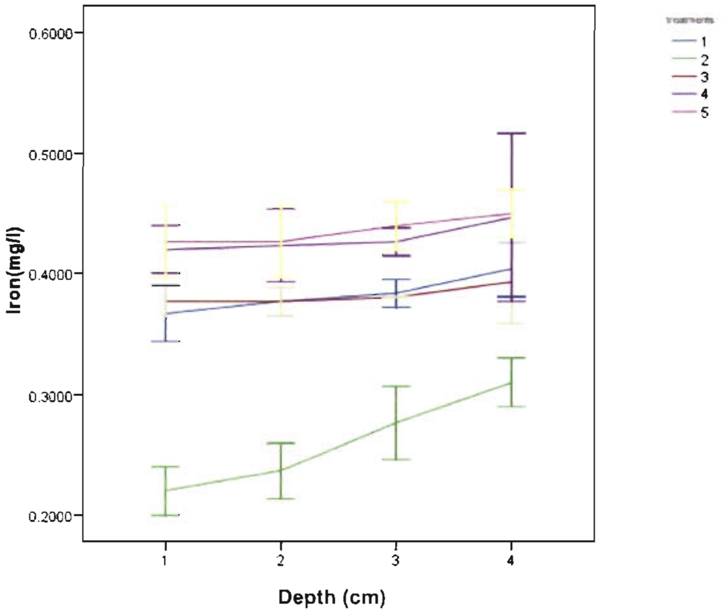

Figure 4.2 Concentrations of soluble iron in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

Table 4.2 Concentrations of parameters in the tank bottom soil, 105 days after stocking *

Depth	Treatment	TAN(mg/g)	EOM (mgC/g)	Soluble Iron (mg/l)	Soluble Mn (mg/l)	TOC (mg/g)	Sulphide (ppm)
0-1 cm	T1- Size 1-4 cm	$0.053^{ab}\pm0.01$	$0.183^{c}+0.01$	$0.376^{b}+0.01$	0.080^{b}	$10.95^{b}+0.09$	$0.090^{a}+0.01$
	T2- Size 5-8 cm	$0.033^{a}\pm0.01$	$0.103^{a}\pm0.01$	$0.220^{a}\pm0.01$	0.060^{a}	$8.86^{a}\pm0.72$	$0.077^{a}\pm0.01$
	T3- Size 9-12 cm	$0.080^{ab}\pm0.01$	$0.140^{b}\pm0.010$	$0.377^{b}\pm.01$	0.080^{b}	$11.13^{b}\pm0.25$	$1.093^{b}\pm0.01$
	T4- Size 13-16cm	$0.117^{b}+0.03$	$0.426^{d}+0.025$	$0.420^{c}\pm.0.01$	0.100^{c}	$19.40^{c}\pm0.33$	$2.343^{c}+0.69$
	T5- Shrimp alone	$0.220^{c}\pm0.05$	$0.420^{d}\pm0.01$	$0.427^{c}\pm0.01$	$0.133^{d}\pm0.01$	$18.32^{c}\pm0.39$	$1.206^{b}\pm0.08$
1-2 cm	T1- Size 1-4 cm	$0.057^{a}\pm0.01$	$0.240^{b}\pm0.02$	$0.377^{b}\pm0.01$	0.070^{b}	$10.95^{b}\pm0.08$	$0.090^{a}\pm0.01$
	T2- Size 5-8 cm	$0.033^{a}\pm0.01$	$0.103^{a}\pm0.01$	$0.237^{a}\pm0.01$	0.060^{a}	$9.55^{a}\pm0.21$	$0.77^{a}\pm0.01$
	T3- Size 9-12 cm	$0.063^{a}\pm0.01$	$0.246^{b}\pm0.01$	$0.377^{b}\pm0.01$	0.070^{b}	$10.95^{b}\pm0.08$	$1.097^{b}\pm0.02$
	T4- Size 13-16cm	$0.097^{a}\pm0.01$	$0.433^{c}\pm0.02$	$0.423^{c}\pm0.01$	0.120^{c}	$19.41^{d}\pm0.32$	$2.197^{c}+0.67$
	T5- Shrimp alone	$0.220^{c}\pm0.05$	$0.423^{c}\pm0.01$	$0.427^{c}\pm0.02$	$0.127^{c}\pm0.01$	$18.32^{c}\pm0.39$	$1.100^{b}\pm0.10$
2-3 cm	T1- Size 1-4 cm	$0.073^{a}\pm0.01$	$0.250^{b}\pm0.03$	$0.383^{b}\pm0.01$	0.060^{b}	$12.43^{a}\pm0.40$	$0.090^{a}\pm0.01$
	T2- Size 5-8 cm	$0.43^{a}\pm0.01$	$0.156^{a}\pm0.05$	$0.277^{a}\pm0.02$	0.050^{a}	$12.43^{a}\pm0.40$	$0.090^{a}\pm0.01$
	T3- Size 9-12 cm	$0.083^{a}\pm0.01$	$0.253^{b}\pm0.01$	$0.377^{b}\pm0.01$	0.060^{b}	$12.30^{a}\pm0.26$	$0.090^{a}\pm0.01$
	T4- Size 13-16cm	$0.113^{a}+0.01$	$0.503^{d}+0.02$	$0.427^{c}+0.01$	$0.127^{c}\pm0.01$	$12.90^{a}+0.78$	$1.957^{b}+0.34$
	T5- Shrimp alone	$0.433^{b}\pm0.06$	$0.423^{c}\pm0.01$	$0.440^{c}\pm0.01$	$0.127^{c}\pm0.01$	$15.78^{b}\pm0.51$	$1.110^{c}\pm0.11$
3-7 cm	T1- Size 1-4 cm	$0.220^{a}\pm0.01$	$0.350^{ab}\pm0.03$	$0.403^{bc}\pm0.01$	0.060^{a}	$12.93^{a}\pm0.21$	$0.090^{a}\pm0.01$
	T2- Size 5-8 cm	$0.087^{b}\pm0.01$	$0.323^{a}+0.01$	$0.310^{a}\pm0.01$	0.050^{a}	$12.93^{a}\pm0.21$	$0.90^{a}\pm0.01$
	T3- Size 9-12 cm	$0.347^{c}\pm0.03$	$0.383^{bc}\pm0.01$	$0.400^{b}\pm0.01$	0.060^{a}	$13.13^{a}\pm0.25$	$0.086^{a}\pm0.02$
	T4- Size 13-16cm	$0.530^{d}+0.01$	$0.313^{a}\pm0.01$	$0.447^{bc}\pm0.04$	$0.120^{b}\pm0.01$	$12.73^{a}\pm0.35$	$1.257^{b}\pm16.5$
	T5- Shrimp alone	$0.550^{d}\pm0.03$	$0.396^{c}\pm0.01$	$0.450^{c}\pm0.01$	$0.180^{c}\pm0.01$	$17.25^{b}\pm0.75$	$1.273^{b}\pm0.17$

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

The presence of shrimp and 5-8cm sized Pearlspot combination (T2) generally showed decreased manganese concentration in 0-1 cm as 0.060^a mg/l versus the higher concentration of 0.133^d±0.01 mg/l in T5 (shrimp alone control). A medium value of 0.080^b mg/l was found in T1 and T3, while it was 0.1^c mg/l in T4. Reduced manganese (Mn^{2+}) followed the same trend in almost all treatments with an exception in the lowermost depth (Fig. 4.3). Highest values were recorded in T4 and T5 in both 1-2 and 2-3 cm layer. In the case of 3-7 cm layer of sediment, lowest values were showed by T1, T2 and T3, where significant differences were not observed ($p>0.05$) among the treatments. However, the manganese concentrations in T4 and T5 were not statistically significant ($p<0.05$) and higher than the above mentioned treatments.

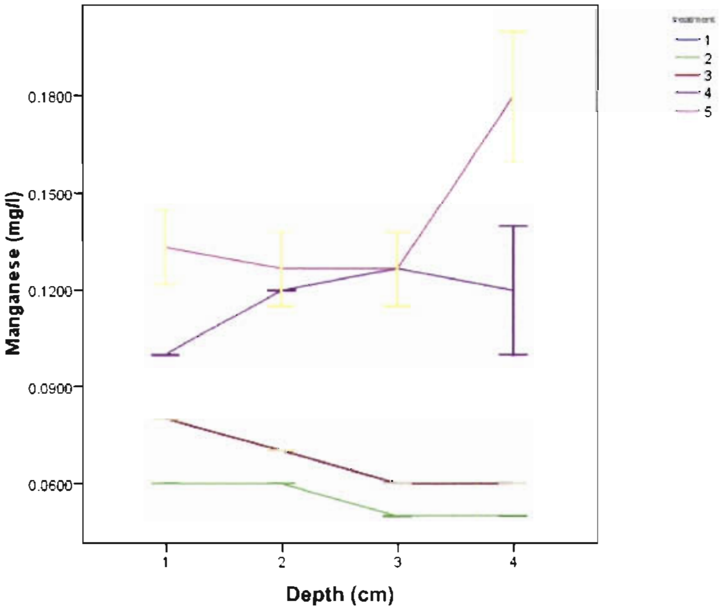

Figure 4.3 Concentrations of soluble manganese in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

Lowest sulphide concentrations (Fig. 4.4) were recorded in T1 ($0.090^{a}\pm0.01$mg/l) and T2 ($0.077^{a}\pm0.01$mg/l) in 0-1cm layer and were not significantly different ($p>0.05$). In T3 ($1.093^{b}\pm0.01$mg/l) and T5 ($1.206^{b}\pm0.08$mg/l) concentrations were higher and were not statistically significant ($p>0.05$). Maximum concentration was observed in the T4 ($2.34^{c}\pm0.69$mg/l). Treatment variability was not observed among the T1 and T2 in 1-2 cm layer ($p>0.05$). In T3 and T5 comparatively higher values were observed, but was lower than in T4. However in the two lower layers (2-3 and 3-7 cm) concentrations of sulphide were found to be decreased in T1 ,T2 and T3,and were not statistically significant ($p>0.05$). In 2-3 cm layer T4 showed values higher than those in T1, T2 and T3. T5 was found to be showing elevated values compared to all the other treatments, while for 3-7 cm layer concentration in both T4 and T5 was higher than that in T1, T2 and T3. T4 and T5 were not statistically significant ($p>0.05$).

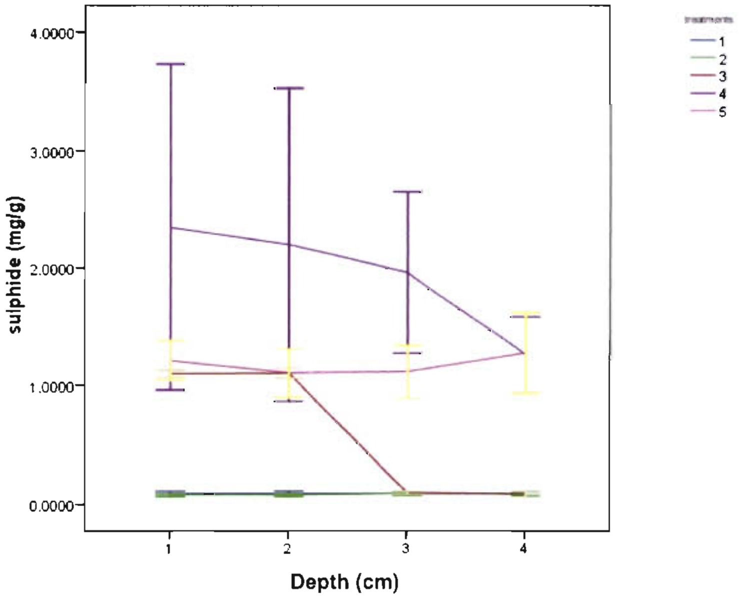

Figure 4.4 Concentrations of sulphide in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

Size variation had profound effects on the concentrations of Easily Oxidized Matter (EOM) and organic carbon (OC) (Fig. 4.5 & 4.6). In 0-1 cm layer as in all other parameters significantly ($p<0.05$) lower EOM values were recorded in T2 (0.103^a±0.01mg/g). Size group was found to be directly proportional to the concentrations of EOM with a concentration of 0.140^b±0.01mg/g (T3) and 0.426^d±0.01mg/g (T4). The smallest size group (T1) also had higher concentrations (0.183^c±0.01mg/g) than treatment 2. In 1-2 cm layer also, the effect of different size groups of Pearlspot were prominent with lowest values of EOM in T2 (0.103^a±0.01mg/g) and highest in T4 (0.433^c±0.02 mg/g) and control (0.423^c±0.01 mg/g). In the T1, T2 and T3 the EOM values showed similar decreasing trend in 2-3 cm with lowest values in T1 (0.156^a±0.05 mg/g) and highest values in T4 (0.503^d±0.02 mg/g). In 3-7 cm layer, the concentrations EOM obtained were entirely of a different pattern with lowest in both T2 (0.323^a±0.01 mg/g) and T4 (0.313^a±0.01 mg/g).

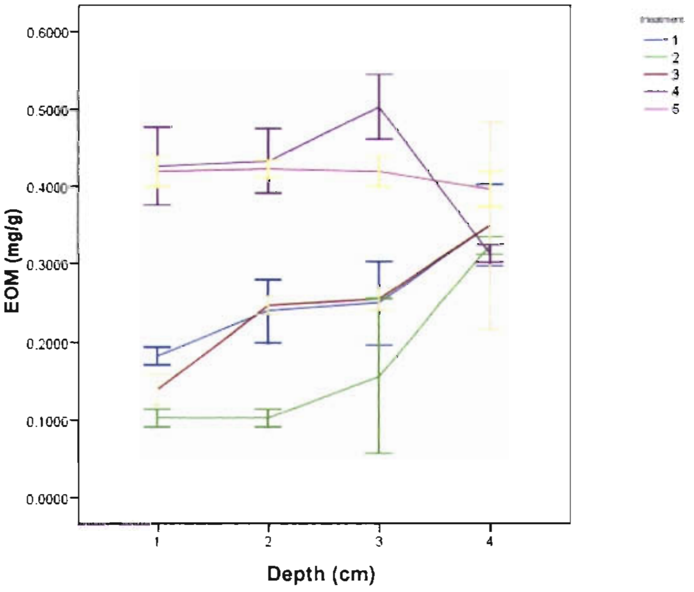

Figure 4.5 Concentrations of easily oxidized matter (EOM) in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

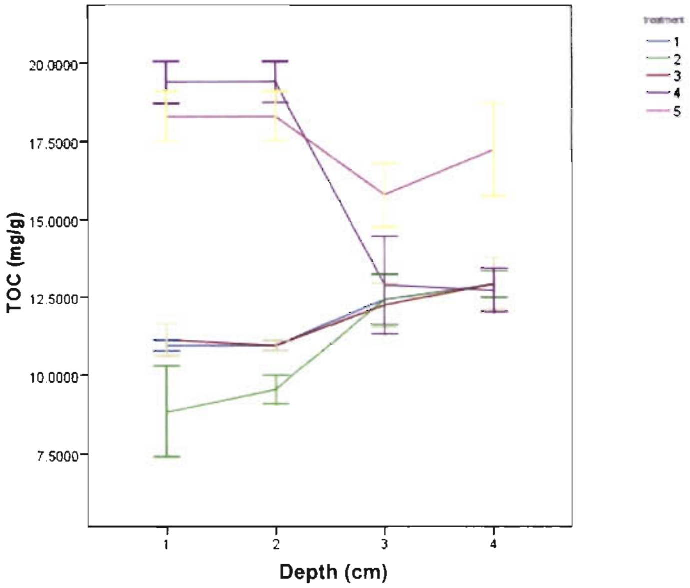

Figure 4.6 Concentrations of total organic carbon (TOC) in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

In both the top layers of the sediment (0-1 cm and 1-2 cm) T2 showed significantly lesser concentrations of organic matter (Fig. 4.6). T1 and T3 showed relatively higher values than T2, but were not statistically different ($p>0.05$). In 0-1 cm layer significantly ($p<0.05$) higher values were observed in both T4 ($19.40^{c}\pm0.33$ mg/g) and T5 ($18.32^{c}\pm0.39$ mg/g), while in 1-2 cm layer, maximum concentrations were observed in T4 ($19.41^{d}\pm0.32$ mg/g). In 2-3 cm and 3-7 cm layers, the concentration of OM was significantly different ($p<0.05$) and higher values were observed only in T5, while other treatments were not statistically significant ($p>0.05$).

4.3.3 Concentrations of nutrients in water column

Table 4.3 summarizes the concentration of the water quality parameters. TAN concentration in the water column was lower in treatment 2 (0.005^{a} mg/l; Fig. 4.7). The order of increase in concentration of TAN were as follows, T2<T1<T3=T4<T5. Significantly higher ($p<0.05$) values of NO_2^--N were observed in T4 ($0.201^{c}\pm 0.17$ mg/l) and T5 ($0.192^{c}\pm0.15$ mg/l), while the concentration was minimum in T2 ($0.113^{a}\pm0.06$ mg/l; Fig. 4.8). NO_3^--N concentrations were found to be reduced in T3 ($4.611^{a}\pm1.90$ mg/l). T1, T2 and T4 showed concentration higher than that of T3, but they were not statistically significant ($p>0.05$) (Fig. 4.9). T5 ($7.721^{c}\pm1.66$ mg/l) showed significantly higher concentrations of nitrate compared to all other treatments. PO_4^--P concentrations followed a different pattern among treatments (Fig. 4.10). Lowest values were recorded in T5 ($0.056^{a}\pm0.02$ mg/l) followed by T2, T3, T4 and were not statistically significant ($p>0.05$). The highest and statistically significant ($p<0.05$) values were recorded in T1 ($0.082^{c}\pm0.01$ mg/l).

Table 4.3 Concentrations of parameters in the water column in different treatments*

Treatments	TAN	NO_2^--N	NO_3^--N	PO_4^{3-}-P
T1- Size 1-4 cm	$0.026^{b}\pm0.02$	$0.153^{b}\pm0.1$	$5.287^{b}\pm2.48$	$0.082^{c}\pm0.01$
T2- Size 5-8 cm	$0.005^{a}\pm0$	$0.113^{a}\pm0.06$	$5.363^{b}\pm2.15$	$0.146^{b}\pm0.08$
T3- Size 9-12 cm	$0.056^{c}\pm0.03$	$0.146^{b}\pm0.09$	$4.611^{a}\pm1.90$	$0.076^{bc}\pm0.01$
T4- Size 13-16cm	$0.059^{c}\pm0.03$	$0.201^{c}\pm0.17$	$5.555^{b}\pm2.68$	$0.066^{b}\pm0.02$
T5- Shrimp alone	$0.083^{d}\pm0.05$	$0.192^{c}\pm0.15$	$7.721^{c}\pm1.66$	$0.056^{a}\pm0.02$

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

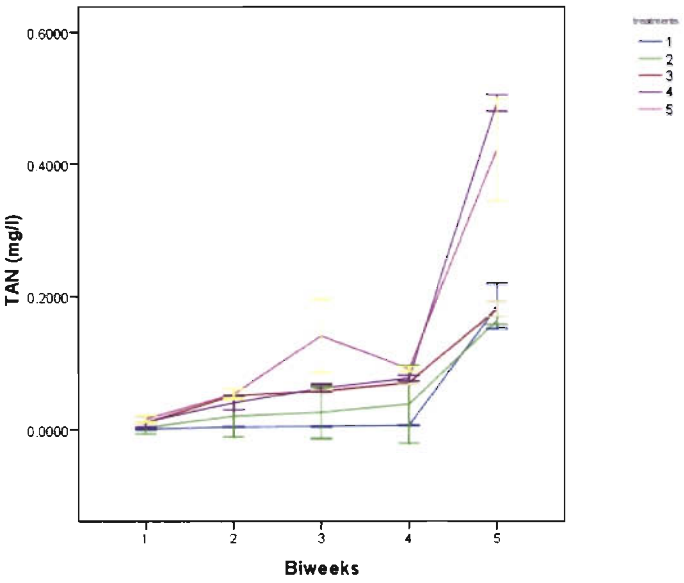

Figure 4.7 Concentrations of Total Ammonia Nitrogen (TAN) in the tank water at different biweeks. Error bars represent standard deviation

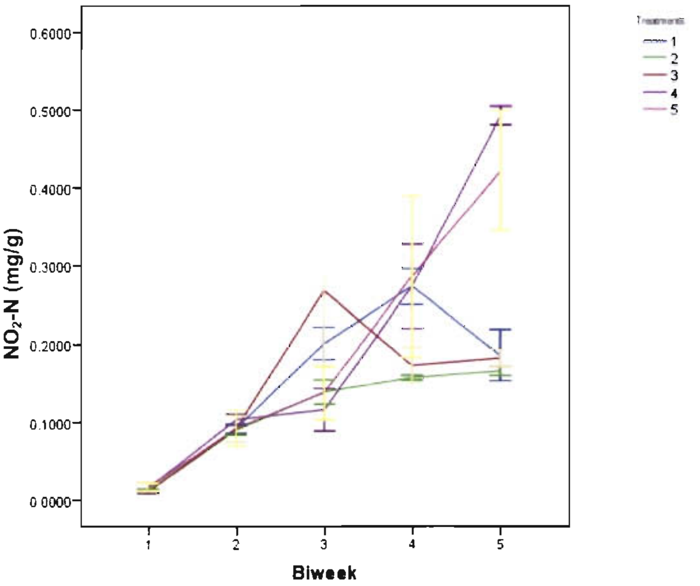

Figure 4.8 Concentrations of Nitrite-Nitrogen in the tank water at different biweeks. Error bars represent standard deviation

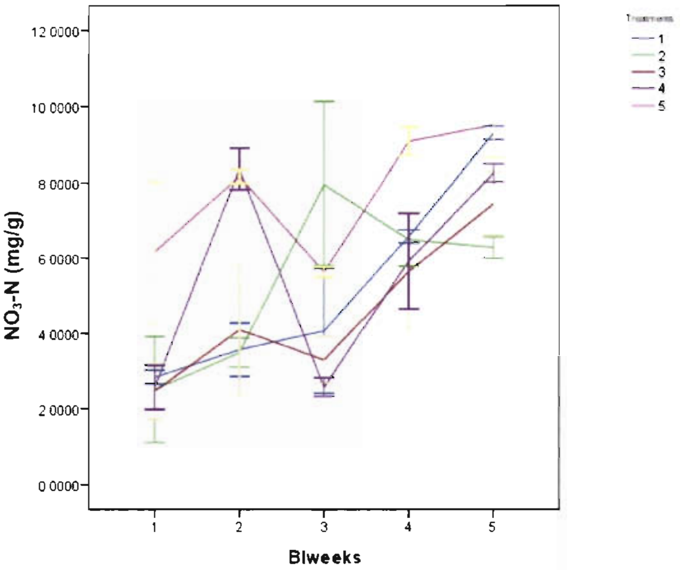

Figure 4.9 Concentrations of Nitrate-Nitrogen in the tank water at different biweeks. Error bars represent standard deviation

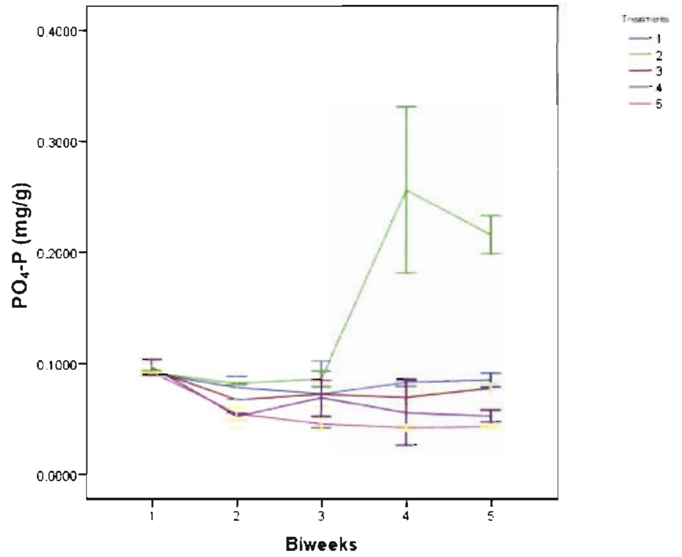

Figure 4.10 Concentrations Phosphate-Phosphorous in the tank water at different biweeks. Error bars represent standard deviation

4.3.4 Growth parameters

Growth, survival and production are shown in table 4.4. The average growth of shrimps varied among different treatments (Fig. 4.11). The highest growth was recorded in treatment 2 and was found to be significant ($p<0.05$), while other treatments exhibited lower growth compared to T2 ($23.43^b \pm 0.53$g) and were not significantly different among them ($p>0.05$). Survival was also found to be higher in Treatment 2 ($83.33^a \pm 5.50$%). However, the treatments did not show statistically significant ($p>0.05$) differences among them (Fig. 4.12). Significantly higher yield was recorded in T2 ($702.50^b \pm 40.01$g), while other treatments showed significantly lower yield (Fig. 4.13). Pearlspot growth parameters in different treatments are represented in table 4.5.

Table 4.4 Growth parameters of shrimp in different treatments *

Treatments	Average weight (g)	Survival%	Yield (g/tank)
T1- Size 1-4 cm	$15.96^a \pm 3.10$	$76^a \pm 12.53$	$428.65^a \pm 40.43$
T2- Size 5-8 cm	$23.43^b \pm 0.53$	$83.33^a \pm 5.50$	$702.50^b \pm 40.01$
T3- Size 9-12 cm	$16.42^a \pm 0.65$	$76^a \pm 8.19$	$447.34^{ab} \pm 33.40$
T4- Size 13-16cm	$14.73^a \pm 4.16$	$78^a \pm 19.29$	$424.69^a \pm 188.56$
T5- Shrimp alone	$13.93^a \pm 0.84$	$74^a \pm 19.08$	$373.45^a \pm 108.54$

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

Table 4.5 Growth parameters of Pearlspot in different treatments *

Treatments	Average weight (g)	Survival (%)	Yield (g/tank)
T1- Size 1-4 cm	$44.67^b \pm 1.53$	$78^d \pm 0$	$622.65^c \pm 21.56$
T2- Size 5-8 cm	$46.67^b \pm 3.51$	$62.67^c \pm 4.51$	$698.33^c \pm 4.90$
T3- Size 9-12 cm	$32^a \pm 1.0$	$47.67^b \pm 5.03$	$461.5^b \pm 43.23$
T4- Size 13-16cm	$28.33^a \pm 2.52$	$33.33^a \pm 2.52$	$340.33^a \pm 46.51$

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

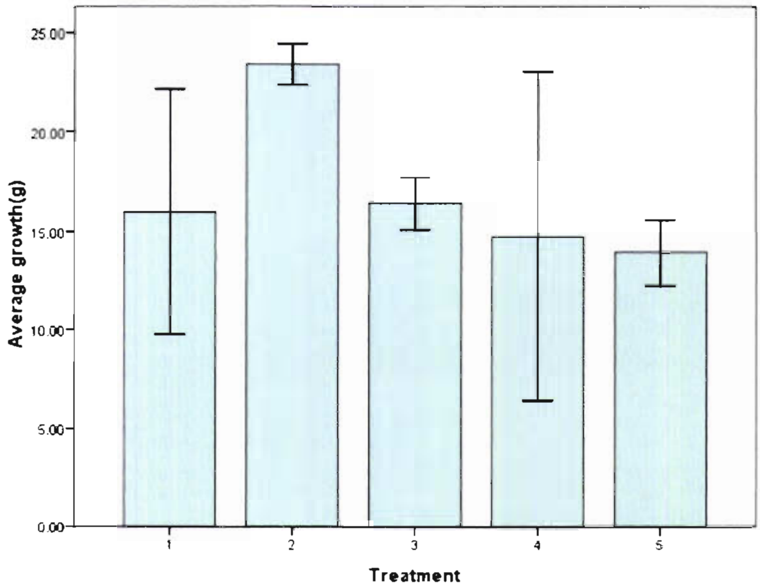

Figure 4.11 Average growth of shrimp. Error bars represent standard deviation

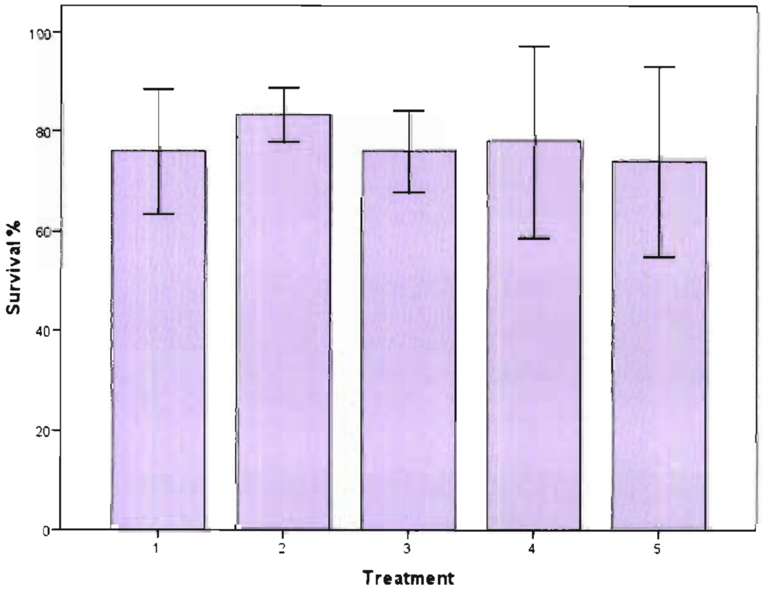

Figure 4.12 Survival % of shrimp. Error bars represent standard deviation

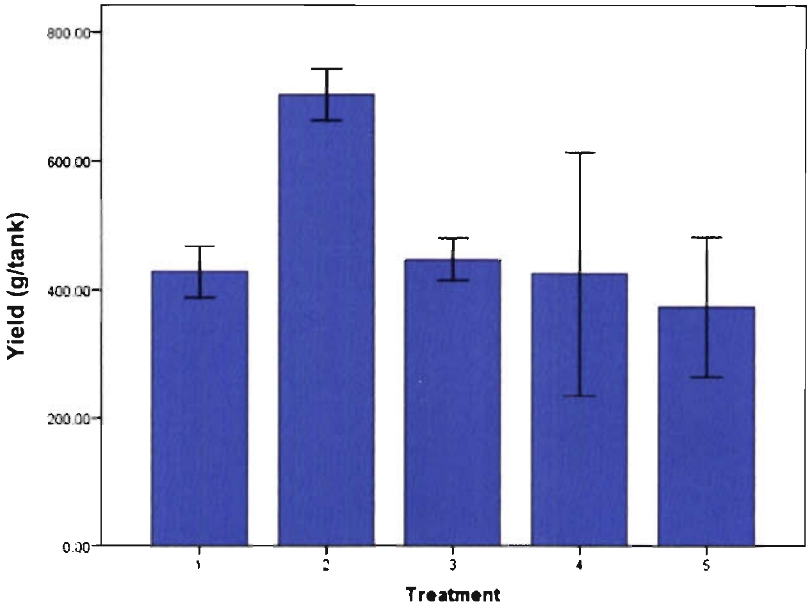

Figure 4.13 Yield of shrimp. Error bars represent standard deviation

4.4 Discussion

4.4.1 Fluorescent counting

The present investigation was undertaken to elucidate the effect of size variation of Pearlspot on bioturbation in the culture system of *P. monodon*. Size related differences in habitat use have been observed in many fresh water fishes (Baltz and Moyle, 1984; Copp, 1990; Sempeski and Gaudin, 1995; Toham and Teugels, 1997). Bioturbation process has a profound influence on the profiles of organic matter (OM), nutrients and oxidized and redox reactions in the sediment (Kristensen and Blackburn, 1987; Bird et al., 2000; Kinoshita et al., 2003). Joyni et al. (2011) reported that in shrimp culture system the presence of Pearlspot produced more bioturbation effect than other brackish water fishes like mullet, milkfish and tilapia. The fluorescent particles were found at maximum depth with maximum intensity in the treatment with 5-8 cm sized Pearlspot (T2). The effect of bioturbation depending on the size of the animal involved was mentioned by Von Boletzky (1996). Weliange and Amarasinghe (2003) observed considerable differences in diet composition of Pearlspot between size groups as well as between the seasons. A change in diet with increase in size of fish was also noticed by Keshava et al. (1988). Menon and Chacko (1956) considered Pearlspot as a bottom feeder as the gut contents were found to contain a fair proportion of substratum material. Similarly Keshava et al. (1988) observed that detritus and sand particles in the gut contents are indicative of bottom feeding habitat of the fish. In general, this fish is a bottom feeding scavenger with a tendency for herbivory. While considering the herbivorus feeding habitat, it might appear that it is predominantly an algal grazer subsisting mostly on filamentous algae and occasionally a small amount of unicellular algae (Bindu, 2006). Grazers pickup large quantities of substrate while searching for feed (Jones, 1968). The main food consists of decayed organic matter (38.61%), filamentous algae (29.15%) and miscellaneous matter (8.04%). Sand (3.89%) also formed a component in the stomach content (Keshava et al., 1988).

4.4.2 Indicators of toxic metabolites in sediment column

Ammonia was one of the important parameters analyzed to verify the effect of bioturbation. Ammonia accumulation in the soil increases under anoxic conditions (Avnimelech and Ritvo, 2003), when nitrification process stops in the absence of oxygen. Ammonia is toxic to shrimp and causes growth retardation and ultimately the death of the organism at higher concentrations. Among the four size groups of Pearlspot evaluated in the present study, all the size groups except the largest (13-16cm) showed a decrease in ammonia concentration compared to control. The size group of 5-8 cm was found to be most effective in reducing the levels of ammonia in sediment column. Nutrient release rates have generally been observed to correlate negatively with fish wet mass; smaller fish release proportionally more nutrients (Lamarra, 1975; Post, 1990; Vanni et al., 2002). Through bioturbation, organic wastes become frequently resuspended in the water column favouring aerobic decomposition over anaerobic decomposition (Cruz et al., 2008). The effectiveness of bioturbation in 5-8 cm size groups might be due to the active movement of fish for foraging and active locomotion of the fish at this growing stage. Fish of 8 cm TL preferred decayed organic matter and micro vegetation, even though larger fish fed on a variety of food. Search for food (decayed organic matter) increases its contact with the bottom possibly resulting in enhanced resuspension. Bioturbation stimulates nitrification and coupled nitrification/denitrification (Kristensen and Blackburn, 1987; Rysgaard et al., 1995; Gilbert et al., 2003) and thus decreases the accumulation of ammonia. Comparatively lesser reduction in the concentration of TAN was observed in smaller (size group 1) and larger (size group 2 and 3) fishes. Since the biomass of Pearlspot in small sized fish incorporated treatment was low, their ability for resuspension and exposure of soil was probably not high enough to produce a substantial effect. This treatment was invariably found to be accompanied by increased levels of ammonia. Kang and Xian (2007) reported that in each group of red eye mullet, the amount of regenerated nutrients increased linearly with body size. However in the present study, the medium sized fishes produced more effect due to their differences in diet selection at different sizes.

Reduced iron and manganese are indicative of toxic metabolites in the sediment column. Reduced inorganic species may affect biological activity and reduced divalent manganese is toxic to fish (Nix and Ingols, 1981). The concentrations of reduced iron and manganese were found decreased in the 5-8 cm size group. In the smaller fish (group 1), the bioturbation activity generated by their movement and foraging, was not sufficient to compensate the wastes produced from the shrimp and fish culture. Avnimelech et al. (1999) illustrated that resuspension, measured in a pond with a small biomass of tilapia was insignificant. Similar results were observed by Ritvo et al. (2004) who reported that very restricted resuspension activity was found for tilapia smaller than 200 g. Intensive feeding was noticed in early mature and spent fish. Feeding intensity appears to be related to spawning activity, besides food abundance. The lesser activity of larger fish observed in the study can be attributed to the divergence of food in larger fish (Keshava et al.,1988) rather than the decayed organic matter. In 5-8 cm size group of Pearlspot integrated treatments, oxygen availability through bioturbation, exerts a measure of control over benthic iron recycling by promoting the reoxidation of Fe^{2+} produced during organic matter mineralization via iron reduction to Fe^{3+} thus closing the cycle by reprecipitating dissolved iron diffusing from deeper sediment layers and approaching the sediment water interface in the oxic zone as iron oxides or oxyhydroxides (Simao et al., 2010). Hence the fish could have a greater impact on the biogeochemical dynamics of benthic iron cycle by introducing a high degree of particles and pore water mixing through bioturbation.

Dissolved sulphide was also found to be less in treatment T2 (5-8cm) compared to the other treatments. The activity of fish in third and fourth treatment was less due the more passive movements of fish; therefore the sulphide concentrations were high in these treatments. H_2S is toxic for aerobic aquatic organisms (Smith et al., 1977). Even low levels of H_2S can harm shrimp (Chien, 1992; Chien and Chen 1992; Bagarinao, 1993).

Wyban et al. (1987) reported that H_2S concentration of even 0.2-0.4 mg/l was toxic to shrimp in culture tanks. Kang and Matsuda (1994) reported the combined effect of hypoxia and H_2S on the early developmental stages of the white shrimp *Metapenaeus monoceros*. Gopakumar and Kuttyammma (1997) found that *Penaeus indicus* avoided a substratum that contained H_2S in a substratum selectivity experiment. However water turbidity in laboratory soil-water microcosms were directly related to size of the organism (Ritvo et al., 1997). Breukelaar et al. (1994) reported a resuspension rate equaling about five times the fish biomass per m^2 per day. Many scientists such as Rhoads (1974), Sandnes et al. (2000), Duport et al. (2006, 2007) and Gilbert et al. (2007) reported that the intensity of sediment reworking can vary according to the size of the bioturbating organism. Lesser bioturbation in the final stages of life cycle is attributed to the fact that fish need very less amount of food for their further growth. Hence, the foraging intensity is found to be decreased in larger fish. Havens (1991) observed that if no feed is applied to the culture system less turbulence is caused and lesser resuspension is expected. In addition, the larger Pearlspot do not show detritivorous feeding behavior. So they may not prefer the sediment surface for their feeding activities.

The total organic matter and EOM concentrations showed a decreasing trend in the size group of 5-8 cm. The accelerated oxidation due to more intensive resuspension compared to all other treatments may have led to a decrease of organic carbon concentration in this particular size group. Resuspension rate is dependent on the fish weight and the number of fish. Ritvo et al. (2004) reported that the degradation of regimenting organic matter was accelerated by carp bioturbation, especially in the upper layer of sediment, as reflected by the reduction in organic carbon, compared to control. Feasibly, in the presence of adequate Pearlspot biomass, organic matter in the pond goes through a continuous cycle of being consumed and recycled, effectively reducing its accumulation in sediment. Further, similar observations were made by Cruz et al. (2008) in tilapia bioturbation experiments.

4.4.3 Water column parameters

Minimum concentrations for the TAN, NO_2^--N, NO_3^--N and PO_4^{3-}-P in water column were observed in the treatment 2. Size class of 5-8 cm Pearlspot in T2 was able to produce maximum bioturbation and resuspension activities due to the high intensity of foraging activities. The increased bioturbation is presumed to maintain the water quality parameters at optimum levels. This phenomenon of decrease in concentration of toxic intermediates by smaller fish also observed in the study of Tarveinen et al. (2005) for total phosphorous, total nitrogen and ammonium. According to Barbrand et al. 1990), roach *Rutilus rutilus,* the smaller fish had higher activity, and the larger benthivorous common carp, *Cyprinus carpio* had lower influence on phosphorous concentration. The increased benthivorous feeding activity of fish for its enhanced growth at the medium size of fish leads to enhanced resuspension and nitrification and subsequently, lower levels of nitrogenous wastes in the water column. The increased feeding activity also led to increased bioturbation by ruffe, *Gymnocephalus cernuus* (Tarvainen et al., 2005). The release of nitrogenous nutrients in water column through bioturbation may have triggered an increase in nitrification and phytoplankton production. In effect, the faster rate of nutrient cycling through the food web may have helped to keep the water quality parameters in an optimum level in T2. In other treatments, the size groups did not effectively maintain the water quality presumably due to the decrease in bioturbation.

4.4.4 Growth and production

Growth, survival and production were found to be increased for those shrimp cultured in combination with Pearlspot of size 5-8 cm. A possible benefit from pond stirring was observed in tilapia ponds where approximately 2.5 higher yields were obtained for stirred pond as compare with unstirred ponds (Costa-Pierce and Pullin, 1989). Jayaprakash and Padmanaban (1985) illustrated the Pearlspot as having different feeding habit and changing feed preference during its life time. Thus different size groups invariably show differences in diet composition. The change in feeding habitat may affect the bioturbation and resuspension activity. The sediment nutrient release may represent a more ecologically important process viz bioturbation, rather than input from external sources because phosphorous released from sediments often contain a large portion of immediately bioavailable Phosphorous (Peters, 1981). Shrimp yield was also directly correlated to soil Fe, Mn, and OM concentrations (Ritvo et al, 1999). Hence the oxidized form of iron (Fe^{3+}) and manganese (Mn^{4+}) is beneficial for the growth, while the reduced form Fe^{2+} and Mn^{2+} are toxic to shrimp. In the present study in control, higher concentrations of the reduced iron and manganese were correlated with a lower growth rate compared to stirred tanks. This special effect of stirring was found in 5-8 cm group of organisms. The release of nutrients was also found to be higher in this group size when compared to all other size groups. Body size of Pearlspot may affect the amount of nutrients released and supplied to phytoplankton. Schaus et al. (1997) reported that Nitrogen and Phosphorous regeneration by detritivorous Gizzard shad positively correlated with fish wet mass. In the case of large fish, only a small part of the food consumption was used for growth (Liu et al., 2005). Decreased amount of feed is consumed causing lesser bioturbation activity. Studies indicated that fish size (Barbrand et al., 1990; Schindler et al., 1993) could alter the rate of fish nutrient regeneration. This increased feeding activity in turn could increase the bioturbation by ruffe (Tarvainen et al., 2005). The steady increase in abundance of phytoplankton after the first month of culture might be due to increased nutrient re-suspension by tilapia of increasing body size (Asaduzzaman et al., 2009). Avnimelech et al. (1999) reported that tilapias can re-suspend sediment at an appreciable level, and such activity is more pronounced in large fish. On the contrary, nutrient regeneration by redeye mullet significantly increased linearly with body size (Kang and Xian, 2007). An abundance of redeye mullet would consume a large amount of detritus and regenerate quantities of nutrients, thus significantly affecting the nutrient cycling and species community in coastal ecosystems. The results of the previous studies indicated that temperature (Schaus et al., 1997), diet (Brabrand et al., 1990) and fish size (Brabrand et al., 1990; Kraft, 1992; Schindler et al., 1993) could alter the degree of nutrient regeneration by fish. Body size may affect the amount of nutrients released and supplied to phytoplankton because smaller fish excrete more nutrients per unit of body mass than do larger ones due to their higher metabolic rate (Kang et al., 2005). Prasadam (1971) in a brief report on the food of Pearlspot recorded that the specimens having a size of 8.5cm in length, feed predominantly on detritus, macrovegetation, filamentous algae and decayed organic matter. In each stage of its life cycle it changes its preference of food items. It would appear that the larvae and fingerlings are omnivores. The present study reveals the significance of size of the bioturbating fish on the growth, survival and production of shrimp. The enhancement of growth of *P. monodon* occurred in 5-8 cm sized Pearlspot added treatment, via the mode of feeding they undertake during this stage of their life history.

4.5 Conclusion

The intensive penetration of fluorescent particles to the 3-7 cm depth layer of soil and decreased concentrations of indicators of metabolites specifies the importance of 5-8 cm size group of Pearlspot as the most effective stage in bringing about the maximum bioturbation. The water quality parameters were found to be in the optimum range in all size groups especially in 5-8 cm size group. The high growth rate and production of shrimp was also characteristic of the bioturbation effect of 5-8 cm size group of Pearlspot. The results of this study indicate that Pearlspot at 5-8 cm size is capable of producing the maximum bioturbation in *P. monodon* culture systems.

Shrimps in experimental tanks after draining water

Shrimp after harvest

***Etroplus suratensis* after harvest**

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Chapter 5

OPTIMIZATION OF THE STOCKING DENSITYOF *ETROPLUS SURATENSIS* IN THE SHRIMP-FISH AQUACULTURE TANKS

Contents

5.1 Introduction

Aquaculture pond bottom soils are recipients of large amounts of nitrogen, phosphorous and organic matter (Boyd, 1990, 1992; Boyd et al., 1994b; Munsiri et al., 1995), and these substances tend to accumulate in bottom soils as ponds age. Pond bottom conditions change with time, affected, to a large extent, by the accumulation of organic matter residues leading to high oxygen consumption and the development of reducing conditions (Avnimelech et al., 2004). High concentrations of organic matter in bottom soil may result in anaerobic conditions in the surface layer of soil and at the soil-water interface (Boyd, 1992). Anaerobic soil does not effectively remove phosphorous from the water, and toxic, reduced substances (nitrite and hydrogen sulfide in particular) produced by microbial activity in anaerobic soil layers may enter the pond water if the soil-water interface is anaerobic (Masuda and Boyd, 1994). Cruz et al. (2008) reported that disease was the most severe problem affecting high density shrimp farms in Negros Island, Philippines. Effluents of high stocking density, which are rich in organic waste, further aggravated the problems of water quality deterioration and creation of anoxic sediment conditions. Moreover, a shift to more reducing conditions on the pond bottom can have deleterious effects, such as the production and release of toxic sulphides, ammonium and other reduced metabolic byproducts (Alongi et al., 1999) to the water column. A direct effect of these has been the growth retardation and less survival due to stressed living condition. Therefore, there is pressing need for the amelioration of waste by optimizing stocking density for each and every system.

The ability of Pearlspot in bringing about of bioturbation and the ensuing increase in growth and production and reduction of nitrogenous waste materials and other toxic substances are already reported by Joyni et al. (2011). The ability of the benthic community to mix sediment depends on the type of organism, its density and its activity (Schmidt et al., 2007) is also reported. The intensity of sediment reworking can vary according to population characteristics such as species density, animal size, biovolume, burrowing depth, density and spacing between the animal burrows (Rhoads, 1974; Sandnes et al., 2000; Duport et al., 2006, 2007; Gilbert et al., 2007), and environmental factors such as temperature and the availability of food (Ouellette et al., 2004; Lecroart et al., 2005; Maire et al., 2007; Nogaro et al., 2008; Braeckman et al., 2010). However, for a lot of bioturbators and bio-irrigators, no matter which functional group they belong to, population density is an important parameter determining the impact on ecosystem functioning, such as nutrient cycling and benthic mineralization (Leno et al., 2006; Bulling et al., 2008; Rossi et al., 2008; Braeckman et al., 2010). Furthermore, dominant species often contribute most to sediment reworking and ecological function (Mugnai et al., 2003; Maire et al., 2007), and the loss or density decline of dominant species might have serious repercussions for ecosystem functioning (Solan et al., 2004). Avnimelech et al. (1999) reported that fish species, size, density and foraging behavior were critical factors affecting sediment disturbances and, therefore, the magnitude of resuspension in the ponds. Even though the ability of Pearlspot in bioturbation has already been established, studies on the stoking density of Pearlspot for maximum and sustainable bioturbation in shrimp culture ponds has not yet been carried out. Stocking density of fish is an important criterion in bringing about the sediment reworking. The previous study also revealed the need and importance of investigations on the optimal ratio of finfish to shrimp. The objective of this study was to optimize the Pearlspot stocking rate in shrimp culture system for generating the maximum bioturbation activity. Since *P. monodon* behavior is closely associated with pond bottom soil, characteristics of the pond bottom soil can directly influence shrimp production (Nimrat et al., 2008).

5.2 Materials and methods

5.2.1 Experimental design

Bioturbation activity in four different stocking densities of *E. suratensis* in combination with shrimp was studied using tank experiments. Five treatments were executed in triplicates (4 experimental and one control) in a completely randomized design. In all experimental tanks a combination of shrimp and Pearlspot with varying stocking density of Pearlspot were used except in control (shrimp alone). In treatment T1, a stocking density of 3 Pearlspot/m^2, in T2, of 4 Etroplus/m^2, 5 Etroplus/m^2 in treatment T3, and in treatment T4 6 Etroplus/m^2 were used. Shrimp alone was used as a control in treatment T5. In all treatments shrimps were stocked at a density of 6 PL/m^2.

5.2.2 Experimental setup

See chapter 2 section 2.2.3

Pearlspot, having a size range of 5-8 cm long were purchased from Puduvypin fisheries research station of Kerala Agricultural University, India and stocked as per the density allotted.

5.2.3 Incorporation of Luminophore (fluorescent tracer) to the sediment column

See chapter 2 section 2.2.4

5.2.4 Sediment sampling and Fluorescent particle counting

See chapter 2 section 2.2.5

5.2.5 Analysis of sediment toxic metabolites and organic carbon

See chapter 2 section 2.2.6

5.2.6 Water sampling and analysis

See chapter 3 section 3.2.4

5.2.7 Growth parameters and statistical analysis

See chapter 2 sections 2.2.7 & 2.2.8

5.3 Results

5.3.1 Fluorescent particle

The effect of stocking density of Pearlspot on bioturbation was measured by the incorporation of fluorescent particle (Table 5.1). The particles were displaced down to a depth of 3-7 cm in all the treatments with fish but intensive displacement occurred in the case of higher stocking density. In 3-7 cm layer of the lower density treatment (T1), only

1.33^{a}±0.58% of particles was displaced and the higher percentage (6.00^{c} ±2.00) of particles displaced in T4. In T2 and T3, the percentage displacements of particles were 2.33^{ab}±0.58 and 4.00b^{c}±1.00 respectively, while in T5 (control), no particles were found in this layer.

Table 5.1 Fluorescent tracer particles (Placed before stocking) along the bottom soil profile (percentage of total number in the soil profile)*

Treatments	0-1 cm	1-2 cm	2-3 cm	3-7 cm
T1- 3 fish/m^2	54.00^{b}±2.65	30.67^{b}±2.08	14.00^{c}±2.00	1.33^{a}±0.58
T2- 4 fish/m^2	52.00^{c}±0.58	32.33^{b}±0	13.33^{c}±0.58	2.33^{ab}±0.58
T3- 5 fish/m^2	36.67^{a}±2.52	43.00^{c}±2.00	16.33^{c}±1.53	4.00^{bc}±1.00
T4- 6 fish/m^2	55.00^{b}±2.65	34.00^{b}±3.61	5.33^{b}±0.58	6.00^{c}±2.00
T5- Shrimp alone	99.33^{d}±0.58	0.67^{a}±0.33	0^{a}	0^{a}

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

In 2-3 cm layer of the sediment T2 showed 13.33^{c}±0.58% and T1 showed a 14.00^{c}±2.00% displacement. T3 and T4 dislocated particles with 16.33^{c}±1.53% and 5.33^{b}±0.58%, whilst in T5 no particles were displaced down to this layer. Particles were displaced down to only 0-1and 1-2 cm in this treatment. Among these two layers most of the particles were found in top layer with 99.33^{d}±0.58% and a very small percentage of 0.67^{a}±0.33 in 1-2cm. In 1-2cm displacement was 30.67^{b}±2.08, 32.33^{c}±2.00, and 43.00^{c}±2.00, 34.00^{b}±3.61 percentage respectively for T1, T2, T3 and T4. In the surface layer lowest percentage was observed in 36.00^{a}± 2.52 (T3) and highest was in control (99.33^{d}±0.58%). T1, T2 and T4 particle displacement was 54.00^{b}± 2.65, 52.00^{c}±0.58, 55.00^{b}±2.65% respectively.

5.3.2 Sediment quality parameters

Sediment quality parameters are represented in table 5.2. Lower TAN concentrations were observed in 0-1 cm layer of treatments T1, T2 and T3 with stocking densities of 3, 4, 5 Etroplus/m^2, while for treatment T4 and T5 with 6 and 0 Etroplus/m^2 the TAN concentrations were significantly higher ($p<0.05$). The lowest concentrations of TAN were recorded in T2 with 4 Pearlspot/m^2 in the 0-1, 1-2, 2-3 and 3-7cm layers of the soil (Fig 5.1). In 1-2 cm layer highest concentrations were recorded in T5 (0.323^c ± 0.06 mg/g) and other treatments showed intermediate effects. In 2-3 cm depth layer also highest TAN concentrations were recorded by T5 (0.465^c ± 0.07 mg/g). In 3-7 cm layer of the sediment highest and statistically insignificant ($p>0.05$) results were observed in T3, T4 and T5 (Fig.5.1).

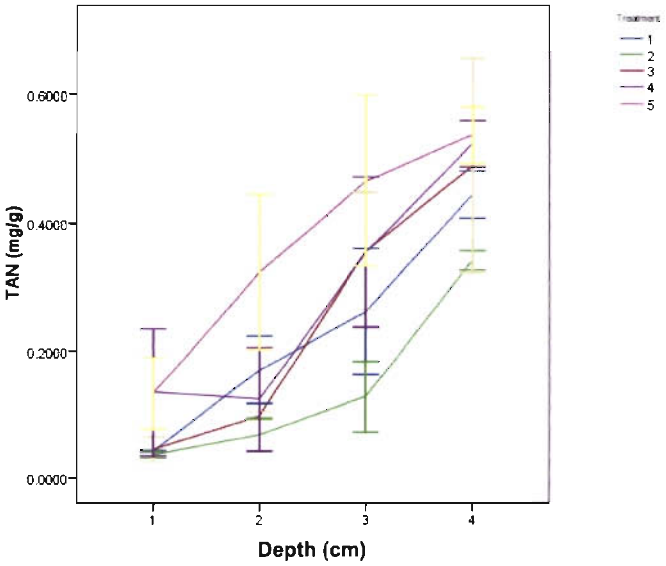

Figure 5.1 Concentrations of Total Ammonia-Nitrogen (TAN) in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

Table 5.2 Concentrations of parameters in the tank bottom soil, 105 days after stocking *

Depth	Treatment	TAN(mg/g)	EOM (mgC/g)	Soluble Iron (mg/l)	Soluble Mn (mg/l)	TOC (mg/g)	Sulphide (ppm)
0-1 cm	**T1- 3 fish/m^2**	0.042^{a}	0.130^{a}	0.307^{a} ±0.01	0.067^{a} ±0.01	9.847^{a}±0.26	0.070^{a}
	T2- 4 fish/m^2	0.036^{a}	0.127^{a}±0.01	0.307^{a} ± 0.02	0.070^{a}	11.550^{c}±0.28	0.383^{a}±0.51
	T3- 5 fish/m^2	0.056^{a}±0.02	0.147^{a}+0.02	0.310^{a} ± 0.02	0.070^{a}	10.816^{b}±0.31	1.307^{b}+0.15
	T4- 6 fish/m^2	0.156^{b}±0.05	0.387^{b}±0.01	0.417^{b} ± 0.01	0.130^{b}	13.723^{d}±0.18	3.820^{c}±0.28
	T5- Shrimp alone	0.133^{b}+0.03	0.413^{c}+0.01	0.417^{b} ± 0.01	0.133^{b} ± 0.01	14.543^{c}+0.20	1.307^{b}+0.15
1-2 cm	**T1- 3 fish/m^2**	0.170^{b}±0.03	0.130^{a}	0.320^{a}	0.067^{a} ±0.01	9.847^{a}±0.26	0.077^{a}±0.01
	T2- 4 fish/m^2	0.068^{a}+0.01	0.130^{a}±0.01	0.317^{a} ± 0.02	0.067^{a} + 0.01	11.550^{b}+0.28	0.090^{a}
	T3- 5 fish/m^2	0.098^{ab}	0.140^{a}±0.01	0.307^{a} ±0.02	0.070^{a}	10.830^{ab}±0.42	1.253^{b}±0.05
	T4- 6 fish/m^2	0.136^{ab}+0.05	0.413^{b}±0.01	0.420^{b} ±0.01	0.140^{b} ±0.01	16.870^{c}+0.95	2.753^{c}±0.16
	T5- Shrimp alone	0.323^{c}±0.06	0.417^{b}±0.01	0.417^{b} +0.01	0.130^{b}	18.890^{d}±0.36	1.307^{b}±0.15
2-3 cm	**T1- 3 fish/m^2**	0.261^{ab}±0.05	0.133^{a}±0.01	0.313^{a} ±0.01	0.067^{a} ±0.01	13.546^{a}±0.1	0.070^{a}
	T2- 4 fish/m^2	0.127^{a}±0.03	0.150^{a}±0.01	0.307^{a} ±0.02	0.077^{a} ±0.01	13.463^{a}±0.01	0.099^{a}
	T3- 5 fish/m^2	0.349^{bc}+0.03	0.137^{a}±0.01	0.303^{a} ±0.01	0.073^{a} ±0.01	13.166^{a}±0.60	1.293^{b}±0.06
	T4- 6 fish/m^2	0.371^{bc}±0.07	0.413^{b}±0.01	0.447^{b}+0.04	0.140^{b} ±0.01	18.133^{b}±0.15	2.753^{c}±0.16
	T5- Shrimp alone	0.465^{c}±0.07	0.423^{b}±0.01	0.420^{b}± 0.01	0.123^{b} ±0.01	18.600^{b}±0.10	1.307^{b}±0.15
3-7 cm	**T1- 3 fish/m^2**	0.444^{b}±0.02	0.147^{a}±0.01	0.387^{b} ±0.01	0.080^{a}	14.460^{a}±0.19	0.077^{a}
	T2- 4 fish/m^2	0.341^{a}±0.01	0.133^{a}±0.02	0.303^{a} ±0.01	0.087^{a} ±0.01	15.610^{b}±0.44	0.150^{a}
	T3- 5 fish/m^2	0.523^{c}±0.02	0.167^{a}±0.02	0.340^{ab}	0.090^{a}	14.666^{ab}±0.57	1.150^{b}+0.07
	T4- 6 fish/m^2	0.533^{c}	0.433^{b}±0.04	0.457^{c} ±0.04	0.183^{b} ±0.01	18.733^{c}±0.40	3.413^{d}±0.14
	T5- Shrimp alone	0.536^{c}±0.22	0.427^{b}+0.03	0.457^{c} ±0.03	0.183^{b} ±0.02	19.666^{c}±0.15	1.610^{c}±0.1

(*) Data not having a common letter are significantly different ($p=0.95$) along columns. (Tukey's HSD test)

Analysis of iron and manganese results indicated both the iron and manganese followed the similar trends in all depth layers of the soil (Fig. 5.2& 5.3). Both iron and manganese were found to be significantly ($p<0.05$) decreased in stocking densities of 3, 4 and 5 Pearlspot/m^2 in all depth layers. Very high stocking density treatment (T4) and control (T5) showed higher concentrations of reduced iron and manganese in all depth layers.

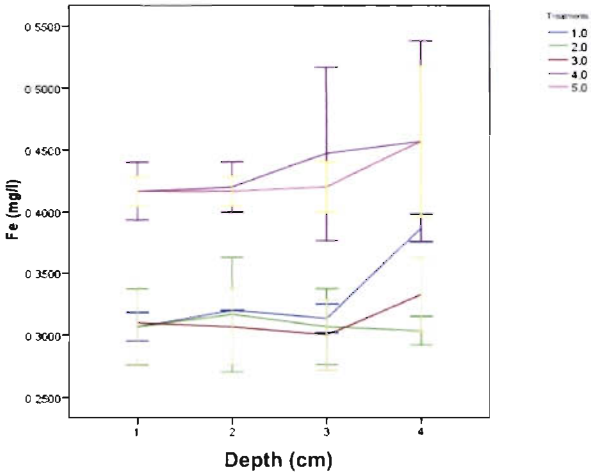

Figure 5.2 Concentrations of soluble iron in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

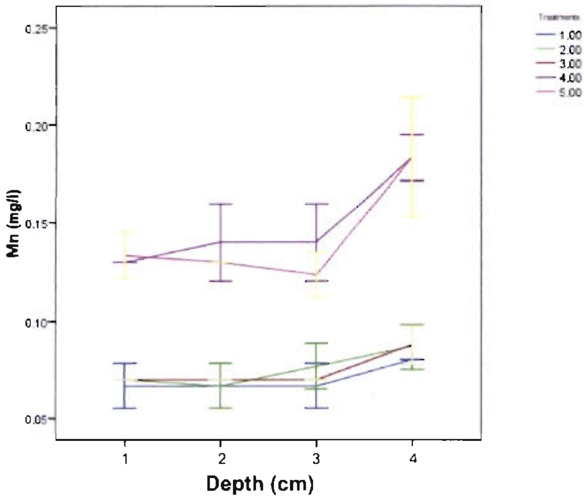

Figure 4.3 Concentrations of soluble manganese in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

Sulphide concentrations were decreased in all depth layers of T1 and T2 (Fig. 5.4). In 0-1cm layer, T3 (1.307^b±0.15mg/l) and T5 (1.307^b ±0.15mg/l) showed intermediate results. Treatment 4 with highest density of fishes (6 Pearlspot/m^2) showed highest concentrations of sulphide (3.82^c ±0.28mg/l). In depth layers of 1-2 and 2-3 cm, as in the 0-1 cm layer, T3 and T5 showed intermediary results and T4 showed higher concentrations. For the deepest layer of 3-7 cm layer the treatments with higher stocking density (T4) led to significantly higher sulphide concentrations (3.413^d ±0.14 mg/l) followed by control (1.610^c ±0.14 mg/l).

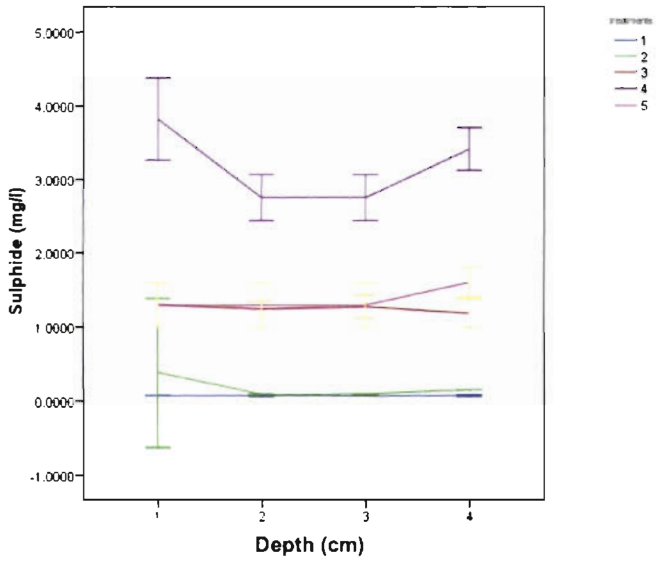

Figure 5.4 Concentrations of sulphide in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation

The total organic matter of the sediment column was considerably low in T1 with 3 Pearlspot/m^2 than other treatments and control (Fig. 5.5). In control the organic carbon was as high as 14.543 mg/g in 0-1 cm layer. Likewise in all depth layers the control showed highest concentrations. Easily Oxidized Matter of sediment were found in lesser concentrations in all depth layers in the treatments with 3, 4, 5

Pearlspot/m^2, while that in the other two treatments, T4 and T5 were found to be higher in all depth layers of the sediment (Fig 5.6).

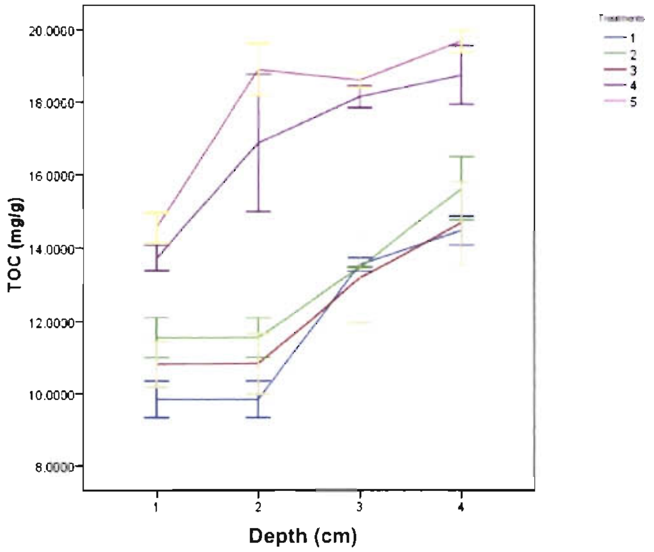

Figure 5.5 **Concentrations of total organic carbon (TOC) in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation**

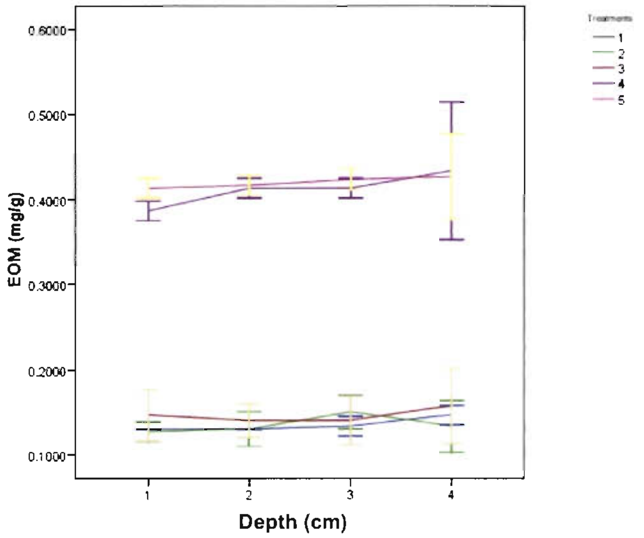

Figure 5.6 **Concentrations of easily oxidized matter (EOM) in the tank bottom soil, 105 days after stocking. Error bars represent standard deviation**

5.3.4 Water column parameters

The mean concentrations of the water quality variables in ponds of the four treatments and the control are provided in table 5.3. Mean concentrations of TAN, NO_2^--N, NO_3^--N and PO_4^{3-}-P were significantly ($p<0.05$) lower in ponds with a stocking density of 4 Pearlspot/m^2 compared to those in control ponds (T5) and other treatments with stocking densities 3, 5 and 6 Pearlspot/m^2 (Fig. 5.7, 5.8, 5.9 & 5.10). TAN concentrations were significantly reduced with the Pearlspot stocking at the rate of 4 Pearlspot/m^2. On the contrary, highest phosphate levels were observed in T2 (0.208^c±0.21mg/l) with 4 Pearlspot/m^2 (Fig 5.10). Phosphate concentrations were found to decrease in treatment 4 (0.092^a±0.03mg/l). Highest concentrations of TAN, NO_2^--N and NO_3^--N were observed in control.

Table 5.3 Concentrations of parameters in the water column in different treatments*

Treatments	TAN	Nitrite-N	Nitrate-N	Phosphate
T1- 3 fish/m^2	0.011^b±0.01	0.246^b±0.26	7.863^{ab}±1.43	0.119^b±0.06
T2- 4 fish/m^2	0.009^a±0.01	0.134^a±0.16	7.217^a±1.41	0.208^c±0.21
T3- 5 fish/m^2	0.011^{ab}±0.01	0.227^b±0.28	8.808^b±2.05	0.148^c±0.10
T4- 6 fish/m^2	0.017^c±0.02	0.531^c±0.36	20.421^c±11.31	0.092^a±0.03
T5- Shrimp alone	0.022^d±0.02	0.678^d±0.47	24.582^d±16.46	0.179^d±0.17

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

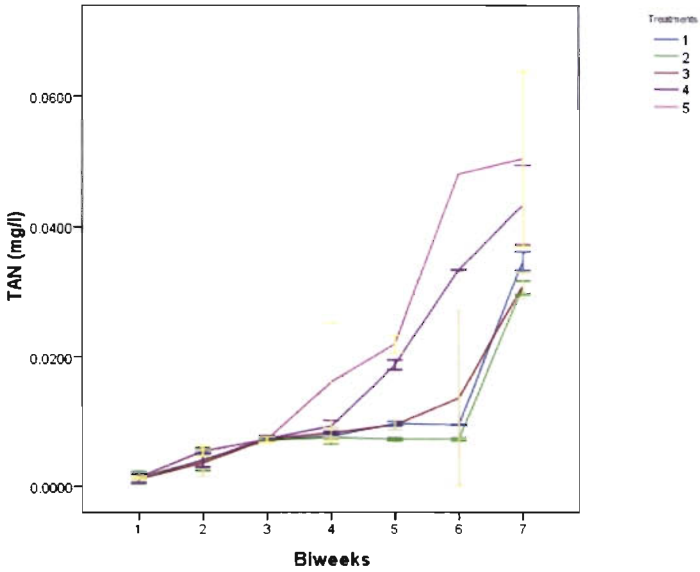

Figure 5.7 Concentrations of Total Ammonia Nitrogen (TAN) in the tank water at different biweeks. Error bars represent standard deviation

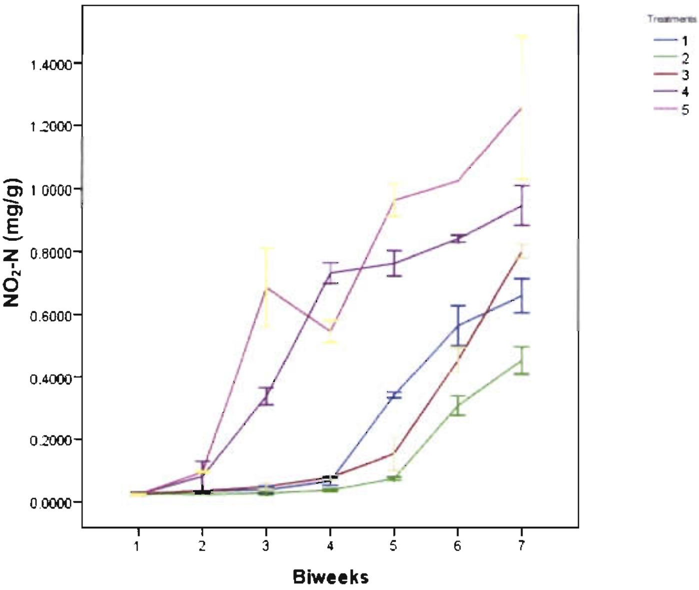

Figure 5.8 Concentrations of Nitrite-Nitrogen in the tank water at different biweeks. Error bars represent standard deviation

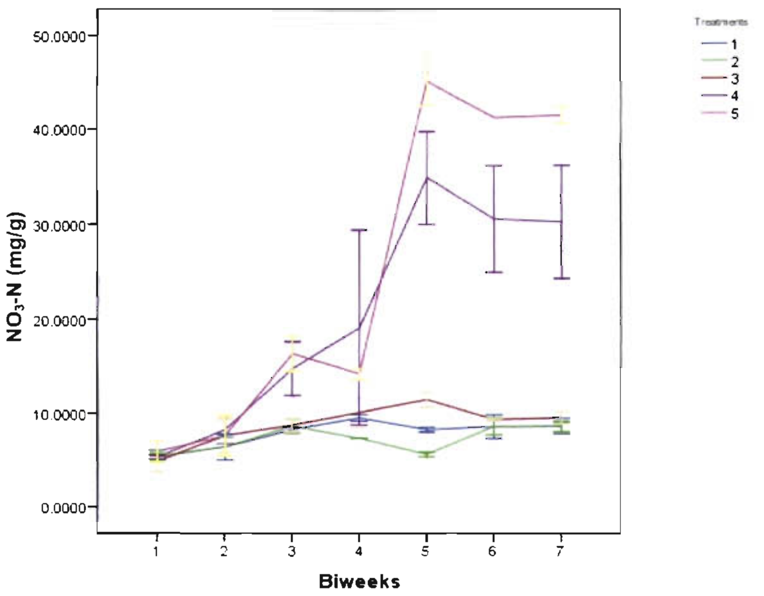

Figure 5.9 Concentrations of Nitrate-Nitrogen in the tank water at different biweeks. Error bars represent standard deviation

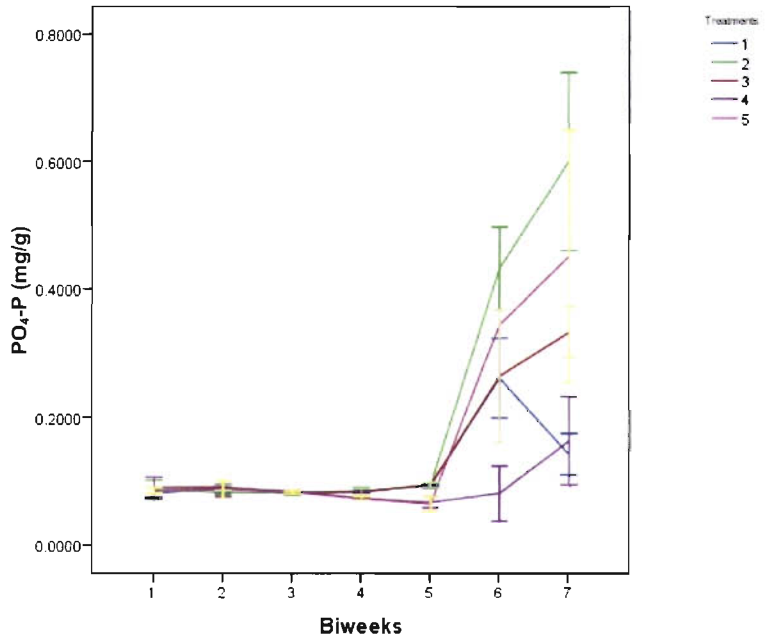

Figure 5.10 Concentrations Phosphate-Phosphorous in the tank water at different biweeks. Error bars represent standard deviation

5.3.5 Growth and Yield

Growth, survival and yield parameters of shrimp are shown in table 5.4. Stocking density of Pearlspot had effects on the average individual harvesting weight, survival and yield of shrimp. Individual average harvesting weight (Fig. 5.11) was 1.5 times higher in T2 (4 Pearlspot/m^2) compared to the control (T5). Total shrimp yield (Fig. 5.13) was found significantly ($p<0.05$) higher (3.4 times) in T2 compared to control ($257.04^c\pm$ 22.12 g/tank). Stocking density of 4 Pearlspot/m^2 (T2) resulted in a 2.2, 1.2, and 2.8 times higher total shrimp yield ($869.33^b\pm62.53$g) than stocking with 3, 5 or 6 Pearlspot/m^2 respectively. The survival of shrimp (Fig. 5.12) was highest in T2 (99%) and lowest in T5 (45%). Other treatments exhibited varying survival rates and were found as 63% (T1), 82.67% (T3) and 48.33% (T4). The overall performance of shrimp was better (highest average individual harvesting weight, 24.36 g; highest survival, 99%; highest total shrimp yield, 869 g/tank) in T2, followed by T3. Shrimp performed better in the presence of 4 Pearlspot/m^2 (T2) compared to the other stocking densities. Growth parameters of Pearlspot are represented in table 5.5.

Table 5.4 Growth parameters of shrimp in different treatments *

Treatments	Average weight (g)	Survival%	Yield (g/tank)
T1- 3 fish/m^2	$17.04^{ab} \pm2.18$	$63^b \pm4.58$	$387.49^a \pm66.81$
T2- 4 fish/m^2	$24.36^c \pm1.41$	$99^d \pm1.73$	$869.33^b \pm62.53$
T3- 5 fish/m^2	$23.90^{bc} \pm4.66$	$82.67^c \pm2.89$	$710.06^b \pm145.91$
T4- 6 fish/m^2	$18.03^{abc} \pm2.80$	$48.33^a \pm8.63$	$306.73^a \pm11.16$
T5- Shrimp alone	$15.72^a \pm0.84$	$45^a \pm1.73$	$257.04^a \pm22.21$

(*) Data not having a common letter are significantly different ($p=0.95$) along columns. (Tukey's HSD test)

Table 5.5 Growth parameters of Pearlspot in different treatments *

Treatments	Average weight (g)	Survival%	yield (g/tank)
T1- 3 fish/m^2	$31.89^{ab}\pm0.50$	$68.33^{bc}\pm2.31$	$521.02^{b}\pm25.32$
T2- 4 fish/m^2	$33.58^{bc}\pm1.80$	$82^{c}\pm6.56$	$662.23^{b}\pm85.88$
T3- 5 fish/m^2	$36.60^{c}\pm1.54$	$62.67^{ab}\pm4.51$	$547.94^{b}\pm14.47$
T4- 6 fish/m^2	$29.59^{a}\pm0.38$	$50^{a}\pm10.58$	$355.73^{a}\pm82.36$

(*) Data not having a common letter are significantly different (p=0.95) along columns. (Tukey's HSD test)

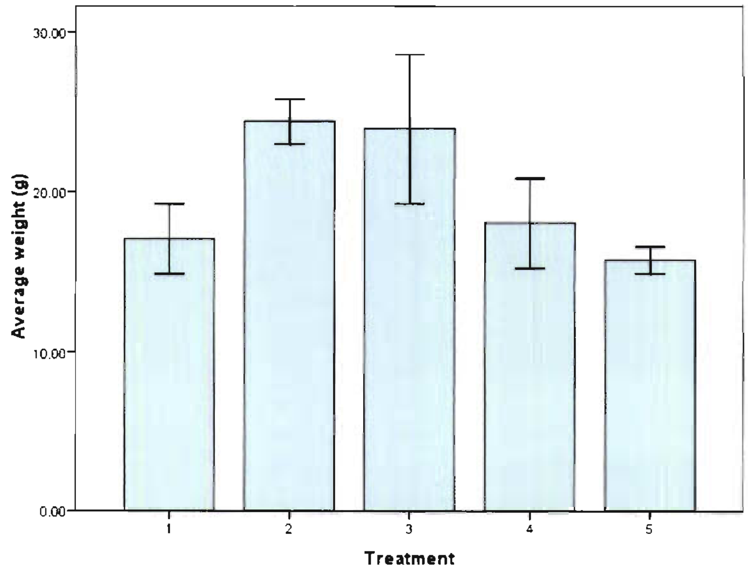

Figure 5.11 Average growth of shrimp. Error bars represent standard deviation

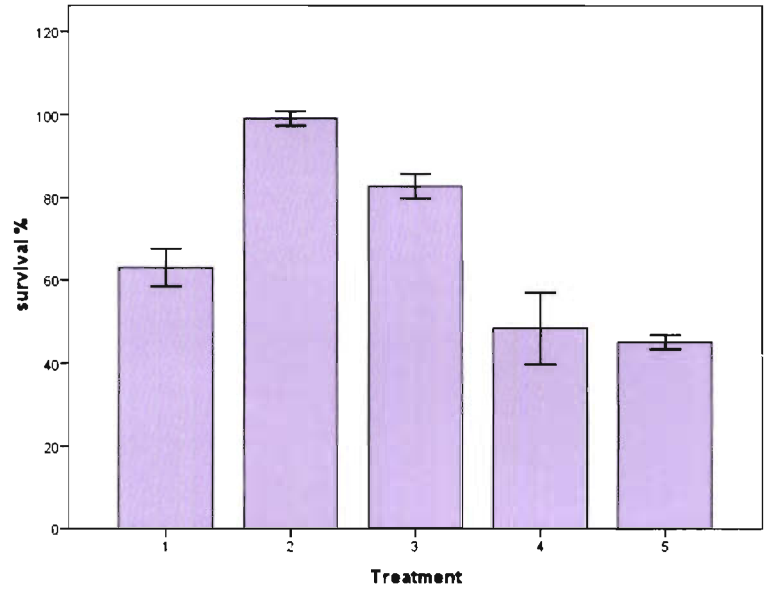

Figure 5.12 Survival % of shrimp. Error bars represent standard deviation

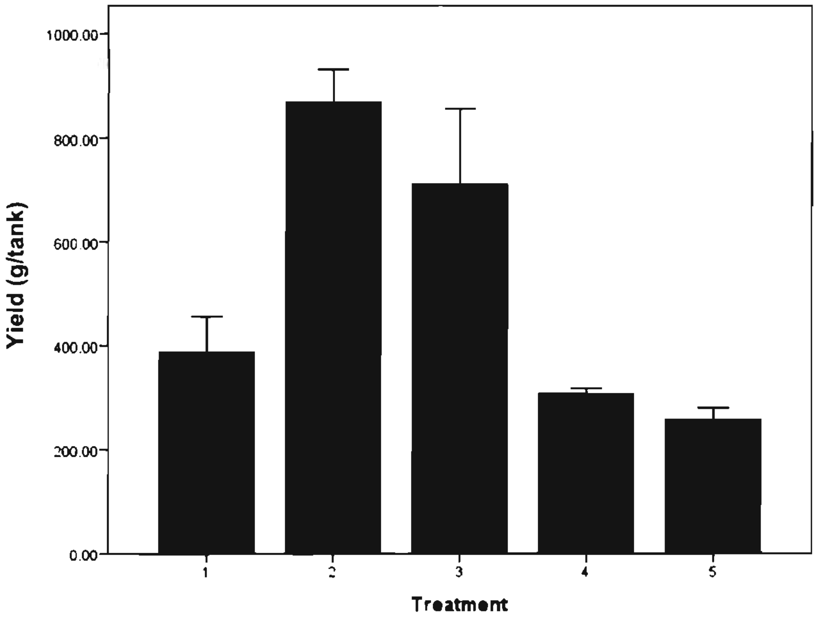

Figure 5.13 Yield of shrimp. Error bars represent standard deviation

5.4 Discussion

Bioturbation is a significant process to accelerate microbial activity, enhance aerobic decomposition of sediment, and increase the resuspension of nutrients (Cruz et al., 2008). Pond bottom sediment quality and quantity reflect pond output and play an important role in mineralization process of organic matter, absorption and release of nutrients to water, influencing water quality and eventually survival of shrimp. The nutrient status, chemical and biochemical process in shrimp pond depend largely on the quantity and quality of bottom sediment, even though the intensity of sediment reworking can vary according to population characteristic such as species density (Rhoads, 1974; Sandnes et al., 2000; Duport et al., 2006, 2007; Gilbert et al., 2007) of bioturbating organism.

The present study was carried out to analyze the effect of varying stocking density of Pearlspot and its effects on bioturbation in shrimp aquaculture system. It had been noted that increasing stocking density of Pearlspot resulted in increasing organic matter in the pond, in T4 and in control. When organic matter accumulated in the sediment through unconsumed feed pellets and excreta of both shrimp and Pearlspot, its degradation produced high concentrations of ammonia. As a result of the process of organic digestion, a layer of anaerobic sediment may form in the ponds, microorganism will consume more oxygen and high quantities of CO_2, hydrogen sulphide, ammonia-N and NO_2^--N will be produced (FAO and NACA,1995). In addition, higher concentrations of ammonium are released as a function of the lower energy yields of anoxic metabolism as compared to aerobic one (Reddy et al., 1986).

Control also produced large quantities of TAN. High ammonium concentration is an indicative of low redox systems (Avnimelech and Ritvo, 2003). Nitrification is inhibited in low redox systems, due to the fact that anaerobic conditions are prevailing in such systems (Joye and Hollibaugh, 1995). Restricted nitrification is another important source of ammonia in the sediment column. Ammonia accumulates in the reduced sediment layer because the biochemical pathway of ammonia transformation requires oxygen. In the treatment two and three with medium stocking density, TAN concentrations were found decreased. This indicates that, in the above treatments with medium stocking densities, Pearlspot bioturbation activities were able to compensate the resuspension and exposure of organic matter produced as a result of shrimp and fish waste. The supply of oxygen to the sediment regulates the distribution of microbial communities (Fenchel and Finlay, 1995) and, in turn, organic matter degradation and nutrient cycling (Mackin and Swider, 1989). Particle resuspension is an important mechanism for nutrient transfer across the sediment water interface of aquatic systems (Anderson, 1974; Ahlgren, 1977; Ryding and Forsberg, 1977; Holdren and Armstrong, 1980; Dillon et al., 1990; Sondergaard et al., 1992). Population density is another important parameter determining the impact on ecosystem functioning, such as nutrient cycling and benthic mineralization (Leno et al., 2006; Bulling et al., 2008; Rossi et al., 2008; Braeckman et al., 2010).The study of Joyni et al. (2011) revealed the ability of Pearlspot to continuously resuspend the accumulated organic matter layer and decreased the levels of TAN of the sediment up to 3cm. In the present study the high stocking density (4 and 5 Pearlspot/m^2) of the fishes were able to influence the TAN concentrations up to a depth of 7 cm. A significant reduction in the TAN concentration by stocking 7 carp (11667/ha) in enclosures in tilapia production pond was reported by Ritvo et al. (2004). It is known that activities of benthivorous fish (Bioturbation) result in ecological benefits as they stir the sediment layer, improving aerobic conditions and enhancing oxidation of detritus (Phan-Van et al., 2008). It has been reported that the genus *Nereis* enhanced both nitrification and denitrification in the sediment surrounding ventilated burrow structures due to their bioturbation activity (Kristensen, 1985; Kristensen et al., 1991; Pelegri and Blackburn, 1995). Nitrogen cycling is faster, and both nitrification and denitrification are enhanced in comparison to azoic sediments, because of ventilation mediated increase in transport of dissolved oxygen and nitrogen compounds (Kristensen et al., 1991; Pelegri and Blackburn, 1995; Sampaio et al., 2002). TAN accumulation may have negative impact on shrimp growth leading to mortality. The toxicity of intermediate oxidation products like, ammonia and nitrite were observed by many authors (Spotte, 1979; Colt and Armstrong, 1981; Chen et al., 1990).

An intermediate effect of decreased TAN concentrations was observed in T1.This is mainly due to the lesser number of fish producing a proportional decrease in the bioturbation activity as reported by Ritvo et al. (2004). In T4 and T5, waste produced by the culturing organisms at higher stocking density prevented the exposure of anaerobic soil to the aerated water column. High concentration of TAN in control tanks and high stocking density treatment can also be explained by the fact that phytoplankton uptake is insufficient to assimilate the large quantity of ammonia generated as a consequence of high feeding rate and stocking density (Hargreaves, 1998).

If available, oxygen is the dominant terminal electron acceptor during the decomposition of organic matter. With the depletion of oxygen, the microbial community reduces other electron acceptors in a characteristic thermodynamic sequence of NO_3^--N, Mn^{4+}, Fe^{3+}, SO_4^{2-} and HCO_3. Reduced compounds or ions that accumulate in the anoxic zone are transported upward with the interstitial water. Ammonium ion (NH_4^+), Phosphate ion (PO_4^{3-}), ferrous ion (Fe^{2+}), manganous ion (Mn^{2+}) and sulphide ion are the examples (Aller and Yingst, 1985; Bird et al., 2000; Edwards, 1958; Henriksen et al., 1980; Kikuchi and Kurihara, 1977; Kikuchi and Kurihara, 1982; Kristensen and Blackburn 1987). Alongi et al. (1999) estimated that in shrimp pond sediments, the contribution to the total carbon oxidation was 41–60% by O_2 respiration,7–22% by Mn reduction,5–25% by Fe reduction, and 13–26% by sulphate reduction.

Sulphate reduction is the dominant anaerobic decomposition pathway in marine sediments (Mackin and Swider, 1989). Sulphide concentrations at the 0-1 cm layer of sediment were reduced up to 0.77 mg/l in the 4 fish co-stocking in comparison to a very high concentration of 1.206mg/l in control. The reduction of sulphide concentration in 4 Pearlspot/m^2 added treatment indicated that the activities of fishes in this treatment were able to compensate the exposure of waste material towards the overlying water column for the oxygenation purpose. Sulphide is a very important fish growth deterrent (Ritvo et al., 2004) and sulphide oxidation appeared to represent the majority of the sediment oxygen demand in shrimp pond bottom soil and accounted for about 84% of the total sediment oxygen demand (Suplee and Cotner, 1996). Since both sea water and saline soils contain high levels of sulphates that can be used as an electron acceptor under anaerobic conditions. Sulphide is a very toxic substance to shrimp even at very low concentration levels of 1 mg/l (Reddy et al., 1986). The decreased levels of sulphide observed in the surface layer of the sediment can be attributed to the activity of fishes at optimal level, since the exposure of feed residues and waste materials to the oxygenated area is important in shrimp ponds. Sulphide concentration dropped very steeply during the exposure of the soil to the air, from an initial value of 0.6 mg O_2 equivalents/g to practically zero within 8-9 days.

Sulfide is not stable at high redox values and is spontaneously oxidized. The sulphide pore water concentrations were high along the whole sediment profile in 6 Pearlspot co-stocked tanks (T4) compared to control and other experimental treatments. This trend can be explicated by the activity of fish causes insufficient exposure of organic matter generated as a result of excretion of both shrimp and fish and excess feed, which facilitated circumstances for the development of anaerobic layer at pond bottom. Entirely different results with very low concentrations of sulphide were obtained in tank with 4 fish stocking (T2) in all sediment layers. An active movement of fish and foraging activity causes the constant stirring of both sediment and water column to maintain the concentrations of sulphide at low levels without affecting shrimp health. Besides, enhanced nitrification coupled denitrification increases the concentration of nitrate in sediment column, and it act as a good conditioner for the reduction of sulphide produced inside the system. In other treatments, increased organic matter and the following anaerobic decomposition pathway influenced the entire culture system in a negative manner. The reduction in sulphide contributed to 60% of the drop in the reducing potential. The additional 40% may be due to oxidation processes of reduced inorganic species (Fe^{2+}, Mn^{2+}) and of rapid oxidation of labile organic compounds.

The ions of both iron and manganese in reduced state in anaerobic sediment form soluble species in the bottom soil pore water (Ritvo et al., 2004) and are sensitive indicators of reduced conditions in pond bottom. Soluble iron concentrations were decreased to 0.220 mg/l in T2, which was considerably less compared to control (0.427 mg/l). Soluble manganese concentrations showed the trend of similar lowered values in treatment 2 with 4 Pearlspot/m^2. Soluble iron and manganese are very toxic to fish at high concentrations (Nix and Ingols, 1981). Mixing of the soil raised the depth and extent of oxygen penetration into the soil (Ritvo et al., 2004). Fauna-induced stimulation of mineralization of organic matter is well known and caused by enhanced microbial activities (Kristensen, 1988). Microbial activity increases owing to enhanced oxidation of the sediment, increased surface area of the organic substrates and decreased the levels of reduced iron and manganese. In agreement with other indicators of toxic metabolites like ammonia and sulphide, the decreased concentrations of reduced iron and manganese highlight the effectiveness of optimum stocking rate in T2 compared to other treatments.

Higher organic matter concentrations were observed in the upper layers of T4 rather than in the upper layers of control. Contrary to this, in deeper layers of soil high organic matter concentrations were recorded in control compared to T4. This may be due to the increased accumulation of organic waste from Pearlspot and shrimp and the less compensation because of the decreased sediment exposure via fish activity. The loss or density decline of dominant species might have serious repercussions for ecosystem functioning (Solan et al., 2004). But concentration of organic matter showed significant lowering in T2 compared to all other treatments. When refractory organic matter is exposed to oxygen during bioturbation, decomposition may increase by one order of magnitude (Kristensen et al., 1995; Kristensen and Holmer, 2001), and its accumulation reduces in pond bottom. The degradation of the sedimenting organic matter was accelerated by carp bioturbation, especially so in the upper layer, as reflected by the reduction in organic carbon (Ritvo et al., 2004). The 0-1 cm sediment layer showed implausible variation in organic carbon, since the bioturbation and resuspension may have enabled the establishment of aerobic conditions and caused the benthic population to thrive, and thus accelerated the organic matter decomposition and degradation at the surface layers. Pearlspot is a continuous resuspender of organic matter in the loosely consolidated upper organic rich layer of soil (Joyni et al., 2011). As a result of optimum bioturbation at medium stocking density, the accumulated organic matter level in the surface soil decreases immediately and nitrification and denitrification process became faster.

The level of organic carbon in soil is rather high and is made up basically of stable organic components, thus it is not a sensitive indicator to the redox processes taking place in ponds (Avnimelech et al., 2004). A fraction of the soil organic matter, EOM, is affected relatively at a faster rate by the environmental conditions and was proposed as a better indicator of pond bottom soil properties and changes (Avnimelech et al., 2004). The EOM, the easily oxidized matter, is the fraction that stresses the redox system and leads to a high oxygen demand (Ritvo et al., 2004). Significantly lower EOM concentrations were observed in the whole depth layers up to 7 cm in the case of 4 fish co-stocking due to the aeration of easily degradable organic matter layer of the soil by enhanced fish activities. Higher values were also observed in the upper layers of T4 and T5. The EOM along the soil profile was reduced up to 24% of the control due to the higher oxygen availability in the 4 Pearlspot density treatment. A reduction of half concentration of EOM occurred in tilapia ponds with a high rate of stocking of carp were observed by Ritvo et al. (2004). Similarly in the present study in 3 and 5 fish co-stocking treatments, the EOM concentrations were reduced to 43.55 and 33% respectively.

Water quality

The physico-chemical properties of pond water are more or less a reflection of the properties of bottom sediment. In the present study, TAN, NO_2^--N and NO_3^--N was significantly ($p<0.05$) reduced with 4 Pearlspot co-stocking than any other treatment including control. The intensity of sediment reworking can vary according to population characteristics such as species density (Rhoads, 1974; Sandnes et al., 2000; Duport et al., 2006, 2007; Gilbert et al., 2007). As a result of redox condition in pond bottom, TAN was found to accumulate in the sediment and eventually diffuse into the water column with comparatively high concentrations in control and 6 Pearlspot/m^2 treatments. Concentrations of sediment pore water or interstitial ammonia may be an order of magnitude greater than those of the water column (Hargreaves, 1998).

Foraging activity of Pearlspot helped to release more nutrients from sediment to the water column compared to the control. This is in agreement with Wahab et al.(1995), Milstein et al. (1988, 2002) who had proven that the browsing and burrowing for food by common carp facilitates the release the nutrients from the bottom into the water column and the nutrients thus released stimulated photosynthesis, leading to increase in phytoplankton and zooplankton biomass. The study conducted by Rahman et al. (2006) revealed that, the effect of common carp addition on phytoplankton and zooplankton biomass was more pronounced with 0.5 common carp/m^2 than with 1 common carp/m^2. Likewise in the present study, the effect of Pearlspot was more prominent in 4 Pearlspot co-stocking than that in 3, 5, and 6 Pearlspot/m^2. The two possible reasons are (1) more fish incorporation increase turbidity (Meijer et al., 1990; Parkos et al., 2003), reducing photosynthesis and hence primary production (Hosseini and Oerdoeg, 1988); (2) higher grazing pressure by fish and zooplankton. The increasing fish density may lead to overgrazing on natural foods, eventually up to the point that recovery is not possible (Steffens, 1990). As the stocking density of Pearlspot increased in T3 and T4 it might have affected the food availability of the shrimp when there are insufficient natural foods available (Spataru and Hepher, 1977). Stocking density influences individual food availability with high densities causing preferred foods to become depleted (Milstein, 1992). The medium stocking density (4 Pearlspot/m^2) led to good results in water quality. Similar results were observed by Rahman et al. (2006) in that optimum turbidity and grazing pressure improved primary and secondary production in treatments with 0.5 common carp/m^2.

Yield parameters

The growth performances of both shrimp and Pearlspot were affected by Pearlspot density. Incorporation of Pearlspot enhanced the growth and production of shrimp through nutrient augmentation in shrimp ponds. The stirring activities of sediments by common carp increases natural food availability by enhancing nutrient flows through the food web (Rahman et al., 2006). In the present study shrimp growth increased when Pearlspot was present, but the effect was more pronounced at a densities of 4 and 5 Pearlspot/m^2. Shrimp production and total production increased almost twice in the presence of 4 and 5 Pearlspot/m^2. When fish density is high, competition for food becomes important. Forester and Lawrence (1978) found that high density of common carp decreased the standing crop of bluegill (*Lepomis macrochirus*, Rafinesque) through food competition, which caused the bluegill to eat their own eggs. Hepher et al. (1989)

reported positive effects at the lower density of silver carp *Hypophthalmichthys molitrix* (Valenciennes) and negative effects at the higher density on its own and other fish species performances.

In the present study, in the presence of 4 Pearlspot/m^2 more natural food was available due to both resuspension and improved primary and secondary production, enhancing the food intake of shrimp. At a density of 5 and 6 Pearlspot/m^2, natural foods became limiting, affecting food intake of both shrimp and Pearlspot. Comparable results were obtained with high stocking density of common carp, which negatively affected rohu ponds due to limiting of food and increased turbidity (Rahman et al., 2006).

5.5 Conclusion

The fluorescent particles were displaced down to deeper layers intensively, as the stocking density increased when compared to control, where displacement occurred only up to 2 cm of sediment. Whilst the only first three treatments with lower stocking density such as 3, 4, and 5 Pearlspot/m^2 were able to decrease levels of indicators of toxic metabolites in sediment column. Yield parameters were high in T2 and T3. The levels of TAN, NO_2^--N and NO_3^--N in water column have shown better results with decreased concentrations only in treatment 2 with 4 Pearlspot/m^2. Strong synergistic effects in terms of availability of food, food intake, growth and production were obtained in shrimp ponds with 4 Pearlspot/m^2. These effects nearly disappeared in treatments with 3, 5, 6 Pearlspot/m^2 and Pearlspot free control. Hence, bioturbated systems with 4 Pearlspot/m^2 and 6 shrimp/m^2 were the best combination which provided better sediment and water quality characteristics without compromising on the growth and production of shrimp.

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Chapter 6

EVALUATION OF THE DIETARY PROTEIN REQUIREMENT OF *PENAEUS MONODON* IN FINFISH BIOTURBATED BRACKISH WATER AQUACULTURE TANKS

Contents

6.1 Introduction

Expansion of shrimp farming in Asian countries has led to concern about potential environmental impacts (Phillips et al., 1993). Nutrients added to the shrimp ponds can affect environmental conditions within ponds and the surrounding marine environments through effluent discharge (Tookwins and Sonsangjinda, 1999). Ecological concerns regarding the interaction between aquaculture and its ecological environment are becoming increasingly important and the extent of problems is such that, they can no longer be ignored. There is a need for better understanding of the environmental interactions, which serves as a basis for improved management of shrimp culture (Barg, 1992). There is a pressing need for the development and dissemination of a range of shrimp culture systems that are both environmentally and ecologically sustainable (Fungesmith and Briggs, 1998).

Protein is one of the major nutrients for shrimp growth and represents one of the primary costs in a compounded feed (Taco and Akiyama, 1997). In addition, protein content of the feed and its availability can affect the water quality via nitrogen excretion. Excess protein will be deaminated and used as an energy source and nitrogen metabolites will be released into the culture medium (Cho et al., 1994). Shrimp excretion and accumulation of feed residue in culture pond can lead to the deterioration of pond conditions and subsequently the shrimp will subject to stress which weakens their immunity (Limsuwan and Junrathakool, 2004). In order to reduce the excretion of nitrogenous compounds and their associated problems, a lower amount of protein can be incorporated into the shrimp pond based on the protein requirements of the species, and adjusted for availability (Jesus et al., 2007). For these reasons there is an interest in optimizing feed input and feed management to improve the economic return in shrimp farms and to reduce the potential of environmental impact. Protein is the most expensive macro-nutrient in shrimp feed, and determination of the optimal dietary protein level is important in formulating the cost effective feed (Wyban et al., 1988). A reduction in haemocytes and immune function were registered when shrimp were fed sub-optimal dietary protein levels. Only 21% of feed nitrogen and 6% of phosphorous added into shrimp grow out ponds are incorporated into harvested shrimp flesh (Stapornvanit, 1993). A large amount of applied feed nutrients remain unused, thus accumulating as organic matter in the bottom sediment. The amount of feed which is underutilized plays an important role in the total nutrient loading and the sustainability of the entire aquaculture system. Because feed settles directly on to the pond bottom, feed wastage can have significant effect on sediment quality and ultimately the health of living shrimp. Chemical, physical and biological processes in pond soil affect water quality, and many water quality problems in ponds originate in the bottom soil (Boyd 1990, 1992). Feed wastage is expensive for farmers who spend up to 50% of their variable costs on feed (Lawrence and Lee, 1997). If pond water is discharged, nutrients and suspended solids can impact on the health of aquatic ecosystems (Naylor et al., 1998; Smith et al., 1999; McKinnon et al., 2002). These harmful effects can be mitigated through processes such as resuspension, chemical transformations and microbial breakdown of organic matter by which the nutrients in sediment could be partially released into pond water. If this is a continuous process, the resuspended nutrients can improve the primary productivity and subsequent fish yield. But very little effort has been made to recover and recycle these nutrients in the sediment for natural food production in ponds and thereby augment shrimp production, which is an area of interest for further research (Rahman et al., 2004).

Therefore, to make shrimp farming more sustainable, pond management with improved strategies to prevent feed wastage through recycling of waste and its incorporation into the harvestable shrimp biomass is essential. Feed recycling in the aquaculture pond thus can minimize the consequential environmental impact and feed cost. Bioturbation and the resuspension of the bottom soil can improve the bottom soil condition and rate of recycling of nutrients into the water column. Bioturbation, i.e. sediment reworking and bioirrigation by benthic fauna is recognized as one of the major processes that can influence the structure and function of aquatic sedimentary environments (Lohrer et al., 2004; Meysman et al., 2006a). Sediment particle reworking results from various activities (i.e. burrowing, feeding and locomotion), and strongly affects the physical, chemical and biological characteristics of marine sediments (Rhoads, 1974; Aller, 1982; Hall, 1994; Rowden et al., 1998; Solan et al., 2008). The recycling of wasted feed as a source of nutrients through the process of bioturbation and resuspension of a bottom feeding fish is important in aquaculture system. Bioturbation and resuspension of Pearlspot is highly beneficial for the improvement of growth and production in shrimp farms (Joyni et al., 2011). Recent research highlights the efficacy of bioturbation using finfish in shrimp ponds in recycling nitrogenous compounds to the overlying water column. Researches on the effect of recycling of excess feed or nutrients in reducing the dietary protein percentage have not been carried out. Therefore, a study was initiated to investigate the effect of addition of Pearlspot on decreasing the use of dietary protein level in the shrimp-fish aquaculture system through recycling of nutrients. Experiments with varying dietary protein levels with and without bioturbation were conducted to understand the effect of bioturbation in bringing down the dietary protein requirement in shrimp aquaculture system.

6.2 Materials and methods

6.2.1 Experimental design

The efficiency of bioturbation activity in decreasing dietary protein levels of shrimp using Pearlspot species was evaluated using treatments in combination with shrimp and Pearlspot and shrimp alone treatments as control. One outdoor experiment with a 2-way factorial design with dietary protein level (24%, 32% and 40%) as first factor and bioturbating fish addition (with or without) as second factor was conducted at the School of Industrial Fisheries, Cochin University of Science and Technology (CUSAT), India. A 24, 32 and 40% protein diet was used, abbreviated as P24, P32 and P40 respectively. Bioturbation is abbreviated as BT. The treatments that received bioturbating fish addition with different protein levels are further referred to as P24+BT, P32+BT and P40+BT. Treatment 1 was P24+BT, T2, P32+BT, and T3 was P40+BT, while in T4, P24 and in T5 P 32 was used. In Treatment T6, P40 was used. Six treatments were executed in triplicates (3 bioturbated and 3 non bioturbated) in a completely randomized design.

6.2.2 Experimental setup

The experiment was carried out in 6 m^3 concrete tanks having an effective bottom area of 6 m^2. All the tanks were provided with a uniform 7 cm thick sediment layer taken from an extensive shrimp culture pond. The soil was consisted of 20.7% clay, 29.8% silt and 49.5% sand. Lime was added initially at 3 kg per tank prior to stocking. Culture tanks were filled with 22 ppt saline water from the Cochin estuary, Kerala State, India. A large closed concrete storage tank was filled as a ready source to fill water losses from the culture tanks. The water level in the culture tanks was maintained at 1 m height during the whole culture period to provide a water volume of 6 m^3. Overflow pipes facilitated the runoff of rainwater keeping mixing with tank water minimal. In addition, it was assumed that the influence of rain on the results is similar for all treatments. To stimulate phytoplankton development, culture tanks were fertilized with urea and super phosphate (Fertilizers and Chemicals Travancore Limited, Udyogamandal, India, Pin 683 501) at the respective rates of 4 and 1 g/m^2/week during the first six weeks of culture. Cattle dung (0.5 kg/m^2) was also added to the tanks before stocking. Twenty-day-old post larvae (PL 20) of *P. monodon* (0.017±0.001 mg) purchased from a commercial hatchery was stocked in the tanks at a density of 6 PL/m^2. Pearlspot seeds, 5-8 cm long sized were purchased from Puduvypin fisheries research station of Kerala

Agricultural University, India and stocked at a density of 4 fingerlings/m^2. Sinking pelleted shrimp feeds containing 24%, 32% and 40% crude protein (Higashimaru Feeds India Limited, Amalgam House, Plot No.9, Bristow Road, Cochin, India, Pin 682 003) were used during the experiment. Treatments were executed in triplicate and were assigned randomly to 18 tanks. Check trays were used to collect shrimps from each tank and wet weight was recorded on biweekly basis. Feeding rate was calculated from the average weight of shrimp for each period. The daily feeding rates were 15% body weight at the start of the experiment, declining gradually to 3% at the end of the culture period. Feed was distributed evenly over the tank's surface, twice daily at 08.00 and 18.00 hours. Shrimps were harvested after draining the tanks 105 days after stocking. Individual length, weight and survival were recorded.

6.2.3 Sediment parameters, water quality and growth indices

Sediment & water quality and growth parameters are measured as described in chapter 3 sections 3.2.3, 3.2.4 & chapter 2 sections 2.2.7

6.2.4 Statistical analysis

Statistical analysis is done as described in chapter 3 section 3. 2.6

6.3 Results

6.3.1 Sediment parameters

Sediment quality parameters are shown in table 6.1. The presence of a bioturbating agent and dietary protein levels are significantly affected the sediment TAN levels ($p<0.05$) in the experimental tanks (Fig. 6.1). TAN gradually accumulated in the sediment in non-bioturbated treatments during the culture period due to reduced resuspension. A gradual reduction in TAN concentration in non-bioturbated tanks from 40%, 32% to 24%

dietary protein levels were noticed. Further decreases in concentrations of TAN were noticed with addition of Pearlspot in varying combinations of varying protein levels.

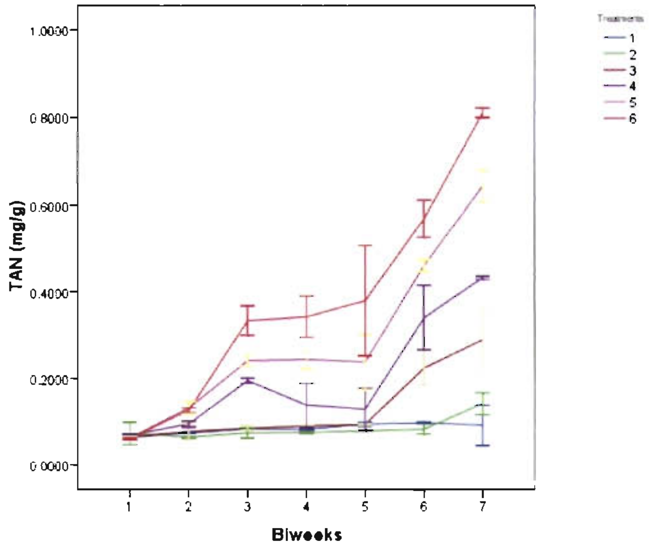

Figure 6.1 Concentrations of Total Ammonia-Nitrogen in the tank bottom soil at different biweeks. Error bars represent standard deviation

The values of TAN were 0.132^{b}±0.08, 0.084^{a}±0.03 and 0.084^{a}±0.19 mg/g respectively in P40+BT, P32+BT and P24+BT (Table 6.1).

However, the Pearlspot addition was not sufficient to make a significant change ($p>0.05$) in the concentrations of NO_2^--N among bioturbated treatments, even though the mean NO_2^--N concentration in the sediment in these treatments were significantly ($p<0.05$) lower than the mean NO_2^--N concentration in non bioturbated tanks (Fig. 6.2). The NO_2^--N and NO_3^--N levels in the sediment were low when compared to TAN.

Table 6.1 Concentrations of parameters in bottom soil of different treatments*

Parameters	T1(P24+BT)	T2(P32+BT)	T3(P40+BT)	T4(P24)	T5(P32)	T6(P40)
TAN (mg/g)	$0.084^{a}\pm0.19$	$0.084^{a}\pm0.03$	$0.132^{b}\pm0.08$	$0.199^{c}\pm0.13$	$0.289^{d}\pm0.19$	$0.375^{c}\pm0.24$
NO_2^--N (mg/g)	$0.055^{a}\pm0.02$	$0.074^{a}\pm0.09$	$0.096^{a}\pm0.05$	$0.190^{b}\pm0.17$	$0.355^{c}\pm0.38$	$0.487^{d}\pm0.45$
NO_3^-- N (mg/g)	$0.076^{a}\pm0.11$	$0.176^{a}\pm0.32$	$0.281^{a}\pm0.56$	$0.542^{b}\pm1.03$	$0.712^{b}\pm1.31$	$1.823^{c}\pm2.61$
PO_4^{3-}-P (mg/g)	$0.006^{a}\pm0.002$	$0.044^{d}\pm0.06$	$0.016^{b}\pm0.03$	$0.061^{c}\pm0.04$	$0.017^{b}\pm0.02$	$0.029^{c}\pm0.03$
TOC (mg/g)	$4.223^{a}\pm0.49$	$4.200^{a}\pm0.054$	$6.656^{b}\pm0.79$	$8.697^{c}\pm1.99$	$10.70^{d}\pm3.48$	$13.39^{e}\pm4.08$
pH	$6.7^{d}\pm0.75$	$6.8^{d}\pm0.02$	$6.2^{c}\pm0.58$	$5.47^{b}\pm0.87$	$4.96^{a}\pm1.3$	$5.04^{a}\pm1.00$
THB (CFU) $\times10^{-3}$	$61.44^{d}\pm20.95$	$58.78^{cd}\pm19.97$	$56.67^{c}\pm19.95$	$42.89^{b}\pm11.27$	$43.33^{b}\pm7.94$	$36.11^{a}\pm6.92$

(*) Means within a row not sharing a letter are significantly different ($p<0.05$; Tukey HSD)

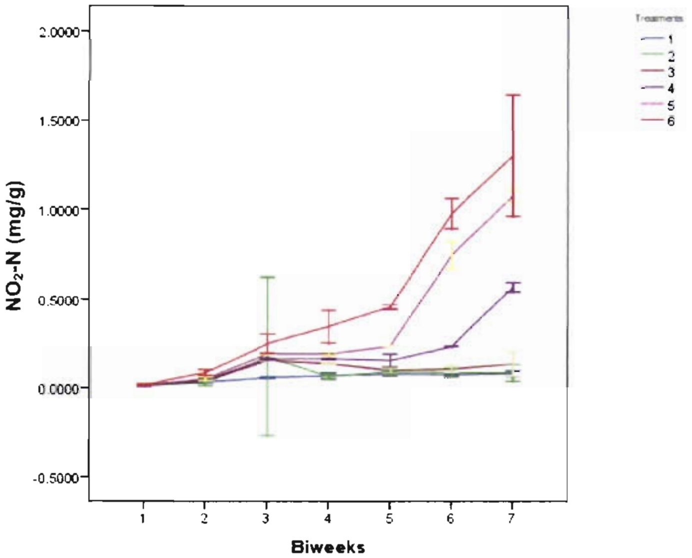

Figure 6.2 Concentrations of Nitrite- Nitrogen in the tank bottom soil at different biweeks. Error bars represent standard deviation

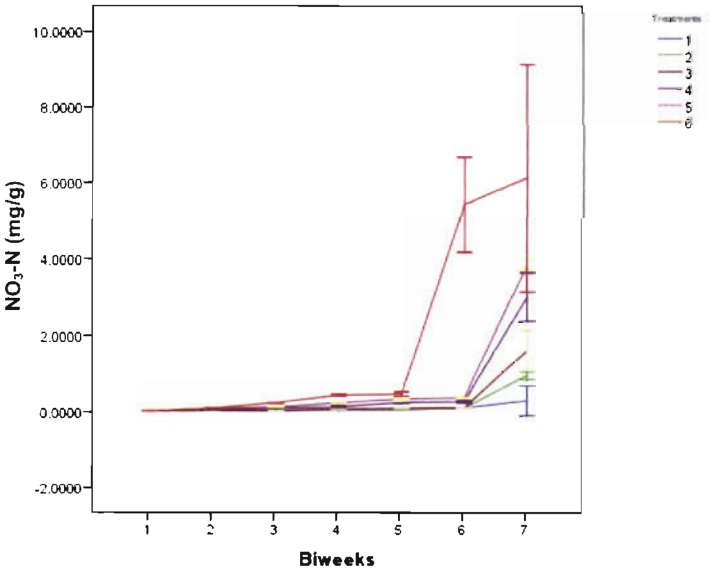

Figure 6.3 Concentrations of Nitrate-Nitrogen in the tank bottom soil at different biweeks. Error bars represent standard deviation

Concentrations of NO_3^--N were also followed the similar trend of diminished levels in bioturbated and enhanced in non bioturbated treatments (Fig. 6.3). However, NO_2^--N and NO_3^--N levels in the sediment increased over time ($p<0.05$).

Slighter disparities were observed in sediment PO_4^{3-}-P levels with that of water levels, where PO_4^{3-}-P (Fig. 6.4) concentrations were contradictory to that of water. In sediment column, lower concentration of PO_4^{3-}-P was observed in P24+BT and higher concentration in P24, followed by P40+BT and P32. The organic carbon content in the sediment were found to be in a position of more than doubled between P24+BT and P24 from 4.22 to 8.69 mg C/g ($p<0.05$) respectively (Fig. 6.5). TOC was highest in P40, followed by P32, it was then found to decrease gradually in the order P24>P40+BT>P32+BT and P24+BT.The highest pH was observed in T1 ($6.7^d \pm 0.75$) where the lowest in T5 ($4.96^a \pm 1.3$) as shown in Fig. 6.6. Higher sediment THB counts were recorded in Pearlspot added treatment with 24% feed ($61.44^d \pm 20.95 x 10^{-3}$CFU) compared to the non-BT- treatments ($36.11^a \pm 6.92$ $x 10^{-3}$CFU) and the mean THB count was found to increase with time in all treatments.

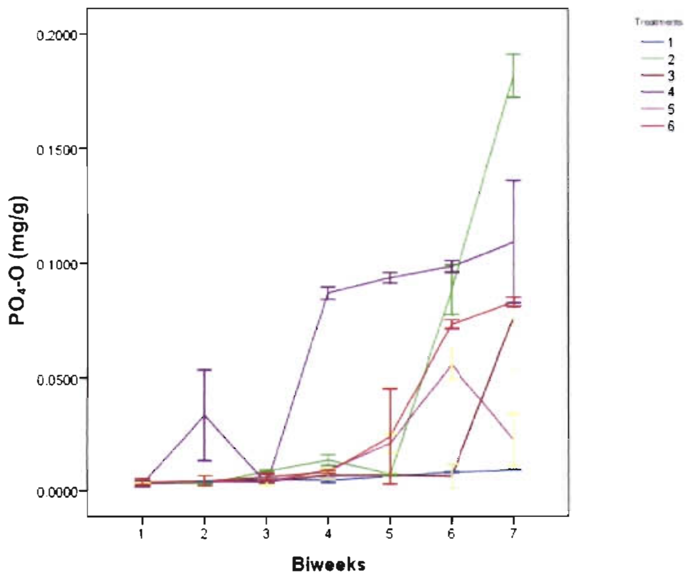

Figure 6.4 Concentrations of Phosphate-Phosphorous in the tank bottom soil at different biweeks. Error bars represent standard deviation

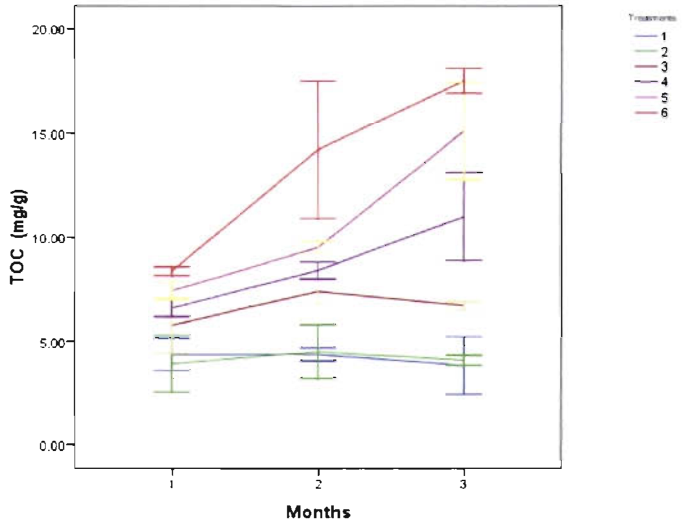

Figure 6.5 Concentrations of Total Organic carbon in the tank bottom soil at different months. Error bars represent standard deviation

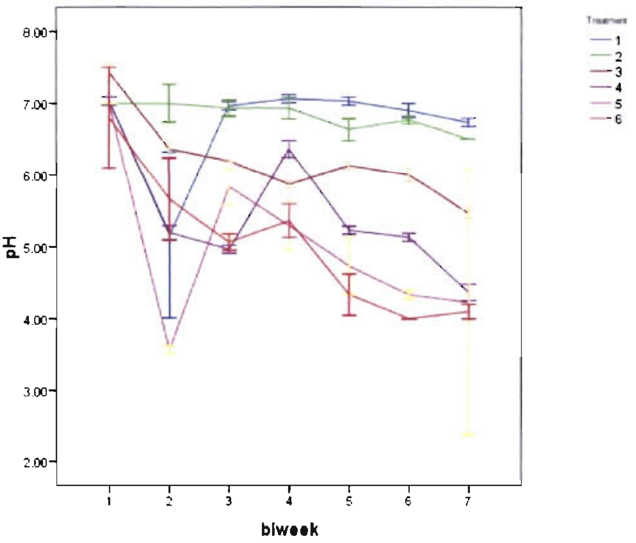

Figure 6.6 pH in the tank bottom soil at different biweeks. Error bars represent standard deviation

6.3.2 Water column parameters

The water quality parameters like temperature, salinity and surface dissolved oxygen were not affected by dietary protein level and Pearlspot addition ($p>0.05$; table 6.2). There was a wide variation in all other water quality parameters like TAN, NO_2^--N and NO_3^--N between treatments with and without bioturbation. The addition of Pearlspot minimised water column TAN to 0.084 mg/l (T1) (Fig. 6.7), whereas the TAN concentrations were significantly ($p<0.05$) higher (0.375 mg/l; T6) in non-bioturbated treatments. The results of the two-way ANOVA showed that besides Pearlspot addition, the dietary protein level significantly influenced the water TAN concentration. The mean TAN concentrations were 0.37, 0.33 and 0.47mg/l in P24, P32 and P40 treatments respectively and varied significantly among sampling dates. The concentrations obtained were also significantly higher ($p<0.05$)) than that in P24+BT, P32+BT and P40+BT. The mean TAN concentration over the days of sampling peaked within one month of commencement of experiment.

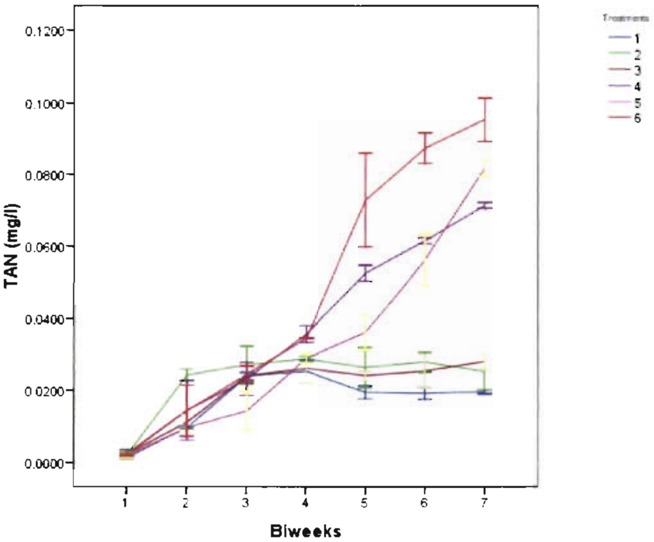

Figure 6.7 Concentrations of Total Ammonia-Nitrogen in water column at different biweeks. Error bars represent standard deviation

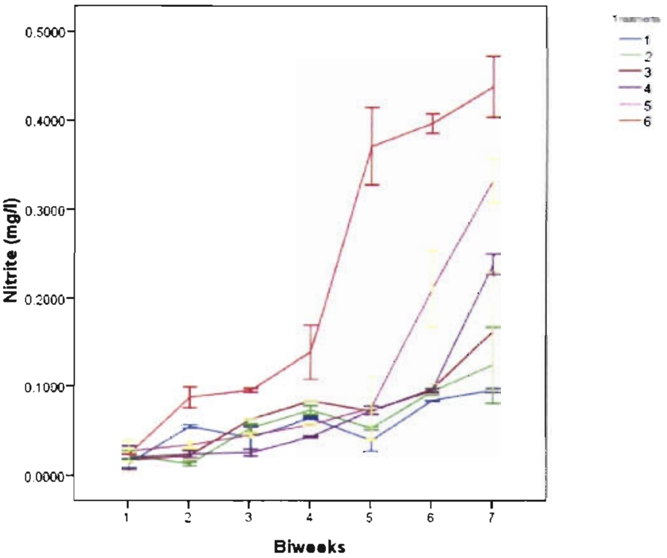

Figure 6.8 Concentrations of Nitrite-Nitrogen in water column at different biweeks. Error bars represent standard deviation

Table 6.2 Concentrations of parameters in the water column of different treatments*

Parameters	T1	T2	T3	T4	T5	T6
Temperature (^{0}C)	$29.06^{a}\pm0.30$	$29.6^{a}\pm0.30$	$29.07^{a}\pm0.30$	$29.08^{a}\pm0.27$	$29.07^{a}\pm0.29$	$29.99^{b}\pm0.33$
Salinity (ppt)	$24.52^{b}\pm0.51$	$24.29^{ab}\pm0.46$	$24.05^{a}\pm0.50$	$24.14^{a}\pm0.57$	$23.95^{a}\pm0.58$	$24.05^{a}\pm0.63$
pH	$7.57^{d}\pm0.42$	$7.38^{c}\pm0.27$	$6.47^{a}\pm0.43$	$6.50^{a}\pm0.53$	$6.70^{b}\pm0.57$	$6.80^{b}\pm0.46$
DO (mg/l)	$7.87^{b}\pm0.33$	$7.88^{b}\pm0.32$	$7.87^{b}\pm0.33$	$7.77^{b}\pm0.42$	$7.51^{a}\pm0.39$	$7.53^{a}\pm0.5$
TAN (mg/l)	$0.07^{a}\pm0.008$	$0.023^{c}\pm0.009$	$0.20^{b}\pm0.009$	$0.037^{c}\pm0.024$	$0.033^{d}\pm0.027$	$0.047^{f}\pm0.035$
NO_2^--N (mg/l)	$0.056^{a}\pm0.03$	$0.061^{a}\pm0.04$	$0.073^{b}\pm0.05$	$0.074^{b}\pm0.07$	$0.111^{c}\pm0.11$	$0.221^{d}\pm0.16$
NO_3^-- N (mg/l)	$2.023^{a}\pm1.52$	$2.698^{b}\pm2.16$	$2.903b^{c}\pm2.04$	$2.726^{b}\pm1.87$	$3.019^{cd}\pm2.09$	$3.218^{d}\pm2.11$
PO_4^{3-}-P (mg/l)	$0.429^{d}\pm0.54$	$0.152^{b}\pm0.13$	$0.144^{b}\pm0.13$	$0.247^{c}\pm0.27$	$0.126^{b}\pm0.13$	$0.072^{a}\pm0.07$
Chl-*a* (mg/l)	$0.61^{c}\pm0.30$	$0.60^{c}\pm0.29$	$0.57^{d}\pm0.30$	$0.40^{b}\pm0.06$	$0.44^{c}\pm0.10$	$0.30^{a}\pm0.03$
THB (CFU)	$17.89^{c}\pm9.21$	$17b^{c}\pm7.61$	$16^{abc}\pm5.63$	$12.44^{a}\pm1.51$	$13.11^{ab}\pm1.83$	$13.78^{abc}\pm1.99$

(*) Means within a row not sharing a letter are significantly different ($p<0.05$; Tukey HSD)

The addition of Pearlspot was observed to cause significant ($p>0.05$) reduction in NO_2^--N concentration. The mean nitrite (NO_2^--N) was highest in P40 ($0.221^d \pm 0.16$mg/l) followed by P32 ($0.111^c \pm 0.11$mg/l) among non-bioturbated treatments, and the differences were statistically significant ($p<0.05$; Fig. 6.8). However, the bioturbated treatments showed significantly lower concentrations than non bioturbated ones and the lowest recorded concentration was 0.056 mg/l (P24+BT) followed by 0.061mg/l (P32+BT), but they were not statistically significant ($p>0.05$). Treatments P24 and P40+BT exhibited a moderate NO_2^--N concentration and were not statistically significant ($p>0.05$).

The mean NO_3^-.N concentration of the different treatments fluctuated between 3.1 and 3.6 mg/l (Fig. 6.9). The lowest NO_3^--N concentrations were recorded by P24+BT ($2.023^a \pm 1.52$mg/l) and the highest was $3.218^d \pm 2.11$mg/l (P40). In treatments P32+BT, P40+BT and P24 the differences were not statistically significant ($p>0.05$), but the values obtained were higher than that in P24+BT, while P32 showed a moderate concentration of $3.09^{cd} \pm 2.09$mg/l. Mean phosphate values were lower in non-bioturbated ponds compared to bioturbated treatments and were statistically significant ($p<0.05$). The variation in PO_4^{3-}-P concentration in time was highly significant ($p<0.05$) and highest values were recorded was 0.49 mg/l in P24+BT treatment (Fig. 6.10).

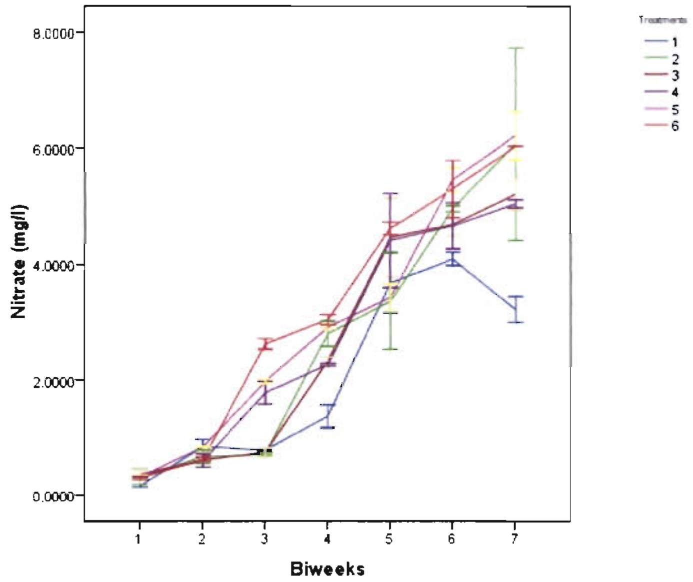

Figure 6.9 Concentrations of Nitrate-Nitrogen in water column at different biweeks. Error bars represent standard deviation

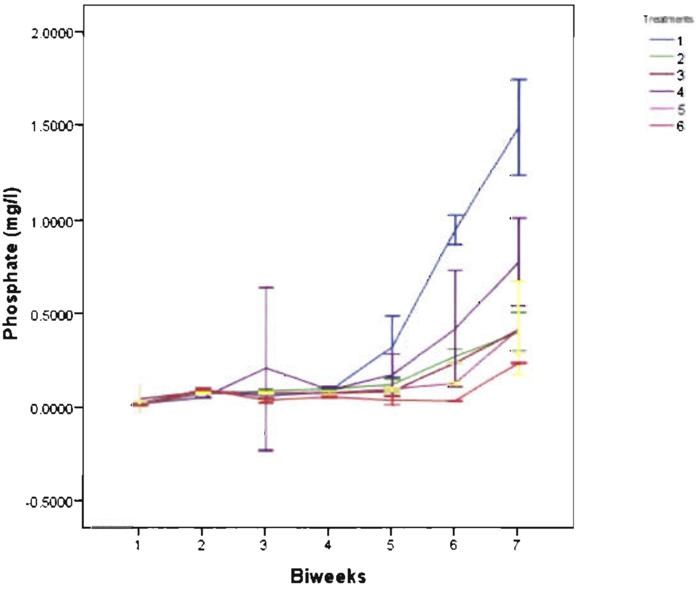

Figure 6.10 Concentrations of Phosphate-Phosphorous in water column at different biweeks. Error bars represent standard deviation

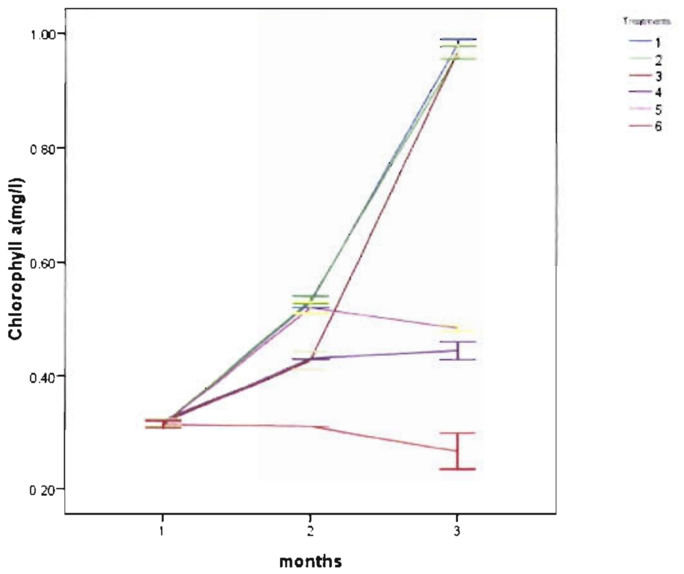

Figure 6.11 **Concentrations of Chlorophyll-*a* in water column at different months. Error bars represent standard deviation**

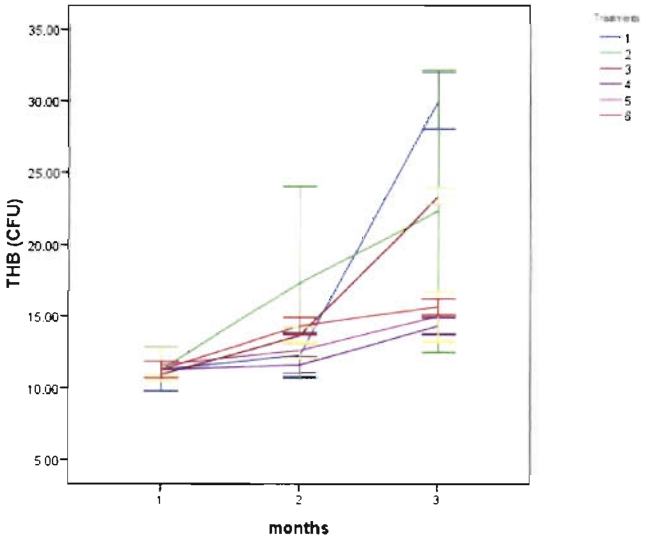

Figure 6.12 **Total Heterotrophic Bacterial counts in the tank bottom soil at different months. Error bars represent standard deviation**

The addition of Pearlspot and dietary protein level had effect on the chl-*a* concentration ($p<0.05$). Moreover, the observations on monthly chl-*a* concentration during the rearing period have shown significant variation ($p<0.05$; Fig. 6.11). Pearlspot addition was observed to increase the THB count ($p<0.05$). The THB count in the water column increased over time in T1-P24+BT ($17.89^{c}\pm9.21$; Fig. 6.12), while it was $13.78^{abc}\pm1.99$ in T5 (P40).

6.3.3 Shrimp growth and yield parameters

The results of growth, survival and production are shown in table 6.3. Significantly higher weight of 13.93-16.82 g was observed in shrimps fed on all protein percentage along with bioturbation.

Table 6.3 Growth parameters of shrimp in different treatments *

Treatments	Average weight (g)	Survival%	Yield (g/tank)
T1 (P24+E)	$16.82^{d}\pm1.73$	$85^{b}\pm14.11$	$518.50^{c}\pm115.06$
T2 (P32+E)	$13.93^{bcd}\pm0.45$	$81.67^{ab}\pm9.07$	$407.86^{abc}\pm33.41$
T3 (P40+E)	$14.62^{cd}\pm3.57$	$93.67^{b}\pm10.97$	$495.91^{bc}\pm156.85$
T4 (P24)	$6.89^{d}\pm1.75$	$80.67^{ab}\pm5.51$	$186.92^{a}\pm43.13$
T5 (P32)	$7.89^{bc}\pm2.84$	$80.67^{ab}\pm5.51$	$231.30^{ab}\pm90.51$
T6 (P40)	$9.80^{abc}\pm2.78$	$52^{a}\pm11.14$	$189.67^{a}\pm95.11$

(*) Means within a column not sharing a letter are significantly different ($p<0.05$; Tukey HSD)

Table 6.4 Growth parameters of Pearlspot in different treatments *

Treatments	Average weight (g)	Survival%	Yield (g/tank)
T1 (P24+E)	$29^{a}\pm0.58$	$70.83^{a}\pm4.17$	$538.67^{a}\pm47.93$
T2 (P32+E)	$32.33^{bc}\pm1.00$	$79.17^{a}\pm b2.41$	$561.00^{a}\pm35.08$
T3 (P40+E)	$32.33^{b}\pm0.58$	$80.56^{b}\pm4.17$	$614.00^{a}\pm23.58$

(*) Means within a column not sharing a letter are significantly different ($p<0.05$; Tukey HSD)

However, no significant differences were observed in 32 and 40% crude protein provided treatments without bioturbation. Individual shrimp weight gain was higher ($p<0.05$) in Pearlspot added treatments compared to T6 (P40) without resuspension and bioturbation (Fig 6.13).

The results of bioturbation experiments conducted in *P. monodon* indicated that all the treatments with bioturbation had a higher survival percentage (more than 80%) and were not significantly different ($p>0.05$; Fig. 6.14). Treatments without bioturbation had shown a survival of 80.67% in P32 and 77% in P24. But lowest survival was recorded with a protein percentage of 40 ($52^{a}\pm11.14$). Pearlspot addition and dietary protein level had a significant effect on net shrimp yield (Fig. 6.15). Higher net shrimp yield ($p<0.05$) was recorded in treatment P24+BT (518g/tank) compared to that in treatment P24 (186.92g/tank), while in treatments P40 (189.67g/tank) and P40+BT (495.91g/tank) moderate yields were obtained. The net yield in P32 and P32+BT was $231.30^{ab}\pm90.51$ and $407.86^{abc}\pm 33.41$ respectively. Growth parameters of Pearlspot are mentioned in table 6.4.

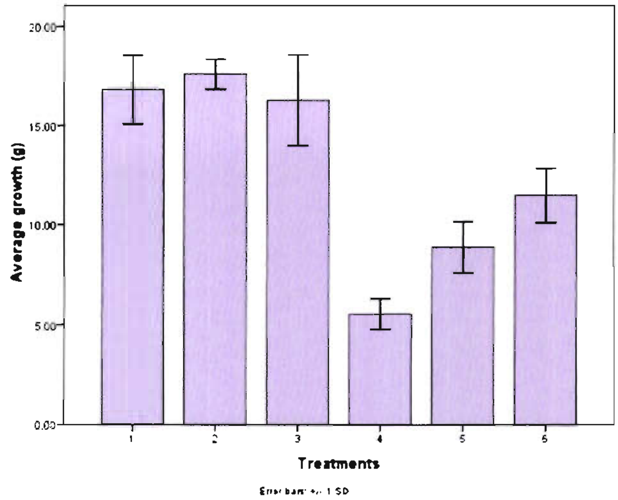

Figure 6.13 Average growth of shrimp. Error bars represent standard deviation

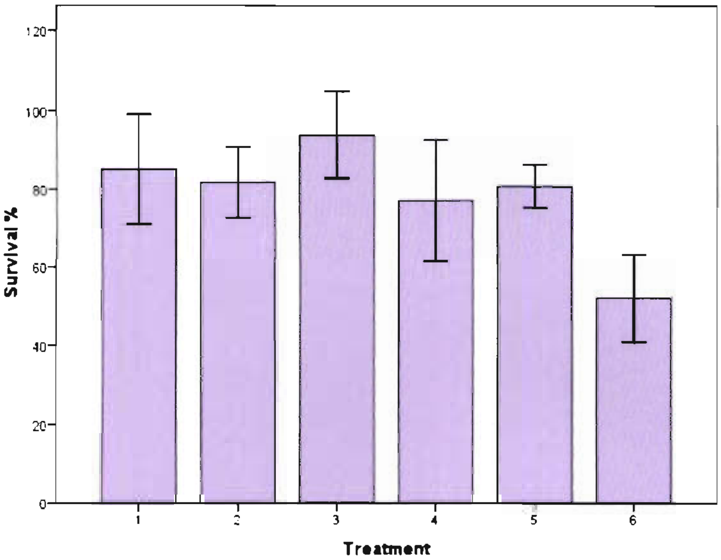

Figure 6.14 Survival % of shrimp. Error bars represent standard deviation

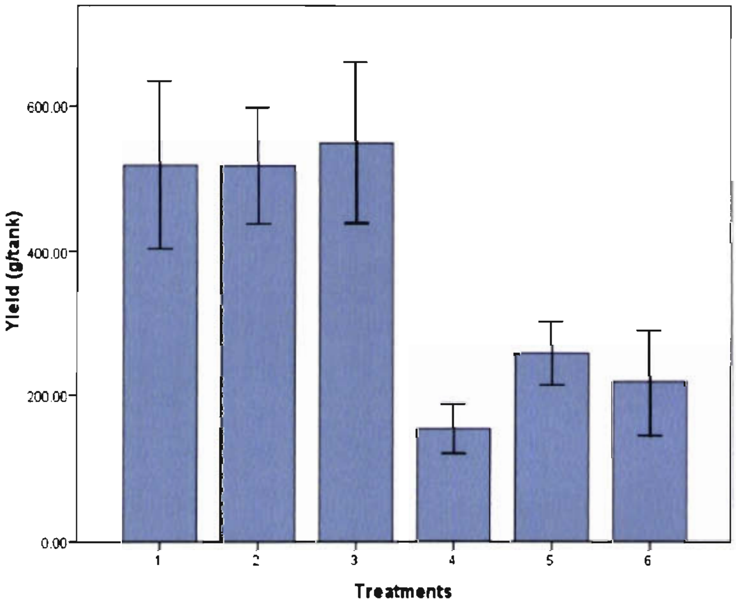

Figure 6.15 Yield of shrimp. Error bars represent standard deviation

Table 6.5 Economic analysis

Cost (IRS.) and economic analysis of Non-bioturbated system of *Penaeus monodon* (40% dietary protein) *Vs* Bioturbated system of *Penaeus monodon* (24% dietary protein) (per m2)

Particulars	Quantity	Rate (IRS.)	Treatments	
			P40	P24 + Btn
Variable cost				
Pond preparation	1 man half days	400.00	200.00	200.00
equipments			138.00	138.00
fertilizer			100.00	100.00
Shrimp seed	110	0.3	27.50	27.50
Pearlspot seed	60	5.00	0.00	300.00
Shrimp feed (40% protein diet)	17.5	64.5	1128.75	0.00
Shrimp feed (24% protein diet)	17.5	25.0	0.00	437.50
Power cost	1080 units	1.65	1782.00	1782.00
Harvest cost (IRS. kg^{-1})	1/2 man days	400.00	200.00	200.00
Fuel cost	2L	44.11	88.22	88.22
Total variable cost			**3664.47**	**3273.22**
Fixed costs				
Interest (5%)			750.00	750.00
Depreciation (5%)			750.00	750.00
Total fixed costs			**1,500.00**	**1,500.00**
Production				
Total shrimp yield (g m^{2-1})			189.67	518.50
Price of shrimp (IRS. g^{-1})			25.00	28.00
production of shrimp			4741.75	14,518.00
Total Pearlspot yield (g m^{2-1})			0.00	538.67
Price of Pearlspot (IRS. g-^{1})			0.00	22.00
Production of Pearlspot			0.00	11,850.74
Total			**4741.75**	**26,368.74**
Economic analysis				
Total production costs			5,164.47	4,773.22
Gross return (IRS.)			4741.75	26,368.74
Net profit (IRS.)			**-422.72**	**21,595.92**
Benefit / cost ratio			**-0.08**	**4.52**

6.4 Discussion

Feed is one of the most important and costly component of the shrimp aquaculture. The protein levels in practical feeds for most of the penaeid shrimp species is in the range of 35-50%. A higher dietary protein requirement of 35-61% was reported by many authors (Lee, 1971; Alava and Lim, 1983; Bautista, 1986; Shiau et al., 1991; Bages and Solane, 1981; Shiau and Chou, 1991; Chen, 1993). However, supplemental feeding not only increases the costs of production but also degrades pond water (Boyd, 1990) and sediment quality. Thus dietary feed protein optimization plays an important role in governing the feed conversion efficiency and production as well as minimizing pond bottom and water quality deterioration. Application of formulated feed constitutes a main input of nitrogen to the semi intensive pond. Considerable quantities of feed related nitrogenous waste is excreted into the system during shrimp aquaculture (Burford and Williams, 2001). Feed loss has long been cited as a major contributor to waste generation. Almost 75% feed nitrogen not retained in the biomass of shrimp can be recovered in the pond sediment (Hargreaves, 1998). Nitrogenous waste accumulates at the pond bottom from the uneaten feed, excreta and other fish waste in the form of organic matter. Hargreaves (1998) has opined that the incorporation of benthivorous fish would favour internal nutrient recycling. The present experiment was conducted to analyze the effect of Pearlspot addition on reducing the dietary protein levels of *P. monodon* without compromising the growth and production, by utilizing their bioturbation (Joyni et al., 2011). Treatments having different protein levels (24%, 32% and 40%), with and without bioturbation were used to assess the effect of bioturbation in the growth and production of shrimp through the enhanced recycling of nutrients. The sediment and water quality parameters were analyzed. The sediment and water column have a significant role in the superior growth and production of shrimp, especially considering their benthic nature.

6.4.1 Nutrients in sediment column

Among the sediment quality parameters analyzed in the present study, nutrient levels were found at higher concentrations in non-bioturbated control tanks than bioturbated tanks. Excretion of nitrogenous compounds by culture organism and microbial decomposition of organic matter due to food leftovers are the major sources of ammonia, nitrates, nitrites, phosphates and other inorganic substances (Neori et al., 1989; Hall et al., 1992).Under anaerobic conditions, micro organisms are able to use nitrite and nitrate as electron accepters instead of oxygen to decompose organic matter this process release toxic metabolites like ammonia and NO_2^--N that are potentially toxic to fish (Blackburn, 1987). Higher TAN and NO_2^--N concentrations were observed in the treatments without bioturbation as the dietary protein levels were increased. Increased ammonia concentration with increasing dietary protein percentages were reported by Li and Lovell (1992). In the bioturbated treatments concentrations of TAN and NO_2^--N decreased considerably. This can be attributed to the bioturbation activity of Pearlspot (Joyni et al., 2011) and its ability to resuspend the sediment. Similar result of reduced levels of ammonia and nitrite achieved by the incorporation of a bioturbating fish was reported by Avinimelech and Ritvo, 2003. Among the bioturbated treatments the lowest nitrogenous matter was observed in 24% protein added treatment. Nitrogen excretion concentration of low protein feed tended to be less than that of high protein, suggesting a better assimilation of protein into shrimp biomass, with better feed (Yaemsooksawat et al., 2009). The reduction in the protein levels in practical feeds makes the feed more cost effective without any adverse effects on growth and production of shrimp. The maximum concentrations of TAN and NO_2^--N were observed in treatment P40, where the accumulation of organic matter was higher due to high protein excretion and feed losses. The higher concentration of organic matter accumulation in the sediment requires large quantities of dissolved oxygen for their degradation, finally leading to anoxic conditions. At a depth of only a few millimeters below the soil surface, the demand for dissolved oxygen by microorganisms will exceed the rate of diffusion of dissolved oxygen to that particular depth, and anaerobic conditions will develop. This in turn causes the accumulation of ammonia in the sediment column due to the anaerobic decomposition of organic matter. Another reason for accumulation of ammonia in the sediment column is restricted nitrification, because oxygen penetration into the sediment is a key factor regulating the nitrification process (Reddy and Patrick, 1984; Rysgaard et al., 1994). In the present experiment, TAN and NO_2^--N concentrations were found higher in non-bioturbated treatment than bioturbated treatments which indicated the accumulation of organic matter and the subsequent utilization of dissolved oxygen which produced anaerobic bottom in the culture system. Further, large quantities of feed used in culture systems also contribute to a considerable amount of the metabolites in the pond (Avnimelech and Lacher, 1979; Chiba, 1986), as in the treatment P40.

Ammonia may be toxic if allowed to accumulate, however ammonia toxicity in aquaculture pond is most likely expressed as the sub lethal reduction of fish growth or suppression of immuno competence than the acute toxicity leading to mortality (Hargreaves, 1998). Chen and Lei (1990) reported that the safe level of ammonia in the culture of juveniles of

P. monodon is 0.37mg/l and for the adolescents a concentration of 4.62 mg/l. Nitrite is another potentially toxic nitrogenous compound that may accumulate in fish culture ponds. Nitrite is released as an intermediate product during nitrification and denitrification. The toxicity of nitrite is expressed through the competitive binding of nitrite to hemoglobin forming methemoglobin, which does not have the capacity to carry oxygen (Hargreaves, 1998). The safe levels for juveniles and adolescents are respectively 0.38mg/l and 10.6 mg/l (Chen and Lei, 1990). The lower TAN and NO_2^--N concentrations recorded in bioturbated treatments is an indication of reduced accumulation of nitrogenous waste products at the pond bottom through active resuspension at regular time intervals. The lowest recorded values were found in treatment P24+BT, where the low protein diet may have resulted in excretion of decreased amounts of nitrogenous wastes, since ammonia is excreted mainly as the end product of protein catabolism (Walsh and Wright, 1995). Feed loss of relatively less protein feed contributed low quantity of protein to the system. Bioturbation actively resuspends nutrients back to the water column which does not allow the accumulation of nitrogen rich organic matter in sediment surface layers. In bioturbated sediments, particles are subjected to various uni- or omnidirectional displacements due to reworking activities of benthic infauna (Rhoads, 1974; Aller, 1982; Berkenbusch and Ashley, 1999; Francois et al., 2001). The low levels of ammonia in P32+BT and P40+BT than their corresponding non bioturbated counterparts proved that resuspension of sediments greatly improved nitrification by the increased availability of oxygen. Stirring up sediment has previously been shown to increase nitrification (Avnimelech et al., 1992). Addition of tilapia decreased the total nitrogen in the sediment due to fish driven oxygenation events (Torres-Beristain et al., 2006). The foraging behavior of different fishes is a critical factor affecting sediment disturbance and nutrient resuspension (Avnimelech et al., 1999; Rahman, 2006). Through the activity of fish the diffusion of nutrient from sediment to water column become faster and causes the levels of ammonia to decrease in sediment column. Ammonium may be oxidized to nitrate by nitrifying bacteria, and nitrate may be used by phytoplankton or denitrified by anaerobic microorganisms in the sediment.

6.4.2 Nutrients in water column

The nutrient concentration in water column showed temporal variation among the treatments. The values were highest in P40 treatment without bioturbation. Feeds and feeding practices have a dramatic impact on ammonia concentration in fish pond water (Hargreaves, 1998). In ponds, sediment remineralization of pond detritus is the dominant source of ammonium in the water column (Hargreaves, 1998; Burford and Longmore, 2001). The highest concentration of nutrients was invariably found in treatment with 40% protein feed. But in all the treatments with bioturbation, the concentrations of nutrients were found reduced. The positive effects of resuspending sediments on enhancing the release of nutrients by stocking benthivorous fish (Jana and Sahu, 1993; Cline et al., 1994) and the consequent recycling of these nutrients through food web have been highlighted in many studies. This process causes a decrease in the levels of nutrients in water column. Jimenez-Montealegre et al. (2002) reported that the addition of tilapia brought some oxygen to the bottom layers by their movements. It has been reported that as resuspension stimulates the N and P flow from sediment to the water column, these elements accumulated lesser in the bottom soil and compared to the water column in the tanks with common carp (Rahman et al., 2008). The observed lower level of nitrogenous compounds in the sediment of bioturbation based ponds was due to enhanced nitrification. The presence of benthivorous fish such as tilapia and mrigal is advantageous, as they channel sedimented organic matter into higher trophic levels through feeding activities and potentially reduce the loss of nutrients in burial or degassing processes, e.g. denitrification and methanogenesis (Riise and Roos, 1997). Nitrogen in soil organic matter may be mineralized to ammonia and recycled to the pond water. In the present study the levels of TAN were low in P24+BT and P32+BT than non bioturbated treatments. Nutrient flow can be increased by fish driven resuspension, which is especially important in semi-intensively managed ponds, where fish production depends nearly entirely on natural food availability (Rahman and Verdegem, 2007). Benthivorous fish influence the nutrient fluxes between water and sediment in several ways as they feed on benthic fauna or detritus (Schroeder, 1978; Zur, 1979, 1980). Mineralization of organic matter and consequent regeneration of nutrients at the sediment-water interface of aquaculture pond is important source of ammonia to the water column (Hargreaves, 1998). Greater resuspension may have resulted in a higher mineralization rate, creating highly eutrophic water with greater NO_3^--N, TAN, TN, PO_4^{3-}-P and TP concentrations in common carp tanks (Rahman et al., 2008). Holmer and Heilskov (2008) reported that tropical shrimp *Alpheus macellarius* stimulated the ammonium release and the nitrate uptake, suggesting that the bioturbation activity of alpheid shrimp had noticeable impact on the nutrient fluxes. An increased disturbance by fish potentially increased the oxygen penetration in the sediment and thus the diffusive distance of nitrate from the water to the anaerobic denitrification zone (Riise and Roos, 1997). Ammonium may be absorbed by phytoplankton, converted to organic nitrogen, and eventually transformed into nitrogen of fish or shrimp protein via the food web. In bioturbation based system, the close linkage between autotrophic and heterotrophic processes in culture system speed up nutrient cycling and positively influences the water quality. In non-bioturbated treatments, the levels were low in the initial stages due to the absence of bioturbating fish and were found to peak suddenly during the final stages of the culture. This is mainly due to the slow diffusion of ammonia during the initial stages of the experiment on account of lesser resuspension. Towards the end of the experiment the accumulated ammonia and nitrite enter to the water column at an elevated rate as toxicants and causes the deterioration of water column. Contrary to this, in bioturbated tanks, a more or less continuous resuspension and redeposition of particles and stirring of the surface sediments by Pearlspot is the dominant mechanism by which, the nutrients are rather uniformly transferred from the sediment column to overlying water throughout the culture period at an optimal level. Avnimelech et al. (1999) reported that resuspension by fish in aquaculture ponds was continuous disturbance mechanism. Another reason for reduced levels of nutrients can be the faster mineralization through nitrification and coupled denitrification and the efficient recycling of nutrients. Higher nitrogen and phosphorous availability in the water stimulated higher photosynthesis rates (Hepher et al., 1989; Poxton, 1991; Milstein and Svirsky, 1996). The levels of ammonium-Nitrogen production (Saha et al., 2001). Bioturbation plays two major roles in semi-intensive production of shrimp it improves fish nutrition through stimulation of natural food and enhances dissolved oxygen levels through photosynthesis, while ammonia levels are reduced through assimilation by phytoplankton (Boyd, 1990). As reported by Jimenez-Montealegre et al. (2002), tilapia driven movements and re-suspension increased the bottom dissolved oxygen availability which lead to better mineralization and stimulated the natural food web. The activities of Pearlspot (such as feed scavenging, mating and building nests on the pond bottom) appeared to agitate the whole water column and demonstrating similar results that were observed due the activities of tilapia in water column (Phan-Van et al., 2008). It is reported that the presence of tilapia improved the aerobic conditions in the water column where intense photosynthesis co-existed without significant adverse seasonal impacts (Phan-Van, 2006). The bioturbation activities of fish would also improve the vertical distribution of temperature and dissolved oxygen in the water column, especially in the lower water layers of stabilization ponds, which lead to better treatment efficiencies of pollutants. Recently, Rasmussen et al. (2005), using Rhodamine tracer, demonstrated that irrespective of flow rate, the presence of fish enhanced the mixing process, with the mixing time in tanks with fish being one-third that for tanks without fish. The in-tank dispersion coefficients and dispersion numbers differed significantly in the presence of fish. The results of the present study corroborates that by Rasmussen et al. (2005) in that fish or any active moving objects in the water, can generate mixing of the water column, thereby enhancing the dispersion/diffusion of dissolved substances throughout its environment. There is evidence that fish bioturbation can impact water layers as deep as the water-soil interface at the superficial layer of pond bottom. In bioturbated tanks the concentration of phosphate was found higher in water column compared to the non bioturbated one during the beginning of the culture period.

NO_3^--N levels were also found reduced in sediment, in tanks bioturbated with Pearlspot. One of the possible reasons for the reduced levels of nitrate is the increased nitrification process in the oxygenated layer of sediment. Although denitrification is an anaerobic process, it is largely dependent on oxygen concentration for the production of nitrate through nitrification (Hargreaves, 1998). Factors that stimulate nitrification like warm temperature, abundant oxygen will also stimulate denitrification. In aquatic sediments in which the nitrate concentration in the overlying water is low, the denitrification rate will be limited by the nitrification rate, which in turn is regulated by the depth of sediment oxygen penetration (Rysgaard et al., 1994; Jensen et al., 1994).

Biodisturbance by benthivorous fish facilitates the penetration of dissolved oxygen into pores of sediment, and this enhances the mineralization of organic P trapped in sediment. Furthermore, PO_4^{3-}-P trapped between sediment particles are liberated into the water when sediment particles are mixed up and re-suspended due to the activities of benthivorous fishes (Rahman et al., 2004). Fish bioturbation enables oxidation of sedimenting organic matter by resuspending it into aerobic upper water layers (Scheffer, 1997), enhances the release of phosphorous (Jana and Das, 1992; Jana and Sahu, 1993; Saha and Jana, 2003), changes the structure of soil particles and decreases the concentrations of total dissolved sulphide, soluble manganese, easily oxidized matter, and exchangeable ammonium at the superficial layer (Ritvo et al., 2004). Smith (1996) reported that in the marine and estuarine sediment, the interstitial phosphorous is found within the pore structure and released by stirring of the sediment.

6.4.3 Chlorophyll-*a*

Chl-*a* count was higher in treatments with bioturbation compared to non bioturbated counterparts. Thus, inspite of lower concentration of nitrogen and phosphorous in these treatments compared to the respective controls, indices of phytoplankton abundance did not decrease. Moreover, TAN and NO_2^--N in the treatments with bioturbation never exceeded the safe range indicating that, during the study period the deleterious nitrogenous waste was effectively removed by phytoplankton and microbial activity (Shilo and Rimon, 1982; Diab and Shilo, 1988). The increased and sustained nutrient influx to the water column through bioturbation (Yusoff et al., 2001) is believed to be responsible for the more stable plankton bloom (Cruz et al., 2008). Yusoff et al. (2001) observed that increase in availability of inorganic carbon in the water column resulted from bioturbation, which stimulated the bloom of green algae. However the frequent disturbance of the bottom sediment through bioturbation, directly suppressed the bloom of harmful blue-green algae by preventing the development of cyanobacterial mats on the sediment (Cruz et al., 2008).

The domination of the beneficial phytoplankton, specifically the green algae group is highly beneficial in shrimp culture system. The predominance of green algae resulted in enhanced levels of dissolved oxygen, reduced ammonia and carbon dioxide, more stable pH, and suppression of cyanobacterial blooms (Burford, 1997). The presence of green algae also plays an important role in reducing the proliferation of potentially pathogenic organisms by outcompeting them for space and nourishment in addition to the improvement of water quality (Cruz et al., 2008). Many beneficial phytoplankton species are now known to have antimicrobial activities against pathogens (Ram et al., 1981). In the present investigation Chl-*a* concentration increased with the progress in rearing, and at the end of the culture, chl-*a* reached as high as 808 mg/l which was higher than the range reported previously for shrimp ponds (Lin, 1986; Martin et al., 1998).

The nutrient released by herbivorous zooplankton has long been recognized as an important source of nutrients to phytoplankton (Goldman et al., 1979; Lehman and Sandgren, 1985), the importance of nutrients released by fish is still being debated. Some investigators concluded that the absolute amount of phosphorous regenerated by fish is unimportant relative to other sources (Nakashima and Leggett, 1980), while others stressed that the magnitude of nutrients released by detritivorous fish and the response of phytoplankton in terms of biomass production and composition may be important (Barbrand et al., 1990; Kraft 1992; Schindler et al., 1993). This release can increase the total nutrient content of the water column and thus can be regarded as a kind of new nutrient that is fundamentally different from nutrients recycled by pelagic-feeding fish or zooplankton (Dugdale and Goering, 1967; Caraco et al., 1992).

Moreover, in the present study, Chl-*a* concentration remained relatively high in all bioturbated treatments from third month of the rearing till the end of the culture, indicating that the system never became nutrient limiting, and thus, in turn, sustained high phytoplankton biomass. Seemingly, dissolved nutrients together with the high light intensity, and warm temperature supported active growth of phytoplankton; which helped to condition the water quality in the tank by the production of oxygen and uptake of dissolved nutrients (Krom and Neori, 1989). Phytoplankton assimilates the nitrogen and phosphorous formed in the pond water; the phytoplankton is then consumed by the living aquatics, which include shrimp (Zhang et al., 1989). Phytoplankton uptake of dissolved inorganic nitrogen from the water column is the primary pathway of nitrogen removal in the aquaculture ponds (Hargreaves, 1998). By feeding on benthic organic detritus and then releasing a portion of the consumed nutrients as excreta, detritivorous fish may provide an important source of nutrients to stimulate phytoplankton growth (Schaus et al., 1997; Moore et al., 2004). In shrimp culture systems, phytoplankton and bacteria play a crucial role in the processing of nitrogenous wastes (Shilo and Rimon, 1982; Diab and Shilo, 1988). Microorganisms mineralize organic matter and return it into the trophic chain as nutrients for phytoplankton consumption (Lalli and Parsons, 1997). In the present study, the contribution of the phytoplankton to the natural food was significantly high among the treatments as indicated by the elevated and sustained chl-*a* concentration throughout the culture period.

6.4.4 Microorganisms

The THB count was found to high in both sediment and water column. The 24% protein feed added treatment with bioturbation showed $61.44^{d}\pm20.95$ x10^{-3}CFU for sediment and $17.89^{c}\pm9.21$ x10^{-3}CFU for water. It suggested that bioturbation brought about by burrowing, bioirrigation, and other basic biological activities influenced a suite of physical, chemical and microbiological processes. It increased the sediment-water interface area for increased sediment oxygen uptake (Svensson and Leonardson, 1996; Hansen et al., 1997; Heilskov and Holmer, 2001; Lewandowski and Hupfer, 2005; Nogaro et al., 2008) and improved transport of other terminal electron acceptors (e.g. NO_3^--N) into the sediment (Andersson et al., 1988; Kristensen and Mikkelsen, 2003; Andersen et al., 2006), and created more hospitable ecological niche for heterotrophic and phosphate solubilizing bacterial consortia (Harper et al., 1999; Cuny et al., 2007). Benthic animals alter the physicochemical characteristics of the sediment as well as production and abundance of bacterial and diversity of infauna (Van de Bund et al., 1994; Widdicombe and Austen, 1998). The increased bacterial load inturn led to higher decomposition rates releasing inorganic nutrients which further stimulated bacterial development (Avnimelech et al., 1989). Enhanced microbial mineralization activities result in more efficient regeneration of nutrients in general, and phosphorous in particular (Heilskov and Holmer, 2001; Banta and Andersen, 2003). Through the activities of the heterotrophic decomposers, nitrogen and phosphorous are recycled effectively to stimulate primary production in all the culture systems (Abraham et al., 2004). The present study revealed higher levels of microorganisms in bioturbated tanks than non-bioturbated tanks. Oxygen penetration (Svensson and Leonardson, 1996; Wang et al., 2001; Heilskov and Holmer, 2001; Fonseca et al., 2003; De Haas et al., 2005) increased the oxygenated area of the sediment-water interface and stimulated microbial growth rates. The augmentation of bacteria reduced soil organic matter by faster decomposition and improved dissolved oxygen concentration and removal of ammonia (Hargreaves, 1998), thus maintaining good condition of health of shrimps and finally higher growth rate at reduced protein percentages.

For bacteria, the finer particles resuspended from the sediment offer large surfaces for attachment and subsequent decomposition (Tjetjen, 1979). The heterotrophic bacterial population utilizes the inorganic N to synthesize bacterial protein and new cells (single cell protein) and it may be utilized as a food source by carp, tilapia (Schroeder, 1987; Beveridge et al., 1989; Rahmatulla and Beveridge, 1993) or shrimp (Burford et al., 2004), thus lowering the demand for supplemental feed protein (Avnimelech, 1999). The utilization of microbial protein depends on the ability of the target animal to harvest the bacteria and its ability to digest and utilize the microbial protein (Avnimelech, 1999). The higher yield reported in the carbohydrate added treatments in the study by Hari et al. (2004) showed that *P. monodon* can well utilize the additional protein derived from the increased bacterial biomass as a result of carbohydrate addition. In the present study also the growth of *P. monodon* is attributable to the utilization of microbial protein derived as a result of resuspension mediated supply of nutrients. Resuspension process mediates the absence of aerobic bacteria which increases due to the increased availability of oxygen and nitrogen uptake through production of microbial protein. This promoted nitrogen uptake by bacterial growth decreases the ammonium concentration more rapidly than that during nitrification (Hargreaves, 2006). Yingst and Rhoads (1980) reported bioturbation by invertebrates potentially affected bacterial communities by modifying the availability of nutrients and O_2 to micro-organisms. The immobilization of ammonium by heterotrophic bacteria occurs much more rapidly because the growth rate and microbial biomass yield per unit substrate of heterotrophs are higher than that of nitrifying bacteria by a factor of 10 (Hargreaves, 2006). Thus the reduction in TAN and NO_2^--N levels observed in the bioturbated treatments of the present study can be attributed to the increased THB population, which immobilized TAN for the synthesis of new bacterial cells (Hari et al., 2004).

6.4.5 Growth and production

Growth and production of shrimps were found to be highest in 24% dietary protein supplied treatments with bioturbation, when compared to the higher dietary protein (40%) exclusive of bioturbation. Enhanced growth rate of shrimps in 24% dietary protein supplied treatments with bioturbation can be elucidated by the following reasons. Lower TAN in the sediment positively influenced the food intake and health of the shrimps (Avnimelech and Ritvo, 2003). Furthermore, bioturbation enhances natural food in culture system through various ways like spore resuspension, phyto and zooplankton development through utilization of resuspended nutrients, providing good environment for diatoms, benthos, macroinvertebrates etc. *P. monodon* is one of the most omnivorous of shrimp species, and has been shown to consume plant and detrital material (Bombeo-Tuburan et al., 1993; Focken et al., 1998). Generally, the presence of diatoms in shrimp ponds is a desirable condition (Phillips, 1984; Shigueno, 1985). The contribution of diatoms to shrimp nutrition may stem from their high concentrations of polyunsaturated acids (Orcutt and Patterson, 1974) which are known to promote growth in crustaceans (Kanazawa et al., 1979). Benthos forms one of the major food sources for shrimp in intensive and semi intensive culture, in addition to artificial feed (Rubright et al., 1981; Allan and Maguire, 1994). Bioturbation activities affect mostly fine sediment particles that are single or joined into aggregates and are organic or non organic. This particulate material plays an important role in the functioning of ecosystem while forming detrital bacterial complexes and providing substrate for protozoan and bacterial colonies. Nutrients are therefore likely to be transferred through the food chain via primary producers to secondary consumers; this link provides a possible mechanism for regenerated nutrients to be assimilated through the food web. This material becomes available to a wide range of larger organisms, including fish and shrimp (Trott et al., 2004). Shrimp rely on natural foods even in fed ponds. Studies using stable isotope have shown that the natural biota can contribute to shrimps nutrition in less intensive systems (Parker and Anderson, 1989; Cam et al., 1991; Burford, 2000). Focken et al. (1998) observed 71% natural food in stomachs of *P. monodon* in semi-intensively managed fed pond. Four to ten percent of nitrogen enriched natural biota was retained by shrimp within 48 h (Burford, 2000). Shrimp culture in the present investigation, being a closed system, witnessed an accumulation of nutrients over time; this might be considered an advantage of a closed system;- since the higher amounts of nitrogen and phosphorous added on within the system could support the growth of natural food organisms contributing ultimately to the shrimp growth. This view is in agreement with the study of Allan et al. (1995) who observed that prawns grow faster in prepared ponds where meiofauna is abundant.

In the non-bioturbated treatments, especially in higher dietary protein applied treatment, growth of *P. monodon* was found to be decreased. This can be attributed to the anaerobic condition developed inside the non-bioturbated tanks. In the treatments with elevated dietary protein percentage (40%), the buildup of nitrogenous waste enhanced due to the accumulation of food residues and faecal matter with higher protein percentage. Accumulation of waste products in farm sediments usually results in dramatic changes in sediment chemistry, and also affects the macro and meio benthic communities in the sediment (Brown et al., 1987; Karakassis et al., 1999). The complete disappearance of macrozoobenthos upon the development of anaerobiosis in the sediment has been observed by Zur, 1980. The unconsumed feed, fecal matter and dead organisms were decomposed by bacteria, hence increasing the amount of toxic compounds such as hydrogen sulphide and which might have affected the composition and abundance of benthos (Rubright et al., 1981; Pillay, 1992; Allan et al., 1995).

Less availability of natural food may substantially affect the growth of shrimp. Besides, reduced sediments retarded growth in P40. Decreased grazing and consequential slow growth of tilapia were reported by Avnimelech and Zohar (1986). Removal of reduced sediment has been recommended to prevent growth-retarding conditions in intensive penaeid culture (Wyban and Sweeney, 1989). Growth suppression of *Penaeus chinensis* with increasing levels of ammonia was reported at a threshold response at 0.6 mg/l TAN (Yang, 1990). The growth of macroinvertebrates during the final part of the culture period has been prevented by the accumulation of the toxic compounds in the sediment in non-bioturbated treatments.

Survival rate was found significantly ($p<0.05$) lower in P40 (52%) than all other treatments. Martin et al. (2007) reported a significantly lower survival of shrimp fed on higher protein diet (40%) compared to those on fed lower protein feeds (25-35%). This observation is likely to be associated with higher concentration of total ammonia nitrogen in pond water when protein levels were increased (Yaemsooksawat et al., 2009). The excessive accumulation of waste or organic matter makes the water environment unfavorable for shrimp growth. It is also reported that higher TAN concentrations lead to lower survival of shrimp in aquaculture ponds. The weight gain in bioturbated tanks was higher than control treatments. Higher survival rates obtained in all treatments with bioturbation showed that water and sediment quality were favorable for *P. monodon* cultivation (Hariati et al., 1996), and suggest that differences in production are related to food quality and food availability. The reduction of protein level from 40% to 24% did not reduce weight gain significantly in shrimp pond with bioturbation indicating that constant and continuous resuspension of nutrients by Pearlspot enhanced protein utilization.

Natural food contains high-quality protein, which ranges between 55%-60% on dry weight basis. In contrast, the supplemental feed used in the present study contained low dietary protein (24%). Therefore, a combination of high-quality protein from natural food with increased utilization of supplemental feed protein recovered in sediment probably led to a protein-sparing action, thus the higher shrimp growth was observed in P24+BT compared to P40. Growth of *Fenneropenaeus merguensis* did not change when diets in which dietary protein content was reduced from 51-34%, while increasing the non protein nutrients (Sedgwick, 1979). The nitrogenous waste generated from low protein feed tended to be less than that of high protein feed, suggesting a better assimilation of protein into shrimp biomass. Feed utilization was better in the group fed lower protein feed.

It is a very amazing fact that the yield of *p. monodon* was higher in low protein levels with bioturbation. Results from this study demonstrate that feed inputs were adequately assimilated in bioturbated tanks with low protein levels, allowing the shrimp to achieve consistently higher yields. For ponds in which fish yields are based on primary productivity, promotion of nutrient recycling within the pond will maximize nutrient availability for phytoplankton (Hargreaves, 1998). Reduction in feed loss and improvements in nutrient conversion efficiency reduce the FCR (Reid et al., 2009). This fact would more significantly demonstrate the double benefit of integrating fish to shrimp culture with waste water stabilization; not only is the shrimp/fish biomass increased but also the waste treatment efficiency enhanced (Phan-Van et al., 2008). The production capacity of shrimp ponds relies ultimately on factors maximizing primary productivity and minimizing nutrient losses (Alongi et al., 1999). The better performance of shrimp in bioturbated treatment was probably because of utilization of uneaten residues of feed and faeces from the accumulated sediment through recycling mediated by Pearlspot and the consequent development of healthier ambient conditions. The overall recycling efficiency of nutrients leads to three to four fold reuse of nutrients in the system (McLusky, 1999). The increase in gross and net yield of shrimp in bioturbated ponds was mainly be attributed to the increased survival rate, besides the individual growth. Addition of fish provided additional natural food along with improvements of environmental conditions through a range of ecological and biological processes. Gonzales (1988) estimated that natural pond biota contributed between 51 and 89% of the total food intake for juvenile (0.02 g/prawn) *P. monodon.* While the reason for decreased growth rate of shrimp in control ponds may be the higher concentrations of toxic substances in the pond bottom which impairs the ability of the shrimp to and effectively scavenge for the available natural food organisms in ponds. The net yield of shrimp was significantly higher with Pearlspot than without Pearlspot, indicating that inter-specific competition between Pearlspot and shrimp does not occur.

Observations such as higher growth rate and shrimp yield in the carbohydrate added ponds can be attributed to the low inorganic nitrogen levels (Wahab et al., 2003) and increased heterotrophic production (Avnimelech, 1999; Burford et al., 2003; Burford et al., 2004). Furthermore, fish bioturbation probably also affects other ecological aspects of the fish culture-integrated waste stabilization system such as the water quality reclamation process, nutrient recycling and variations of food web structure (Phan-Van et al., 2008). It is reported that the bioturbatory activities of tilapia can over ride the vertical distribution of water temperature in stabilization ponds to a depth of 1 m during the dry season. At the same time it can bring some DO downwards to the lower layers of the water column, improving aerobic conditions on the pond bottom (Phan-Van et al., 2008).

The realistic economic analysis (Table 6.5) performed in the study showed that Pearlspot added treatments with low protein feed substantially reduced the feed cost when compared to high protein feed. Feed data on economic analysis indicated that the bioturbated treatments (P24+BT, P32+BT and P40+BT) were more profitable than the non-bioturbated ones (P24, P32, and P40). The analysis also revealed that shrimp-Pearlspot polyculture with a stocking density of 6 shrimps and 4 Pearlspot/m^2 in bioturbation based culture system would prove to be a very profitable endeavour. These findings are supported by those of Liti et al. (2005), who reported higher positive returns in integrated than non-integrated treatments. Furthermore, the combined effect of high shrimp yield and higher market price for the shrimps increased the income and the net profit in the case of 24% feed added treatment with bioturbation when compared to the high protein feed of 40% without bioturbation.

6.5 Conclusion

The technology used in the present investigation is much simpler and works at low cost with easy management. Its economic efficiency is superior not only in reducing dietary protein levels, increasing yield, and survival rate and also in maintaining better water and sediment conditions both due to reduced protein levels and by the bioturbation activity. The bioturbated fish-mediated resuspension ponds contributed substantially in increasing the direct yield.

It is also characterized by high ecological efficiency in terms of utilization rate of input feed and nitrogen. Water purification is very important for closed culture and this is an exemplary of enhanced water quality. The Pearlspot resuspension ponds showed decreased concentrations of TAN, NO_2-$^-$

N, NO_3^--N and organic carbon to a great extent. The mechanisms of water purification of these ponds include resuspension and bioturbation via feeding and movements of the reared Pearlspot. Since the NO_2^--N and NO_3^--N concentrations increased pronouncedly at the end of the production cycle, the concentration of TAN was at a low level and at even undetectable levels near the production end. Therefore, the co-function of added Pearlspot in reduction of nutrient in sediment column with increased bioavailability (enhanced mostly nitrifiers) to target organism are clear.

Results of the study clearly demonstrate the effect of bioturbation in improving the utilization of input materials and in increasing profit from culture along with reduced dietary protein levels. The bioturbation technology provides a sustainable method to maintain water and sediment quality in aquaculture systems and moreover concomitantly fish feed is produced through the recycling of excreted nitrogen. Since the purchase of commercially prepared feed in fish culture has a share of 50% or more in the production costs, an effluent treatment technique that maintains water quality and simultaneously produces in situ fish feed has a large asset over other techniques (Crab et al., 2007). Furthermore the efficacy of the technique in retention of excess protein and excreted nitrogen and its conversion into harvestable products make this system more attractive because nitrogen retention and removal from sediment and water column occurs within the culture system without any external agent.

In summary, addition of Pearlspot (4 Pearlspot/m^2) benefited brackish water shrimp production (6 post larvae/ m^2) through reducing toxic inorganic nitrogenous compounds in water and sediment, enhancing the availability of plankton and benthic macro invertebrates thus reducing the protein percentage demand by shrimp for supplemental feed and improving survival, production and economic benefit gain by the utilization of recycled feed. The result of the present study could be useful in improving the sustainability of tiger shrimp farming in terms of ecological, social and financial benefits. Economic sustainability could still be further enhanced by conducting on-farm trials, and is subject to further research. It is suggested that this achievement be translated into mass production of fish/shrimp in vast coastal areas of the maritime states of India.

Shrimps and Pearlspot after harvest

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Chapter 7

SUMMARY AND RECOMMENDATIONS

A general introduction to the problems facing in the shrimp culture due to waste formation and its consequent environmental hazards and production problems of Giant tiger shrimp, *Penaeus monodon* is gives in **chapter 1**. Besides, the backgrounds of the study are also described. An exhaustive review of literature on studies carried-out so far on the bioturbation technology in general and its application in aquaculture, and the waste and nutrient recycling using finfishes are encompassed in this chapter. Bioturbation is an effective technology in shrimp farming to ameliorate waste and for nutrient recovery within the culture system for its effective utilization to enhance production and maintain ecological sustainability.

Chapter 2 presents the results of evaluation of the effect of bioturbation of four finfish species on the culture system of *Penaeus monodon* (Tiger prawn). The objective of the present work was to assess the potential of brackishwater finfish to improve bottom soil conditions and thereby increase the growth and production of *Penaeus monodon*. Experimental tanks with a bottom area of 6 m^2 were stocked with shrimp (6 shrimp/m^2) and finfish (3 fish/m^2), and fed with a commercial diet. Five treatments (four experimental and one control) were maintained in triplicate. The four finfish species selected for the experiments were *Etroplus suratensis* (pearlspot), *Mugil cephalus* (mullet), *Chanos chanos* (milk fish) and *Oreochromis mossambicus* (tilapia).

Pond bottom soil properties indicative of mixing and redox conditions were evaluated. Bottom soil cores were taken from the experimental tanks and sectioned to 0-1, 1-2, 2-3, and 3-7 cm layers. Concentrations of TOC, EOM, TAN, total dissolved sulfides, soluble manganese and soluble iron were determined as the indicators of anaerobic metabolites. Fluorescent stained sand particles (luminophores) were added to the bottom soil as bioturbation tracers. Results were analysed by two-way ANOVA and post hoc analysis were done following Tukey's HSD test using SPSS Version 18. Tracer analysis results indicated that particles were displaced down to 7 cm depth in tanks with tilapia. In Pearlspot and shrimp combination tanks, particles were displaced to depths of 3 cm, suggesting that the stirring activity of tilapia and Pearlspot was effective down to 7 and 3 cm, respectively. Evaluation of chemical parameters revealed a significant decrease in the concentrations of anaerobic metabolite indicators for all fish co-stocking, but particularly in Pearlspot and shrimp combination tanks. In addition, co-stocking, especially with Pearlspot, led to more intensive degradation of the organic matter and significant lowering of the EOM fraction. Assessment of growth parameters also revealed higher growth rate ($p<0.05$) and survival of shrimp (97 ± 4^{d}) in the Pearlspot-shrimp combination treatment. These results confirmed the effect of finfish on bottom soil bioturbation, especially with Pearlspot co-stocking. Two types of bioturbation were identified: 1) site specific digging and soil displacement in the case of tilapia, and 2) continuous stirring, re-suspension and resulting oxidation in the case of Pearlspot.

The third chapter deals with the effectiveness of fish driven bioturbation in the resuspension of nutrients, effect on water quality and utilization of resuspended nutrients in shrimp-fish aquaculture ponds. Five treatments (four experimental and one control) were maintained in triplicate. The four finfish species selected for the experiments were *Etroplus suratensis* (pearlspot), *Mugil cephalus* (mullet), *Chanos chanos* (milk fish) and *Oreochromis mossambicus* (tilapia). Results of sediment and water quality parameters were analysed by two-way ANOVA and growth parameters were analysed by one-way ANOVA. Post hoc analysis was done following Tukey's HSD test using SPSS Version 18.

In the absence of finfish, high concentrations of TOC, TAN and nitrite were found to accumulate in the soil and water column. TAN and NO_2^--N were decreased significantly in sediment with fish incorporation, particularly in the presence of Pearlspot due to the resuspension of the lighter organic rich particles up to the aerated water column. Concentrations of TAN and nitrite were also found to be less in water column in resuspended tanks due to the effective and timely recycling of nutrients. Growth, survival and total phytoplanktonic biomass were found high in all the tanks with fin fishes especially in Pearlspot co-stocked tanks due to the incorporation of recycled nutrients/nitrogenous waste products into the biomass of culturing organisms and plankton.

Bioturbating effects of various size groups of *Etroplus suratensis* in shrimp-fish brackishwater aquaculture ponds were attempted and the results are presented in **chapter 4.** The bioturbation efficiency of various size groups of Pearlspot was found to be entirely different. Five treatments (four experimental and one control) were maintained in triplicate. In treatment 1, fishes having a size of 1-4 cm were used. In treatment 2, the size used was 5-8 cm. In treatment 3, 9-12 cm sized fishes were used and in treatment 4, 13-16 cm sized fishes were used. Shrimp alone was used as a control in treatment 5.The results showed that the fish having a size range of 5-8 cm was found to be more efficient in resuspension of nutrients and reduction of toxic metabolites in sediment and water column. Tracer analysis results revealed that the fluorescent particles were found in deeper layers and more mixing was found in 5-8 cm sized organisms incorporated treatments. Hence most efficient and suitable stage of Pearlspot along with *P. monodon* to produce maximum bioturbation is found to be the fish having a size range of 5-8 cm.

Optimization of the stocking density of *Etroplus suratensis* in the shrimp-fish brackishwater aquaculture ponds was attempted and the results are given in **chapter 5**. In this regard the experiment was carried out with the objective to delineate the best stocking density suitable for the shrimp-fish brackishwater aquaculture systems. The stocking densities maintained were 3, 4, 5 and 6 Pearlspot/m^2 and the control treatment was maintained without fish. Among the various treatments, the better results for reduction of waste and maximum production was obtained with an initial stocking density of 4 fishes/m^2, which was found to be an optimal in shrimp-fish brackishwater aquaculture system.

Evaluation of the protein requirement of *Penaeus monodon* in shrimp-fish brackishwater ponds provided with bioturbation was studied and the results are given in **chapter 6**. The dietary protein percentage of treatments with and without *Etroplus suratensis* were 24, 32 & 40%. The objective of the study was to evaluate the effect of bioturbation mediated resuspension of Pearlspot in reducing the dietary protein percentage of shrimp in Shrimp-Pearlspot brackishwater culture system.

The results revealed that sediment parameters like TAN, NO_2^--N and TOC were significantly lower in treatments with Pearlspot having 24 and

32% of protein. Water quality parameters were found better in treatments with Pearlspot as a component and showing a significant reduction in TAN & NO_2^--N especially in treatment fed with 24% protein diet. Treatments without Pearlspot have significantly higher values for all toxic metabolites in sediment and nitrogenous waste products in water as compared to the treatments with Pearlspot. In all the treatments with the Pearlspot, there was a remarkable increase in the production and growth of *P. monodon* when compared to the treatments without Pearlspot and was almost independent of the percentage of dietary protein supplied. But in treatments without Pearlspot, the production and growth of *P. monodon* were different and their increases were directly proportional with protein percentage. In conclusion, the treatment provided with lower protein of 24% with bioturbation have shown better results in both shrimp production and survival when compared to the 40% dietary protein supplied treatment without bioturbation.

The salient findings of the present study are summarized in **chapter** 7. This is followed by the references cited in the thesis and list of publications originated from the present study.

Based on the results of the present study, the following recommendations are made:

- Bioturbation with Pearlspot is a excellent natural and internal control against the metabolic toxins developed inside the aquaculture systems, which is also ecologically sustainable.
- Pearlspot is a very good resuspender of nutrients temporarily clogged in the upper sediment column. No external materials are required to ameliorate the waste in bioturbation systems.

- Zero water exchange system can be used to reduce the risk of disease transmission which happens through the water exchange. The wastes produced inside the tank are effectively used inside the tank itself. The risk of discharging waste water of culture system to the surrounding environment is evaded.
- Nitrogenous waste produced in the culture system can be converted into the harvestable biomass by the bioturbation.
- Bioturbation can be used to increase the growth of *Peneaus monodon* via nutrient recycling.
- Bioturbation assists in the production of natural food in culture system and helps to decrease protein percentage of feed of *Peneaus monodon* to 24% compared to the normal 40%. This contributes highly to the economic profit of farmer and is economically sustainable.
- Clear and practical indicators to characterize the effect of bioturbation on bottom soil properties, as demonstrated in the present work, may help and ease further studies in this direction.
- Economic sustainability could still further be enhanced by conducting on-farm trials, and is subject to further research.
- It is suggested that this achievement be translated into mass production of fish/shrimp in vast coastal areas of the maritime states of India.
- Further studies are recommended to ensure the effect of bioturbation in various fresh water polyculture systems as *E. suratensis* can survive both in fresh water and brackish water.

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