CHAPTER **2. Farming of *Macrobrachium rosenbergii***

CHAPTER. 2. FARMING OF *MACROBRACHIUM ROSENBERGII*

2.1. INTRODUCTION

Aquaculture as an innovative step to economic strategy of any country hardly needs emphasis, it includes all aspects of production of fresh, brackish and marine water aquaculture organisms in captivity either some or all stages of their life cycle up to marketable sizes. Lone Khalid (1988) has described “Aquaculture as an underwater agriculture”. FAO (2002), “Farming of aquatic organisms including fish, molluscans, aquatic plants and crustaceans. Farming implies some form of interventions in the rearing process to enhance production such as regular stocking, feeding, protection from predators etc”. Aquaculture has been defined as “the rearing of aquatic organisms under controlled or semi-controlled conditions”. “Aquaculture has also been stated the same objective of agriculture and stock breeding mainly to increase the production by all possible means than the natural wild level of production”. Further, the new encyclopedia Britannica, has defined aquaculture on “The exploitation of a natural or artificial body of water for the growth of food products, such as fish, mollusks, crustaceans and seaweed”.

The freshwater prawn *Macrobrachium rosenbergii* was the first species to be studied extensively and farmed commercially which is indigenous in the whole of South and South East Asian countries as well as Northern Oceania and Western Pacific islands. It has been transferred extensively within its natural range and has been introduced into many countries where its farming has been established (Nandlal and Pickering, 2005). Among all the freshwater prawn, scampi is the largest known species and grows to a maximum size of 750 gm.

2.1.1. Systemic position of *Macrobrachium rosenbergii*- Nomenclature (New, 2002)

The giant freshwater prawn, *Macrobrachium rosenbergii* (De Man, 1879), was one of the first species of the *Macrobrachium* genus. The family tree of the giant freshwater prawn is:

Kingdom	Animalia - animals
Phylum	Arthropoda - (insects, spiders, crustaceans etc.)
Subphylum	Crustacea - (crabs, lobsters, shrimp, etc.)
Class	Malacostraca
Order	Decapoda
Sub-order	Pleocyemata
Family	Palaemonidae
Subfamily	Palaemoninae
Genus	*Macrobrachium*
Species	*rosenbergii* (DeMan, 1879)
English	- Giant River prawn
Tamil	- Mandai erral
Telegu	- Neela kanta royyi
Malayalam	- Atta kondu
Commercial name	- Scampi

In India, the major commercial species are *M. rosenbergii* and *M. malcomsonii.* In India, the giant freshwater prawn inhabits most of the tidal rivers, along both the coasts, in the west coast from Indus delta to Malabar Coast and on the east coast from the South to Mahanadi delta and also in deltanic Bengals (Chandrasekaran and Sharma, 1997).

Chandrasekaran and Sharma (1997) and Mariappan (2000) reports on the prawn fisheries in the longest rivers such as Godavari, Krishna, Ganga, Hooghly and Cauvery.

2.1.2. Economic value

Farmed production of freshwater prawns in India increased from 7140 mt in 1999-2000 (financial year April 1999 to March 2000) to 30450mt, valued at Rs.584.6 crores (US $1.3 million) in 2002 -2003 (MPEDA, 2001 and 2004). The production of farmed marine shrimp in India in 2002-2003 is estimated at 115320 mt, valued at Rs. 3346.96 crores (US $7.438 Million) (Kutty, 2005). The average farmed prawn production for India is 879 kg/ha/year, which is higher than the corresponding value for shrimp (758 kg/ha/year) (MPEDA, 2004).

Prawns are considered a delicacy and therefore have a huge demand in domestic and foreign markets. They are well known as a high protein, low fat food and containing protein (16 – 19%), total lipid (1.0 – 2.2%) and gross energy 85 -90 kcal (Gopalan *et al.*, 2000). These are exported to as many as 70 countries all over the world (Bhojan, 2003). For example India alone carried about INR 6100 crore, earned by the export of prawn and shrimps. Giant freshwater prawn alone contributed INR 444.1 crore (US$ 925 millions) (Murthy and Thanuja, 2005).

2.1.2.1. Status of freshwater prawn farming

Giant freshwater prawn farming is a major contributor to global aquaculture, both in terms of quantity and value. By 1987, global production of farmed *M. rosenbergii* was estimated to be around 27,000 tons per annum (New, 1990). In 1993, the overall production was 17,164 tons, worth US$ 116,799,000 and in 2005 it reached 205,033 tons with a net value of US$ 896,263,000 (FAO, 2007). China is by far the leading producer with over 128,300 tons. Vietnam was the second in the list with 28,000 tons. However, even if a very modest expansion of 10 percent year^{-1} occurs, global farmed production of *M. rosenbergii* will have significantly exceeded 400,000 tons by 2010.

2.2. REVIEW OF LITERATURE

2.2.1. History of freshwater *M. rosenbergii* aquaculture systems

Primitive methods of prawn culture had been practiced for centuries in some Asian countries, especially in India and Bangladesh. Ling (1962) first studied the life cycle and Fujimura (1966) demonstrated mass rearing techniques of juveniles of *M. rosenbergii*. The first juvenile prawns were produced in June, 1962 and within a period of about ten years, worldwide interest in freshwater prawn culture was generated and research and development started practically in all the Asian and far Eastern countries (Ling and Costello, 1976). Burma, Bangladesh, India, Indonesia, Kampuchea, Malaysia, Philippines, Sri Lanka, Thailand and Vietnam have their own native stock. However, Australia, England, Hawaii, Japan and Singapore obtained their initial stock from Malaysia, Israel, Taiwan, Province of China, imported stocks from Thailand (Ling and Costello, 1976).

After successful commercial rearing of *M. rosenbergii* larva by Ling (1969), many attempts have been made towards the production of seeds with artificial, live and microencapsulated diets (Nelson *et al.,* 1977a; Kanazawa *et al.,* 1982; Ang *et al.,* 1987; Rao 1994; Dhert and Sorgeloos, 1995; Alam *et al.,* 1996; Murthy, 1998; Tiwari and Sahu, 1999; Debabani *et al.,* 2001; Kovalenko *et al*., 2002; Das *et al*., 2007; Velu and Munuswamy, 2007 and Nhan *et al*., 2010). Attempts were also made to improve the nutritional quality of *M. rosenbergii* with different feeds with probiotics. In this regard, contributions were made by Ravishankar and Keshavanath (1988); Sheen and D'Abramo (1991); Das *et al*. (1996); Harparz (1997); Tidwell *et al.* (1998a, 1999, 2000); Gonzalez-

Pena *et al*. (2002); Du and Niu (2003); Felix and Sudharsan (2004); Giap *et al*. (2005); Lan *et al*. (2006) and Gupta *et al*. (2007).

2.2.2. Soil composition

For the farming of *M. rosenbergii*, good pond soil and availability of water are two important prerequisites. The soil texture and compounds such as organic carbon, pH and nutrients varies in different ponds. The soil characteristics of aquaculture ponds are reported by Boyd (1995), Chien (1992), Clifford (1992) and Hattori (1994). Smith (1996) studied soil texture, trace metals, total nitrogen and phosphorus in Australian freshwater prawn farming area. Mukhophadyay *et al*. (1997) reported 20.2% clay, 13.5% silt and 66.5% sand in low saline *M. rosenbergii* culture ponds. Paulraj (1999) studied the accumulation of organic matter, nitrogen and phosphorus content of the soil during fourth month of culture. Correia *et al*. (2003) studied the effect of pond on natural food availability and growth of *M. rosenbergii*. Wudtisin and Boyd (2006) recorded 36.2 %, 63.6 % and 0.2 % of clay, silt and sand, respectively, in freshwater prawn ponds.

2.2.3. Soil bacteria

Bacteria are the most dominant group of microorganisms in the soil and its population depends upon physical, chemical and biological conditions of the soil (Alexander, 1983). Fonseka (1990) and Smith (1996) studied the total microbial population in freshwater prawn farm in Sri Lanka and Australia, respectively. Abraham *et al.* (1995) studied occurrence of luminescent bacteria in penaeid shrimp grow-out system. Nabi *et al*. (1996) reported bacterial colony forming units of *P. chinensis* and *M. rosenbergii* in summer and winter monsoon periods.

Pond water, pond sediments and receiving water are compared with bacterial load in *P. monodon* by Tendencia and de la Pena (2001). Phatarpekar *et al.* (2002) investigated the quantitative and qualitative of bacterial flora associated with larval rearing. Sahul Hameed *et al*. (2003) studied the bacterial load in larvae and post larvae of *M. rosenbergii* and their resistance to various antibiotics used in aquaculture. Abraham and Palaniappan (2004) studied luminous bacterial load and its species composition in commercial penaeid shrimp farms. Total heterotrophic bacterial counts were studied in modified extensive and semi – intensive shrimp culture system in west Bengal, India by Abraham *et al*. (2004). Lalitha and Surendran (2004) studied water canal sediment bacterial samples of *M. rosenbergii* culture pond. Jeyasekaran *et al.* (2006) explained bacteriological quality of *P. indicus*, Tuticorin, Tamilnadu, India. Jana *et al.* (2007) studied bacterial changes in water quality attribute to the polyculture of *M. rosenbergii*. The diverse range of bacteria has been examined as probiotics for possible use in aquaculture by Kesarcodi-Watson *et al*. (2008).

2.2.4. Soil fungi

Fungi are viable in a variety of habitat. Mostly all aquatic fungi are heterotrophicin nature, require free oxygen, some grow in acid as well as in alkaline waters, at pH values of 3.0 - 9.5. Manoharachary and Ramarao (1983) isolated 47 fungal species from two freshwater mud ponds in Hyderabad. Singh and Wadhwani (1989) reported the fungal population of flowing and stagnant aquatic habitats.

The diversity of freshwater fungi has been investigated in different ecological habitats such as, ponds, streams, lakes, reservoirs and rivers. Okaemo and Olufemi (1997) and Okpokwasilli *et al.* (1998) studied fungal species in tilapia and catfish pond, respectively, in Nigeria. Further, Girivasan *et al.* (1998) and Koilraj *et al*. (1999) isolated fungal species in peat soil and caves, respectively. Paulraj (2002) isolated 12 and 7 genera of mesophilic and thermophilic fungi in the culture ponds of *M. rosenbergii* respectively.

2.2.5. Phytoplankton

Phytoplankton forms the basic link in the food chain of fishes in aquatic biotope. Many investigators have studied phytoplankton and their role in the freshwater ponds (Sharma and Saini, 1991). MacLean *et al.* (1994) reported the phosphorous and nitrogen are the most important limiting nutrients for the phytoplankton growth. Akpan and Okafor (1997) reported the diversity and abundance of plankton in response to fertilization with fresh piggery and poultry dungs in two freshwater ponds in Nigeria. Johnston *et al*. (2002) studied water quality parameters and plankton diversities in shrimp pond in Mekong delta of Vietnam. The effects of different densities of caged *Oreochromis niloticus*, on water quality, phytoplankton populations, were evaluated in *M. rosenbergii*, production ponds (Danaher *et al*., 2007). Rahman *et al*. (2008) reported water quality, nutrient accumulation and plankton and benthos were high in common carp pond.

2.2.6. Zooplankton

Zooplankton forms an important link in the transfer of energy from producers to carnivores. The consumption of zooplankton by juveniles of shrimp in aquaculture ponds was suggested in earlier studies (Moriarty and Barklay, 1981 and Chen and Chen, 1992). Further, Boyd (1990) and Sharma and Saini (1991) reported peak zooplankton population coinciding with or followed by the maximum release of nutrients. Hills *et al*. (1997) and Tidwell *et al*. (1995, 1997a) showed that the benthic fauna of *M. rosenbergii* culture pond plays a major role in determining its production. Further, Martinez-Cordova *et al*. (1997) reported the presence of larvae of copepods, polychaetes and ostracods in the digestive tract of *P. vannamei*. The importance of live feed in aquaculture was reviewed by many investigators (Neelakantan *et al.* 1988 and Lavens and Sorgeloos, 1996). Sivakumar and Altaff (2001) reported diversity of rotifers species in Dharmapuri district in Tamilnadu, India. The copepod and cladocerans population of fifty freshwater bodies are studied in Dharmapuri district by Sivakumar and Altaff (2004). The abundance and species composition of zooplankton assemblage were examined in *P. monodon* pond in Australia by Preston *et al*. (2003).

The diversity of copepods of Muttukadu and Ennore of Chennai coast were recorded 33 species from March 2002 to February 2003, in Chennai, Tamilnadu, India by Altaff *et al*. (2004). Sivakumar and Altaff (2005) reported diversity of zooplankton in around Chennai, India. Coman *et al*. (2006) studied zooplankton and epibenthic invertebrates of *P. monodon* pond, for entire growth period. The largest fractions of N and P inputs accumulating in fish, phytoplankton and zooplankton observed in common carp ponds with artificial feed to fertilize in rohu, *Labeo rohita* pond (Rahman *et al*., 2008).

2.2.7. Physical and chemical parameters of the culture pond water:

2.2.7.1. Water Quality

Probiotics was used to supply beneficial bacteria strains to rearing water that will help to increase microbial sp. composition in the environment and to improve water quality. Probiotics is considered to be able to make cultured animals healthier by inhibiting the growth of pathogenic bacteria in the same habitat. This led to new strategy for prevention of disease outbreaks and improvement of seed quality (Maeda, 1999, Oanh *et al*., 2000, Verschuere *et al*., 2000 and Rengpipat *et al*., 2003). The major source of nutrients in intensive prawn culture pond is feed. Excess feed, fecal matter and other metabolites become available in large quantities for the growth of algae and micro-organisms. Sudden increase or decrease of algal and microbial population can cause drastic changes in water quality parameters, which inturn affect the growth of the cultivable animal.

2.2.7.2. Water depth

Average pond water depth and water movements are two important factors that can affect numerous aspects of pond environment. New and Singholka (1985) recommended 0.9 - 1m depth for freshwater prawn farming. A water depth of 40.7 - 110 cm was reported by Rao (1986b) in *M. malcolmsonii* culture pond. Recently D'Abramo *et al*. (2000) studied water volume and exchange rate in *M. rosenbergii* juvenile growth. Apart from this, water quality with different manures (MacLean *et al.,* 1994), range of salinity (Ignatius and Thampy, 1991), trace metals (Abdennour *et al.,* 2000) and probiotics (Wang *et al.,* 2005) of shrimp/prawn culture ponds were also reported.

2.2.7.3. Turbidity and Transparency

Water transparency refers to the quantity of suspended material which interfers with light penetration in the water column of about 35 - 45 cm is considered to be normal. If it is below 30 cm it indicates high phytoplankton density whereas above 45 cm indicates low phytoplankton density. High turbidity raises temperature and enhances the dissolved oxygen stratification in ponds and also clogs the gills of the prawn. Rao (1986b) recorded a turbidity level of 24.2 to 38.7 cm in *M. malcolmsonii* culture pond. Sadek and Moreau (2000) recorded 35 ± 15 cm and 37 ± 10 cm, in *M. rosenbergii* and *P. semisulcatus* culture ponds respectively. Wang *et al*. (2005) reported the final transparency of the commercial probiotic treated ponds of *P. vannameii* was higher (26.5± 2.1cm) than (6.7± 0.9cm) the control ones 56.5 ± 8.6 cm of transparency were recorded in *M. rosenbergii* cage culture by Cuvin –Aralar *et al*. (2007).

2.2.7.4. Temperature

Normally a temperature range of 25 - 30°C supports normal growth of prawns/shrimps, (Thang, 1995). Optimum growth of *M. rosenbergii* at 27°C temperature was reported and recommended (Smith and Sandifer, 1982; New and Singholka, 1985). Ra'anan *et al.* (1990) observed mortality of *M. rosenbergii* in the culture pond of Israel at 19°C. Temperature ranges from 24.8 to 29°C for *M. malcolmsonii* (Rao, 1986b), 19 to 33°C for *M. rosenbergii* (Langer and Somalingam, 1993) and 27.7 - 29.5°C for *P. monodon* (Hariati *et al.* 1996) was suggested. Hoq *et al.* (1996) and Sadek and Moreau (2000) recorded the temperature ranges from 27.5 to 30.5°C, and 26 ± 2.9°C in *M. rosenbergii* polyculture system, respectively. Herrera *et al*. (1998) and Manush *et al*. (2004) reported critical thermal maxima and minima in post larvae and juvenile of *M. rosenbergii* acclimated at 10 to 41.6°C. VanArnum *et al*. (2001) reported influence of temperature in food consumption of ***M.*** *nipponense* increased with temperature ranges from 10 - 30°C. Niu *et al*. (2003) studied the effect of temperature on feed, consumption, growth and metabolism in *M. rosenbergii*. Wang *et al.* (2005) recorded the temperature of probiotic applied ponds of *P. vannamei* of about 22.2 to 34.8°C. 28.9 – 32.5 °C of temperature were recorded in low-cost diet experiment by Hossain and Paul (2007) in *M. rosenbergii*.

2.2.7.5. pH

Water pH is influenced by accumulation of carbon dioxide during night, which makes water pH to fall to its minimum, at dawn. According to New and Singholka (1985), fresh and marine water resources used for prawn hatchery should have pH ranges from 7.0 – 8.5.

Generally a pH range from 7.5 – 9.0 was reported in the monoculture ponds (Rao, 1986b; Durairaj *et al.*, 1992; Langer and Somalingam, 1993 and Vasudevappa *et al.*, 1998). However, pH range from 7.4 - 8.5 was reported in *M. rosenbergii* polyculture system (Hoq *et al.*, 1996; Hassan and Bandhopadhyay, 1997 and Sadek and Moreau, 2000). Straus *et al*. (1991) reported high pH caused mortality in *M. rosenbergii*. Cheng and Chen (2000) tested with four different pH levels at 28° C, different temperature levels at pH 7.5, different salinity levels of 7.5 – 7.8 at 28°C and 0.6% feeding rate in different temperature of 7.5 pH. Cheng and Chen (2002a) and Chen and Chen (2003) reported feeding rate was reduced in *M. rosenbergii* exposed to pH 6.8 and lower. High pH level decreased the last zoea stage of *M. rosenbergii* larval rearing (Mallasen and Valenti, 2005). Hossain and Paul (2007) recorded 6.4 -7.7 pH in different low-cost feeding regimes in their experiment.

2.2.7.6. Dissolved oxygen

Oxygen concentration in pond water exhibits a diurnal pattern with maximum occurrence during the peak of photosynthesis in the afternoon, minimum occurring at dawn due to high respiration. Low dissolved oxygen in ponds is one of the most common causes of mortality and growth reduction in prawn. Dissolved oxygen range from 2.5 to 10.2 ppm was recorded in *M. rosenbergii* culture ponds (Durairaj *et al.,* 1992; Raman, 1992; Langer and Somalingam, 1993 and Vasudevappa *et al.*, 1998). Chen and Kou (1996) studied oxygen consumption related to temperature and excretion. Taylor *et al*. (2002) studied the oxygen consumption which inturn influence the metabolic rate in *M. rosenbergii* post larvae. Cheng *et al.* (2003b) investigated the physiological parameters of *M. rosenbergii* exposed to various dissolved oxygen (DO) levels. Manush *et al*. (2004) tested externa and internal maxima and minima rate of oxygen consumption in adult *M. rosenbergii.* Lan *et al*. (2006) recorded 3.48 ± 0.24 to 4.45 ± 0.46 mg/L of DO in rotational rice-prawn system at different density in *M. rosenbergii* culture. Hossain and Paul (2007) reported 8.1-8.5 ppm of dissolved oxygen in different low cost feeding regime in *M. rosenbergii*.

2.2.7.7. Alkalinity and hardness

In general, alkalinity ranged between 30 - 300 mg/l in freshwater aquaculture system (Chand, 1999c and Adhikari, 2000). Alkalinity is closely related to hardness. Bartlett and Enkarlin (1983) and New and Singholka (1985) reported hardness level of 40 - 150 ppm as normal for *M. rosenbergii* culture. However, occurrence of high total hardness was studied in many cultures ponds of *M. rosenbergii* (Vasquez *et al.*, 1989; Brown *et al.*, 1991; Sadek and Gayer, 1995; Hoq *et al.*, 1996 and Sadek and Moreau, 2000). Further, Rao (1986b) recorded an alkalinity range from 141 to 194 ppm in *M. malcolmsonii* culture ponds. Variations in the levels of the total alkalinity of *M. rosenbergii* culture ponds were reported (Durairaj *et al.*, 1992; Langer and Somalingam, 1993; Sadek and Gayer, 1995 and Hassan and Bandhopadhyay, 1997). Sadek and Moreau (2000) recorded a total alkalinity range between 200 - 220 mg/l in *M. rosenbergii* polyculture system.

2.2.7.8. Ammonia and nitrite

Ammonia is released by excretion and bacterial decomposition. Ammonia is more toxic in alkaline water. When ammonia is combined with nitrite, it affects the animal growth. At the same time total ammonia is toxic when dissolved oxygen concentration is low. Chen *et al.* (1990) studied the effect of ammonia and nitrite on *P. monodon* juveniles. Straus *et al*. (1991) recorded high ammonia value cause mortality in *M. rosenbergii* culture pond. Chen and Kou (1996) revealed that Ammonia- N excretion and total nitrogen excretion decreased with increased pH level in *M. rosenbergii*. Higher level of ammonia was reported in many *M. rosenbergii* culture ponds (Langer and Somalingam, 1993; Vasudevappa *et al.*, 1998 and Sadek and Moreau, 2000). The ammonia in water decreases the virulence of *Enterococcus* and reduces the immune resistance of *M* *rosenbergii* (Cheng and Chen, 2002b). Higher level of ammonia decreased the last zoea stage of *M. rosenbergii* (Mallasen and Valenti, 2005). Ammonia- nitrogen toxicity studies was carried by Naqvi *et al*. (2007) in *M. rosenbergii* juveniles in culture pond.

2.2.8. Farming

The giant freshwater prawn can be cultured alone or in polyculture with fishes in pond. In tropical areas, prawns were cultured and selectively harvested on a regular basis from continuous production ponds (Fujimura, 1974), whereas in temperate areas ponds were drained and harvest was carried out (Smith *et al.*, 1976). Many attempts were carried out to increase the production and yield of *M. rosenbergii* with different stocking densities, water and soil qualities (Boyd, 1990; Clifford, 1992; Langer and Somalingam, 1993; Sadek and Gayar, 1995 and Adams and Thompson, 2011), artificial and natural feed (Rao, 1992, 1994, 1998; Alam *et al.*, 1993a, b and Murthy, 1998) in mono and polyculture systems (Buck *et al.*, 1981; D'Abramo *et al*. 1986; Karplus *et al.,* 1986a; MacLean *et al.*, 1994; Sadek and Moreau, 1998, 2000, Tidwell *et al*., 2004a, b; Kutty, 2005; Uddin *et al*., 2007; Kunda *et al*., 2008 and Uddin *et al*., 2008).

2.2.8.1. Monoculture

Monoculture of *M. rosenbergii* was carried out by many investigators in different stocking densities (Brody *et al.*, 1980; Limpadanai and Tansakul, 1980 and Smith and Sandifer, 1982). Further, Subramanyam (1984) obtained 700 kg/ha of *M. rosenbergii* in 180 days with a stocking density of 30,000/ha. Karplus *et al*. (1986a) reported 1 - 4 nos/m^2 in *M. rosenbergii* culture pond. Similar type of experiment was carried out by Stwalley and Beasley (1987), Wang *et al*. (1987) and D'Abramo *et al*. (1989). Raman (1992) recommended a stocking density of 1.7 - 2.5 nos/m^2 for *M. rosenbergii* culture. A stocking density of 6 and 12 nos/m^2 was experimented by Sadek and Gayer (1995), Sadek and Moreau (1998) and Tidwell *et al.*, (1999).

Giap *et al*. (2005) studied the effect of different feeding and fertilization regimes on rice and *M*. *rosenbergii* production. 28.8 ± 3.2 to 49.8 ± 2.8 percentage of survival was achieved at different stocking density of *M*. *rosenbergii* (1, 2, 3, and 4 PL m^2) using pellet and pellet with snail meat by Lan *et al*. (2006). Three experimental diets were formulated using fish meal, meat and bone meal, mustard oilcake, sesame meal and rice bran in different combinations in *M*. *rosenbergii* (Hossain and Paul, 2007) and Moraes-Valenti and Valenti (2007) investigated the feeding habit, growth, and production and population structure of *M*. *amazonicum*. Singh *et al*. (2008) studied the growth performance and Schwantes *et al*. (2009) reviewed the production performance of *M*. *rosenbergii* in Thailand. Nhan *et al*. (2010) investigate the effects of larval stocking density and feeding regime on larval growth, survival and larval quality of *M*. *rosenbergii*.

2.2.8.2. Polyculture

The advantage of polyculture of prawn over monoculture is that it requires less prawn seed and feed, therefore lower investment. Wohlfarth *et al*. (1985) cultured *M. rosenbergii* with common carp, Chinese carp and tilapia. Same types of experiments were conducted by Costa-Pierce *et al*. (1987) using silver carp, grass carp and gray mullet in *M. rosenbergii* polyculture system. Karplus *et al*. (1990) obtained 81% survival in *M. rosenbergii* polyculture with carps. Similar study was also conducted by Granados *et al.* (1991), Langer and Somalingam (1993) and Hoq *et al.* (1996).

Further, Sadek and Moreau (1996) reported *M. rosenbergii*, *Oreochromis niloticus, Cyprinus carpio* culture with different stocking densities. Ahmed *et al.* (1996) stated that polyculture of *M. rosenbergii* will not affect the production of carps. Hassan and Bandhopadhyay (1997) revealed fish and prawn culture practices in rain fed coastal soils.

Sarangi *et al*. (1998) studied the possibility of polyculture of *M. rosenbergii* in Andaman Island. The production potential of *M. rosenbergii* in polyculture system was described by Nair and Murthy (1999) and Sadek and Moreau (2000). Garcia-Peerez *et al*. (2000) compared the yield of monoculture and polyculture production of *M. rosenbergii* in Pueto Rico.

Hossain and Islam (2006) workout for optimized stocking density of *M. rosenbergii* with carps for 3 months in 10 experimental pond of 80 m^2. Optimized the stocking ratios of tilapia and freshwater prawn in periphyton based systems and compared tilapia monoculture and its polyculture with freshwater prawn by Uddin *et al*. (2006). Kunda *et al*. (2008) and Wahah *et al*. (2008) reported stocking density of *M. rosenbergii* with small fish 'mola' *Amblypharyngodon mola* in rotational rice-fish/ prawn culture systems in Bangladesh. Mohanty (2009) also studied *M. rosenbergii* with carps in rice-field in India. Asaduzzaman *et al*. (2010) studied two carbohydrate sources compared in 40m^2 ponds stocked with *M. rosenbergii* juveniles, 20 *Orecochronis niloticus* and rohu, *Labeo rohita* in three different combinations.

2.2.9. Probiotics

Recently many workers proved probiotics as a better choice to incorporate in the feed and aquaculture environment. Suralikar (1996) reported the use of *Lactococcus lactis* subspecies *cremoris* as probiotic for *M. rosenbergii* post-larvae. Rengpipat *et al*. (1998) reported *P. monodon* larvae reared using the Bacillus-fortified *Artemia* probiotic as a feed. Himabindu (1998) observed that a significant growth rate was recorded when probiotic was fed to *M. rosenbergii* post-larvae. Gatesoupe (1999) clearly reviewed probiotic terminology applied in the aquatic environment and needs for further research. Oanh *et al.*

(2000) reported the effects of probiotics in the culture of post larvae of freshwater prawn *M. rosenbergii.*

The feeding with live bacteria can be an effective treatment for improving the growth in pond condition was reported by Rengpipat *et al*. (2000) in *Penaeus monodon.* Abidi (2003) reviewed probiotic application in Nellore district, where farmers using both water and feed probiotic in *M. rosenbergii* culture. Indulkar and Belsare (2003) examined 90 to 95 % survival of post-larvae of *M. rosenbergii* when administrated probiotic mixed diet. Vaseeharan and Ramasamy (2003) results indicated that probiotic treatment offers a promising alternative for the use of antibiotics in shrimp aquaculture. Gullian *et al*. (2004) and El-Dakar and Goher (2004) found the enhanced growth was generally obtained in shrimp fed diets with *B. subtilis* inclusion. Lin *et al*. (2004) used a probiotic strain (*Bacillus* sp.) in the culture of *Liptopenaeus vannamei.* Venkat *et al*. (2004) conducted a study of probiotics treatment in the post-larval diet of *M. rosenbergii* using *Lactobacillus acidophilus* and *L. sporogenes* for 60 days. Wang *et al*. (2005) tested the effectiveness of water quality, population density of bacteria and shrimp productions in ponds treated with commercial probiotics in *P. vannamei.*

Farzanfar (2006) reviewed the use of probiotics in shrimp aquaculture. Vine *et al*. (2006) also reviewed probiotics in marine larviculture. A significant improvement of growth of *M. rosenbergii* occurred when the feed included a mixed culture of *Bacillus* strain, (Deeseenthum *et al*., 2007). Keysami *et al*. (2007) studied by using *Bacillus subtitles* bacterium, on larval growth and development rate of *M. rosenbergii* in Malaysian hatchery. Wang *et al*. (2007a) analysed the diversity of bacteria in shrimp ponds. Decamp *et al*. (2008) reported the performance of *Bacillus* strains, using data from Asian and Latin American hatcheries with *P. monodon* and *Liptopenaeus vannamei*. Gatesoupe (2008) updated the importance of lactic acid bacteria and probiotic treatments in polyculture farming. Kesarcodi – Watson *et al*. (2008) reported the need, principles, mechanism of action and screening processes of probiotic application in aquaculture. Sahu *et al*. (2008) reported the selection of the potential probiotics, their importance and future perspectives in aquaculture industry. Zhang *et al.* (2008b) identified the potential probiotic in shrimp *F. chinensis.*

Saad *et al*. (2009) investigated the impact of adding probiotics (Biogen) in the diet of *M. rosenbergii* during the post larval growth. Sansawat and Thirabunyanon (2009) studied the characteristic activity and antagonistic ability of the novel probiotic strain of *B. subtilis* isolated from the gastro intestinal tract of *M. rosenbergii.* Qi *et al*. (2009) discussed mainly the application about species, effects, mechanism, problems and prospect of probiotics used in aquaculture in china. Though, several studies have shown that the probiotic concept has potential with aquaculture sector, much more work is still needed.

2.2.10. Aim of the study

The perusal of the literature indicates the importance of freshwater prawn culture and a number of factors governing the successful culture of *M. rosenbergii*. Earlier reports indicated a variation with regard to soil parameters, water parameters and plankton in different ponds. Stocking density, culture duration and harvest also showed variation in different places. Though, culture of *M. rosenbergii* was studied extensively in many countries like America, England, Australia, Bangladesh, Israel, Egypt, Brazil, Thailand, Taiwan, Philippines, Malaysia, China, etc. and also many parts of India, only a few reports are available from Tamilnadu (Durairaj and Uma Maheswari, 1991 and Durairaj *et al.*, 1992).

Further, it is evident that most of the probiotics are used for shrimp culture practices except Suralikar (1996), Himabindu (1998), Indulkar and Belsare (2003) and Venkat *et al*. (2004) reported larval rearing of *M. rosenbergii* in India, but no reports on farming trial. However, probiotic specific to freshwater conditions have not been developed, the commercial probiotics currently used in marine shrimp farms are from soil, water, intestine and terrestrial group. The effect of these bacteria or their spores in the environment or to the cultured animals has not so far been investigated in a comprehensive manner. In this concept the present study was carried out on detail, of intensive culture and growth of *M. rosenbergii* in two adjacent ponds along with soil, water and feed commercial probiotic applications.

2.3. MATERIALS AND METHODS

2.3.1. Pond location

The *M. rosenbergii* culture farm selected for the present study is situated at Vishnuvakkam 56 km away from Chennai, Tiruvallur District, Tamil Nadu, India. This farm consists of two ponds: control pond (fig.1) and probiotic experiment pond of 0.603 ha (length and width, 298 x 213 m) (fig.2). Depth of these ponds is about 1.5 m. Control pond is separated from probiotic experiment pond by a bund of 80 - 95 cm width. All the other three sides of the ponds also have bund of same width. These ponds are surrounded by agriculture field and are provided with a sluice gate measuring 2 x 1.5 m (length and width) in order to drain the water. There are three screens at the sluice gate with a mesh size of 0.5, 1.0 and 1.5 cm in order to prevent the escape of animals at the time of drainage of water. In addition to the sluice gate, two emergency pipes of 8 inch diameter with valves were also installed for letting out water during rainy seasons. In order to prevent cannibalism, shelter and hideouts (country tiles and coconut leaves) were

Fig.1. Control pond of *Macrobrachium rosenbergii* culture

Fig. 2 . Probiotic experimental pond


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Fig.3. Dewatered and dried pond

Fig .4. Ploughed pond


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provided at the bottom of the pond.

2.3.1.1. Pond preparation

As a first preparatory measure, the ponds were dewatered and dried. The soil surface was exposed to sunlight till it develops deep cracks (fig. 3). The ponds were then ploughed using a tractor to tilt the soil up to a depth of 10 - 15 cm (fig. 4). This was followed by the manual application of agricultural lime, (100 kg/ha) to each of the ponds in order to decompose the organic matter of the pond soil. Twenty four hours later, water was pumped to a height of 15 cm and allowed to stand for 48 hrs and at the same time 50kg of bleaching powder were applied to kill the microbes and fish eggs, thereafter it was drained. Subsequently to this, the ponds were filled with ground water pumped through two 15 HP motor from a bore-well. Filling up of water to a height of one meter was achieved by pumping water for two weeks.

2.3.1.2. Pond fertilization

After filling water, the ponds were fertilized with microbial mixture and inorganic fertilizers for a period of 10 days in order to provide nutrients for the growth of microbes, algae and zooplankton. First, microbial mixture [rice bran (15 kg), groundnut oilcake (5 kg), jaggery (1kg) and yeast (100 gm)] was concentrate applied to the ponds, whenever there is depletion of plankton bloom again this microbial mixture was diluted and apply to the ponds, when the animals are noticed in juvenile conditions. Simultaneously 10 kg/ha of superphosphate was applied, subsequently 5 kg/ha of urea were also applied. At the same time Soda mix [Composition of soda mix- Ca^{++}, Mg^{++}, Na^{+}, K^{+}, Cr and So2] (Mineral mix from C.P. Aquaculture (India) Pvt. Ltd., Chennai, Tamilnadu, India) were applied to the pond to improve the mineral level in pond water. Further, Super PS (C.P.) Aquaculture (India) Pvt. Ltd., Chennai, Tamilnadu, India) also mixed with sand and applied to pond for 20 days once upto end of the culture in probiotic pond only (*Rhodobactor* Sp., *Rhodococcus* sp., at concentration of 10^9 CFU/ml).

2.3.2. Postlarval stocking and acclimatization

The postlarvae (60,000) were obtained from Aqua Nova (P) Ltd., Kannathur, Chennai, which is situated 106 km away from the culture farm. Five hundred healthy and active postlarvae (PL-15) (mean length 12.8 ± 1.1 mm and mean weight 1.2 ± 0.2 mg) were packed in each polythene bags (40 x 80 cm) containing two liters of water (fig.5) and the bag was inflated with oxygen and closed tightly with the help of a rubber band (fig.6). *Artemia* nauplii were added to the polythene bags as food for the postlarvae while transportation.

Larvae were carefully transported during the evening hours after sunset by a van. The polythene bags containing postlarvae were placed in the ponds for about an hour for acclimatization. The polythene bags were then opened with least disturbance and pond water was allowed to enter into it by slowly opening the mouth of the bags. The postlarvae were slowly released and introduced in both ponds (fig. 7, 8, 9). The stocking density of *M. rosenbergii* in control pond and probiotic experiment pond was 1.3/m^2.

2.3.3. Physical and chemical parameter of water analysis

Physical and chemical parameters of water samples of both the ponds were analysed one week prior to the stocking of postlarvae, on the day of stocking of postlarvae, as well as weekly and monthly samples were analysed during the culture period. The physical and chemical parameters such as odour, colour, transparency (Secchi disc), water level, pH (C.P. pH kit), salinity (Refractometer), dissolved oxygen (C. P. DO kit), temperature (Mercury thermometer-atmospheric and water) were analysed weekly in the culture farm.

Fig .5. Measuring post larvae

Fig.6. Packing of Post larvae with aerated bags


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Fig.7. Packed postlarvae ready for transportation to culture ponds.

Fig.8. Post larvae packing introduced in the culture pond

Fig.9. Acclimatization of post larvae in the culture ponds


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Monthly collection of water samples from control and probiotic experimental ponds were made without overlapping the days of weekly sample analysis and the various parameters of water analysis were analysed in the laboratory by adopting standard procedures of APHA (1995).

2.3.4. Soil analysis

Monthly analyses of the soil samples of both ponds were carried out during the culture period. Soil samples from nine places in each pond were collected in a zigzag pattern and the soil was mixed well before analysis. All the studied soil parameters were tested in the "Soil testing and Technology Advisory Centre, Department of Soil Science & Agricultural Chemistry, Tamilnadu Agricultural University, Coimbatore, Tamilnadu, India.

2.3.5. Soil bacterial analysis

. For the culture of soil microbes, culture media were sterilized in an autoclave at 103 kpa for 15 minutes. The glassware's were sterilized in a hot air oven at 160°C for 3 h. Pour plate was used to enumerate total heterotrophic bacterial population in the soil samples. Nutrient agar medium was used to culture the bacteria. Composition of the nutrient agar medium per 100 ml distilled water is as follows (pH 7.2):-

Peptone	- 5.0 g
Beef extract	- 3.0 g
Yeast extract	- 2.0 g
Agar	- 15.0 g
Sodium chloride	- 1.0 g

Ninety-nine ml and 9.0 ml of sterile saline (0.85% NaCl) blanks were prepared for the serial dilution of the sample. One gram of soil sample was homogenized and then transferred to sterile saline and thoroughly mixed. The samples were then serially diluted using 9.0 ml of saline water blanks.

One ml of aliquotes from each samples were pipetted out into sterile petriplates and 15 - 20 ml of sterile nutrient agar medium was poured into the petriplates and the plates were rotated clockwise and anticlockwise. The plates were inverted after the medium got solidified. Duplicate plates were maintained for each dilution and the plates were incubated for 24 - 42 hrs at 37°C. After incubation period the bacterial colonies were counted using a bacteriological colony counter. Petriplates containing 30 - 300 bacterial colonies were selected for the enumeration of bacterial colonies. The bacterial populations were expressed as number of colony forming units (CFU) per gram of the sample analysed.

2.3.6. Generic composition of bacterial strains

Isolated bacterial colonies with different morphological growth characteristics were selected at random. The selected bacterial isolates were sub-cultured by streaking in nutrient agar plates to check the purity of the strains. The pure strains were then selected and stored in nutrient agar slants at 4°C. All the isolates from both ponds sediment were identified upto generic level. The bacterial isolates were identified after Shewan *et al.* (1960) and Bergey's manual (1986).

2.3.7. Soil fungal analysis

The mesophilic fungi were isolated from soil samples using different culture medium at different temperatures. For the present study, Czapek-Dox-Agar (CDA) medium was used for isolation of mesophilic fungi.

2.3.7.1. Composition of Czapek-Dox-Agar medium/1000 ml distilled water

Sodium nitrate	- 2.0 g
Potassium dihydrogen phosphate	- 1.0 g
Magnesium sulphate	- 0.5 g
Potassium chloride	- 0.5 g
Ferrous sulphate	- 0.01 g
Agar	- 20.0 g
Sucrose	- 30.0 g

One gram of soil sample was dispersed thoroughly in 10 ml of sterile distilled water termed as stock solution. From this, 1 ml was transferred to 9 ml of sterile water and mixed well. From this, the stock solution 1 ml was pipetted out into 9 ml of sterile water and mixed well. From this solution, 1 ml was transferred into sterile petriplates containing antibiotic amended agar medium (CDA) (10^3 dilutions). Streptomycin sulphate was used as an antibiotic to prevent the bacterial growth in the medium.

The petriplates were incubated at 29 ± 1°C for one week. Six replicates were maintained for each sample of mesophilic fungi. Fungi were mounted using lacto-phenol cotton blue stain and were observed under light microscope. The fungi were identified using Standard Manuals (Cooney and Emerson, 1964; Gilman, 1967; Barnett and Hunter, 1972 and Onions *et al.*, 1981). Percentage contribution and colony forming unit of the fungi were calculated using the following formulae:

$$\text{Percentage contribution (PC)} = \frac{\text{Total no. of colonies of a species}}{\text{Total no. of colonies of all species}} \times 100$$

$$\text{Colony forming unit (CFU)} = \frac{\text{Average no. of colonies / plates}}{\text{Total no. of colonies of all species}} \times \text{dilution factor}$$

2.3.8. Plankton analysis

2.3.8.1. Collection of sample

Monthly collections of plankton sample were made during 6.30 - 7.30 am from both the ponds during culture period using plankton net of bolten silk mesh (size 50 µm). Plankton samples were collected by towing the net horizontally at a depth of 1.5 feet for about 40 - 50 times. The collected samples were narcotised with 20% ethyl alcohol and were preserved in 5% neutral formalin.

2.3.8.2. Phytoplankton analysis

Qualitative analysis of phytoplankton was carried out by observing different morphological characters under compound microscope and was identified following the description of Venkataraman (1969) and Anand (1998).

2.3.8.3. Zooplankton analysis

2.3.8.3.1. Qualitative analysis

The different groups of zooplankton were separated under stereoscopic binocular dissection microscope. Temporary and permanent mounts of the whole plankton were prepared following the methods of Altaff (1990). They were dentified based on the minute morphological details and key characters described by Dussart and Defaye (1995) for copepods; Raghunathan (1989), Murugan *et al.* (1998) and Sureshkumar (2000) for cladocerans; Chandrasekar and Kodarkar (1995) and Dhanapathi (2000) for rotifers; Victor and Fernando (1979) for ostracods**.** The eggs, neonates, copepodids and naupliar stages were also identified and recorded.

2.3.8.3.2. Quantitative analysis

Hundred liters of water samples was collected from the ponds and separately filtered through the plankton net and plankton were narcotised using 20% ethyl alcohol and carefully transferred without any loss to a plastic bottle and preserved in 5% neutral formalin. For quantification of zooplankton the plankton sample was made up to 10 ml and enumerated using a Sedgewick-rafter counting chamber. The plankton sample was thoroughly mixed and 1 ml of the sample was drawn using a wide mouthed pipette and transferred to the counting chamber. The number of copepods, cladocerans, rotifers, ostracods, eggs, neonates, copepodids and nauplii in ten randomly selected squares of the counting chamber were counted under a compound microscope. The number of plankton per liter was calculated using the formula of Santhanam *et al.* (1989):

$$\mathbf{N} = \frac{n \times v}{V}$$

N = Total number of plankton per liter
n = Average number of plankton in one ml of plankton cell
v = Volume of plankton concentrated (ml)
V = Volume of the total water filtered

2.3.9. Feeding schedule

Artificial pelletized feed was given to the postlarvae from the second day onwards. The feed provided was "C.P. Scampi feed" C.P. Aquaculture India Pvt. Ltd., Chennai, India. The size of the pellets was ranged from 0.4-0.6 mm. Biochemical composition of the pellet was 30 % crude protein, 3.5% fat, 12% moisture and 8 % fiber. Fish meal, soya meal, shrimp shell meal, groundnut meal, sunflower meal, cotton seed meal, vitamin and mineral mix were the ingredients of the feed.

Six hundred grams of feed was broadcasted at 6.30 am and 5.30 pm for two days, in the afternoon 10 liters of microbial mixture was applied to each pond. C.P. scampi feed schedule was followed as per company standardized chart. For broadcasting feed, four poles were erected in the pond corners and connected with rope. Using a boat connected with the rope, food was broadcasted slowly so as to reach uniformly throughout the pond.

The post larvae after stocking into the culture ponds were left undisturbed, however regular observations are carried out. Continuously probiotic mixed feed were broadcasted to the probiotic experimental pond. Simultaneously vitamin and mineral mixture also mixed along with feed and applied during night feeding. During the culture "sodamix" were applied to the pond for 20 days once to equalize the mineral requirement to the water. The feed assessments were done in both ponds by trial netting. The feeding schedule was given in the table.1.

2.3.10. Probiotic feeding

The feed additives are Lact-Act (*Lactobacillus sporogens* with a concentration of 1500 million viable spores per gram of powder) and Thionil (mixture of bacterial culture) (Poseidon Biotech, Chennai, Tamilnadu, India) and Mutagen (C.P. Aquaculture (India) PVT. Ltd., Chennai, Tamilnadu, India) (fig.10.). Composition of mutagen includes vitamin A, D, E, K, B_1, B_2, B_6, B_{12}, Biotin, Ascorbic acid, Iron, Manganese, Copper, Zinc, Iodine, Calcium, Magnesium, B.H.T., Immunostimulant and aminoacid were mixed in the feed as per company feed direction during the night and broadcasted to the probiotic experimental pond.

2.3.10.1. Procedure adopted for probiotic mixing:

The known quantity of C.P. feed and the Lactact 10g/kg, Thionil 20g/kg and Mutagen 15g/kg were mixed with water and to this 30 ml of affinity gel also mixed and kept for 10 minutes, dried in the shade for 20-30 minutes (fig.11, 12 and 13), then feed was broadcasted as per feeding schedules. Probiotic mixed feed was offered during night time to the experiment pond animals (4 times/day), during rainy seasons and cloudy times probiotic feed offered in the afternoon time (3 times/day).

Table.1.Feeding schedule of *Macrobrachium rosenbergii* during the culture period in control and probiotic experimental pond

S.no	Period	Feed broad cast time (hrs)			Quantity of Feed broad cast(Kg)
1.	5.2.08 – 20.2.08	6.30-7.30	17.00	-	0.6
2.	21.2.08 – 30.2.08	6.30-7.30	17.00	-	1.0
3.	31.3.08 – 3.4.08	5.30-6.30	16.00-16.30	22.30-23.00	1.5
4.	4.4.08 – 25.4.08	5.30-6.30	16.00-16.30	22.30-23.00	2.0
5.	26.4.08- 25.5.08	5.30-6.30	16.00-16.30	22.30-23.00	3.0
6.	26.5.08- 25.7.08	5.30-6.30	16.00-16.30	22.30-23.00	5.0
7.	26.7.08 – 31.8.08	5.30-6.30	16.00-16.30	22.30-23.00	7.0
8.	1.9.08 - 28.10.08	5.30-6.30	16.00-16.30	22.30-23.00	10.0
9.	29.10.08-23.12.08	5.30-6.30	16.00-16.30	22.30-23.00	4.0

Fig.10. Packages of commercial probiotics

Fig.11. Pouring of probiotics with pelletized feed

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Fig. 12 Mixing the probiotics with the feed

Fig, 13. Drying of commercial probiotic mixture


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After 65th day, based on the trial netting and assessment of biomass of prawn, the quantity of the feed was increased, the feed broadcasting also increased to thrice/four a day at 6.30 am, 11.30 am, 4.30 pm and 9.30 pm. Trial netting was done on 65th, 89th, 117th and 145th day of the culture to assess the biomass of *M. rosenbergii.* Feed increase was affected based on the following formula:

Feed increased = Average weight x approximate survival x percentage of body weight

2.3.11. Fish stocking

Due to very high bloom of zooplankton population and depletion of dissolved oxygen level in both the ponds, fish fingerlings were stocked in these ponds. Catla (*Catla catla*) and Silver carp (*Hypophthalmichthys molitrix*) 300 and 220 numbers, respectively were introduced in each pond. The mean length and mean weight of *Catla catla* was 4 ± 0.5 cm and 4.2 ± 1.0 g respectively, while the mean length and mean weight of *Hypophthalmichthys molitrix* was 3.5 ± 0.5 cm and 3 ± 0.5 g respectively.

2.3.12. Predator's control

Water birds, crabs and tadpoles are the chief predators of the *M. rosenbergii* during culture period. Crackers were used to clear bird population in and around the vicinity of the ponds. Further, hunters were brought to the ponds and made hunting the water birds when the birds are higher number in the ponds. Crabs and tadpoles were removed manually and also by hand netting.

2.3.13. Growth measurement:

Total length (cm), body weight (g) of harvested prawns in both ponds was measured four times in a month during the harvest time. The specific growth rate (SGR), feed conversion ratio (FCR), Protein efficiency ratio (PER), Benefit cost ratio (BCR) and Feed efficiency (FE) were calculated according to Sweilum (2006) as follows:

$$SGR = \frac{(\text{Final weight} - \text{Initial weight})}{\text{Culture period in days}} \times 100 \text{ days}$$

$$FCR = \frac{\text{Quantity of feed consumed}}{\text{Total weight gained}}$$

$$PER = \frac{\text{Weight gain (g)} \times \text{No. of prawns}}{\text{Protein intake}}$$

$$BCR = \frac{\text{Total benefit return}}{\text{Total cost}}$$

$$FE = \frac{\text{Total weight gain (g)}}{\text{Quantity of food consumed}}$$

2.3.14. Harvest

After 119th day of culture, the prawn was harvested in control and probiotic experiment pond and subsequent month's partial harvest were done. During the partial harvest above 60, 50, and 40gm animals were harvested by drag net (fig. 14, 15 and 16). One day prior to harvest, water level was lowered to 0.5m. The complete harvest was done within 4 days (15-20th December 2008). Every day, harvesting was done from 5.00 am - 10.30 am and 3.00 pm - 6.30 pm. Hand picking was also done as a post harvest procedure to accomplish 100% harvest. Fish population was also harvested **(f**ig.17**)**. After complete harvest, animals were weighed and separated according to the size and were ice packed (fig. 18 - 21). The stunted prawns were segregated during harvest and cultured in a separate pond to study the growth status (Chapter. 5).

2.3.15. Economics Analysis

Harvested prawns were sold in the Chennai export market whereas fishes were sold in the local market. Seed, feed, fertilizers, power, labour, harvest and trial netting

Fig.14 and 15.   Harvesting of prawn by tracking and by hand picking


14


15

Fig.16. Harvesting by netting

Fig.17. Harvesting of prawns and fishes

16

17

Fig. 18 and 19. Harvested Adult mature Prawn


18


19

Fig. 20. Weighing the harvested prawns

Fig. 21.Icepacking

20

21

costs were accounted and compared with the results of control and probiotic experiment pond were shown in table. 33 and 34. The operational cost, net income and profit were calculated. The cost of leasing of pond was not included. The cost of production was based on the wholesale market price (2008-2009) for the input used.

2.3.16. Statistical analysis

Statistical analysis was carried out for the resulted data on soil texture, water parameters, fungal and plankton populations were analysed using 't' test (Systat Version 10.0). DO, pH and Temperature were also analysed using Correlation, Regression and ANOVA at 5% level. The growth relationship (positive/negative) between the control and experimental pond cultured prawn was calculated (SPSS Inc. 2010).

2.4. RESULTS

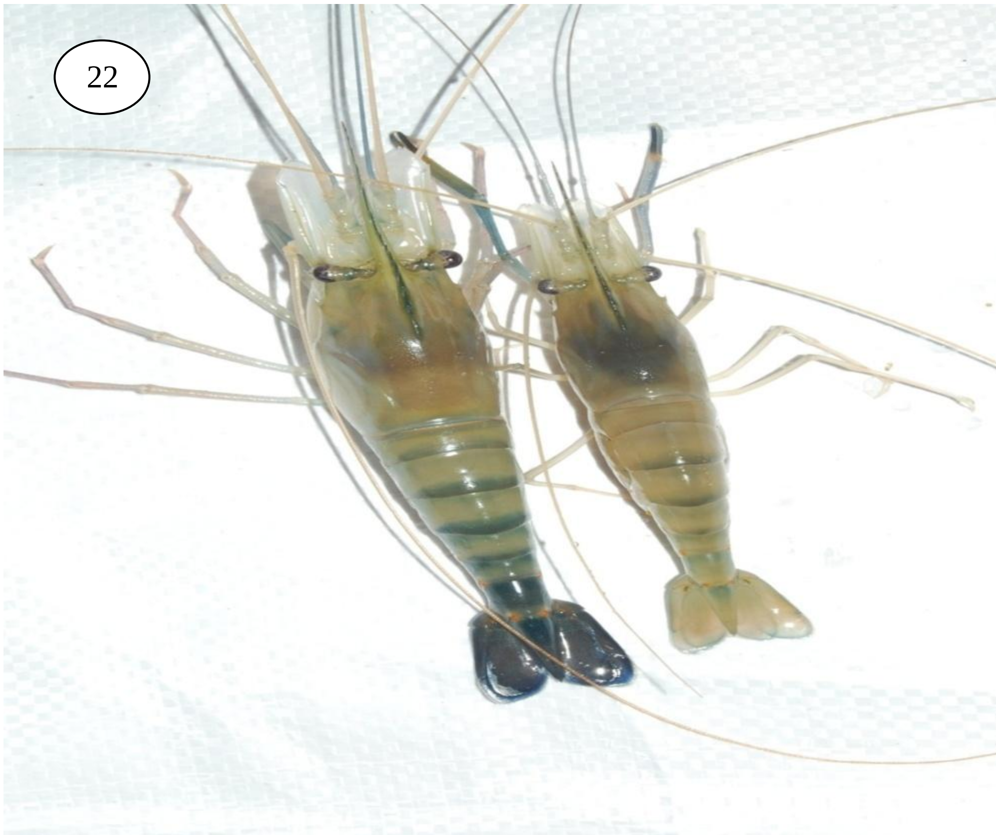
2.4.1. General description of *M. rosenbergii*

The sexes are separate. The whole body of *M. rosenbergii* was divided into 20 segments known as Somites. There are 14 segments in the head which are fused together and invisible under a large dorsal and lateral shield known as the Carapace (fig.22). The carapace is hard and smooth except for 2 spines on either side: one (the antennal spine) is just below the orbit and the other (the hepatic spine) is lower down and behind the antennal spine. The carapace ends at the front in a long beak or rostrum which is slender and curved upwards. The rostrum extends further forwards then the antennal scale and has 11–14 teeth on the top 8 – 10 underneath (fig. 23 and 24). The colour of the bodies of the prawn tends to be brighter in the younger animals and generally darker and blue or brownish in older prawns.

Fig. 22. Adult Male and Female *M. rosenbergii*

Fig. 23. Dorsal views of Adult male *M. rosenbergii*

Fig. 24 Ventral views of Adult male *M. rosenbergii*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23


24

Mature male prawns are considerably larger than females and 2nd chelipods much larger and thicker, the abdomen is narrower (fig.22). The head of the mature female and 2nd walking legs are much smaller than the adult male.

2.4.2. Soil analysis of pond:

The studied soil texture analysis of the freshwater prawn *M. rosenbergii* culture in control and probiotic applied pond at Vishnuvakkam, Tiruvallur district, Tamilnadu, India are presented in table. 2.

The soil pH throughout the study period was more or less same except in the month of July and November (8.2) in control pond and in the month of November (8.2) in probiotic experiment pond (fig.25**)**. The range of pH 7.4 - 8.2 was observed in the present study. The electrical conductivity was higher in the month of June (1.93µs/m) in control pond and in the month of March and June (1.8µs/m) in probiotic experimental pond. Lower level of EC was noticed (0.47µs/m) in the month of September and October in control and in the month of October (0.7µs/m) in experiment pond. The percentage of slit, clay and sand content are not showed much variation between the control and probiotic experiment pond throughout the study period but there was some fluctuations noticed between the months in both ponds (fig.26). Available mean values of nitrogen (29.454 ±1.485, 41.09 ± 1.423), available phosphorus (3.945 ±0.166, 4.654 ±0.228), available potash (105.909 ± 8.182, 129.272 ± 8.543), copper (0.8218 ± 6.397, 0.7555 ± 6.227 ppm), Iron (4.432 ± 0.213, 4.351 ± 0.185 ppm) were recorded in control and probiotic experiment pond **(**table. 2a**)** **(**fig.27**).** The manganese in the month of July (6.35 ppm) showed higher level in probiotic applied pond when compared to control pond.

Significant values ($P < 0.05$) were observed for all the soil texture parameters. The correlation co-efficient (r) values of soil texture parameters of control and experiment

Table-2: Soil texture analysis of control and Probiotic experiment pond of freshwater prawn *M. rosenbergii* culture (February - December 2008)

Soil Parameters	Feb		Mar		April		May		June		July		Aug		Sep		Oct		Nov		Decr	
	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
pH	7.6	7.4	7.6	7.8	7.7	7.8	7.4	7.6	7.6	7.6	8.2	7.6	7.6	7.6	7.6	7.6	7.4	7.4	8.2	8.2	7.6	7.6
Electrical conductivity (µs/m)	1.7	1.7	1.7	1.8	1.35	1.4	1.7	1.7	1.93	1.8	1.03	1.4	0.92	1.2	0.47	0.9	0.47	0.7	0.92	0.9	0.92	0.9
Clay (%)	23.4	26.4	23.6	26.8	24.5	27.2	24.8	28.2	25.2	28.4	25.6	29.8	26.1	29.1	26.8	29.8	27.4	30.2	27.7	31.4	28.4	31.9
Slit (%)	30.2	31.2	30.6	32.4	31.6	33.8	31.8	34.2	31.2	34.9	30.6	34.8	32.1	35.2	31.8	35.4	32.6	35.6	32.8	38.4	33.1	40.1
Sand (%)	27.2	28.4	27.3	28.6	27.4	28.8	28.2	28.8	28.4	29.2	28.3	29.3	28.6	29.4	28.2	29.6	28.8	30.1	28.8	30.9	29.4	32.4.
Organic Carbon (%)	0.12	0.12	0.14	0.13	0.13	0.16	0.15	0.14	0.12	0.14	0.14	0.15	0.15	0.16	0.17	0.16	0.16	0.15	0.17	0.16	0.16	0.17
Available Nitrogen (mg/100 g soil)	25	35	27	36	26	35	26	38	28	40	30	41	30	42	32	45	36	46	34	46	36	48
Available Phosphorus (mg/100 g soil)	3.2	3.5	3.5	3.8	3.5	3.9	3.5	4.2	3.6	4.4	3.8	4.6	4.1	5.1	4.2	5.2	4.4	5.2	4.8	5.5	4.8	5.8
Available Potash (mg/100 g soil)	75	85	72	92	84	108	88	116	90	120	98	128	110	134	126	141	134	150	140	158	148	160
Copper (ppm)	0.78	0.50	0.78	0.75	1.24	0.78	0.78	0.77	1.24	0.85	0.79	1.24	0.79	0.85	0.53	0.77	0.53	0.75	0.79	0.55	0.79	0.50
Manganese (ppm)	6.33	4.35	6.33	4.37	5.02	5.05	6.33	5.05	5.02	6.24	4.33	6.35	4.33	6.25	6.24	6.20	6.24	5.10	4.37	4.30	4.33	4.27
Iron (ppm)	5.34	3.30	5.34	4.35	3.31	4.40	5.34	4.56	3.31	5.10	4.37	5.30	4.37	4.58	4.32	4.36	4.32	4.32	4.37	4.30	4.37	3.30

C- Control **E - Experiment**

Fig. 25. pH of the soil in control and probiotic experiment ponds of *M. rosenbergii* culture

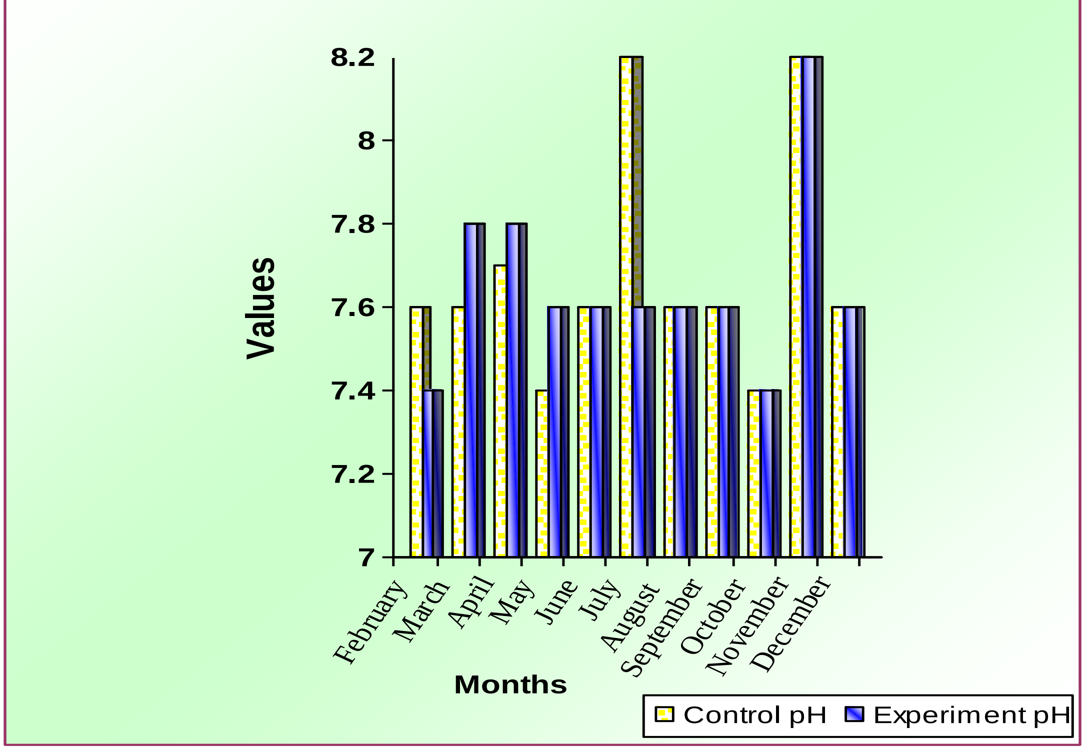

Fig.26. Mean values of Clay, Slit and Sand content of the soil in control and probiotic experiment ponds of *M. rosenbergii* culture

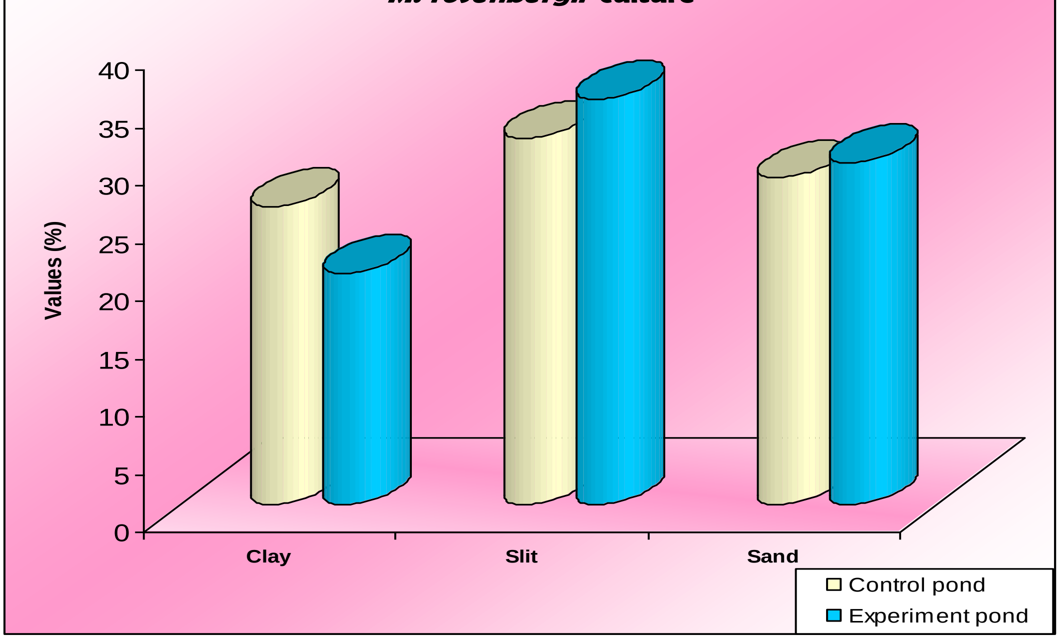

Table-2a: Levels of soil texture of Control and Probiotic experiment pond (T-test analysis)

Parameters	Ponds	Mean ± SEM*	T-test values	P-value
P^H	Control	7.681 ± 8.182 E – 02	93.889	0.000
	Experiment	7.654 ± 6.656 E – 02	115.008	0.000
Electrical conductivity	Control	1.1918 ± 0.154	7.701	0.000
	Experiment	1.3091 ± 0.123	10.564	0.000
Clay	Control	25.772 ± 0.5018	51.357	0.000
	Experiment	20.018 ± 0.545	53.193	0.000
Slit	Control	31.672 ± 0.2873	110.229	0.000
	Experiment	35.090 ± 0.746	46.980	0.000
Sand	Control	28.236 ± 0.209	135.095	0.000
	Experiment	29.590 ± 0.3541	83.577	0.000
Organic Carbon	Control	0.1464 ± 5.439 E-03	26.908	0.000
	Experiment	0.1491 ± 4.564 E-03	32.670	0.000
Available Nitrogen	Control	29.454 ±1.485	19.828	0.000
	Experiment	41.090 ± 1.423	28.865	0.000
Available Phosphorus	Control	3.945 ±0.166	23.705	0.000
	Experiment	4.654 ±0.228	20.399	0.000
Available Potash	Control	105.909 ±8.182	12.943	0.000
	Experiment	129.272 ± 8.543	15.132	0.000
Copper	Control	0.8218 ± 6.397 E- 02	11.849	0.000
	Experiment	0.7555 ± 6.227 E- 02	12.133	0.000
Manganese	Control	5.351 ±0.282	18.960	0.000
	Experiment	5.230 ± 0.263	19.848	0.000
Iron	Control	4.432 ± 0.213	20.726	0.000
	Experiment	4.351 ± 0.185	23.498	0.000

* : Mean sample 11 months Significance at the 5 % level ($P<0.05$)

Table-2b: Correlation co-efficient (r-value) of soil texture of control and probiotic experiment pond

Parameters	Correlation (r-value)	Significance
P^H	0.619	0.042•
Electrical conductivity	0.946	0.000†
Clay	0.972	0.000†
Slit	0.866	0.001†
Sand	0.859	0.001†
Organic Carbon	0.646	0.032•
Available Nitrogen	0.866	0.001†
Available Phosphorus	0.965	0.000†
Available Potash	0.937	0.000
Copper	0.113	0.741
Manganese	- 0.208*	0.540
Iron	- 0.382*	0.247

• : Significance at the 0.05 level * : Negative correlation † : Significant at the 0.01 level

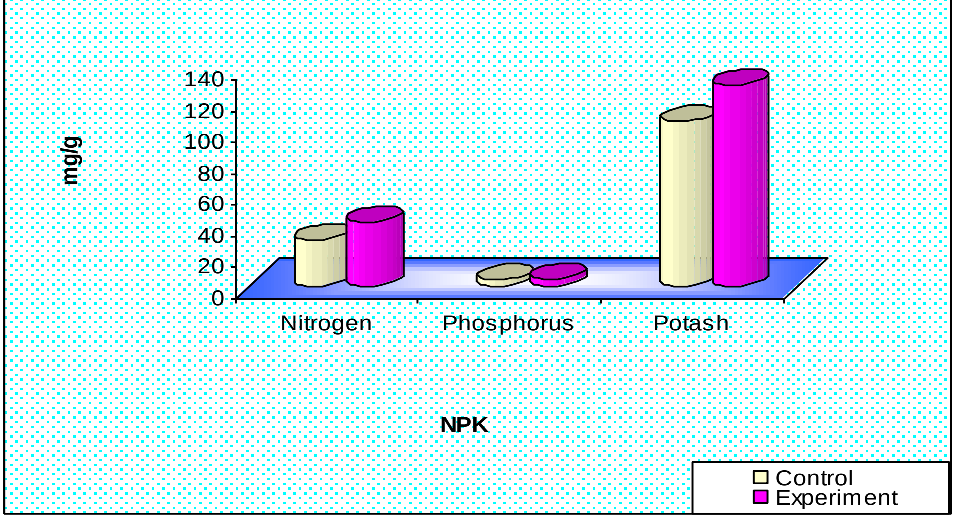

Fig. 27. Mean values of NPK content of the soil in control and probiotic experiment pond of *M. rosenbergii* culture

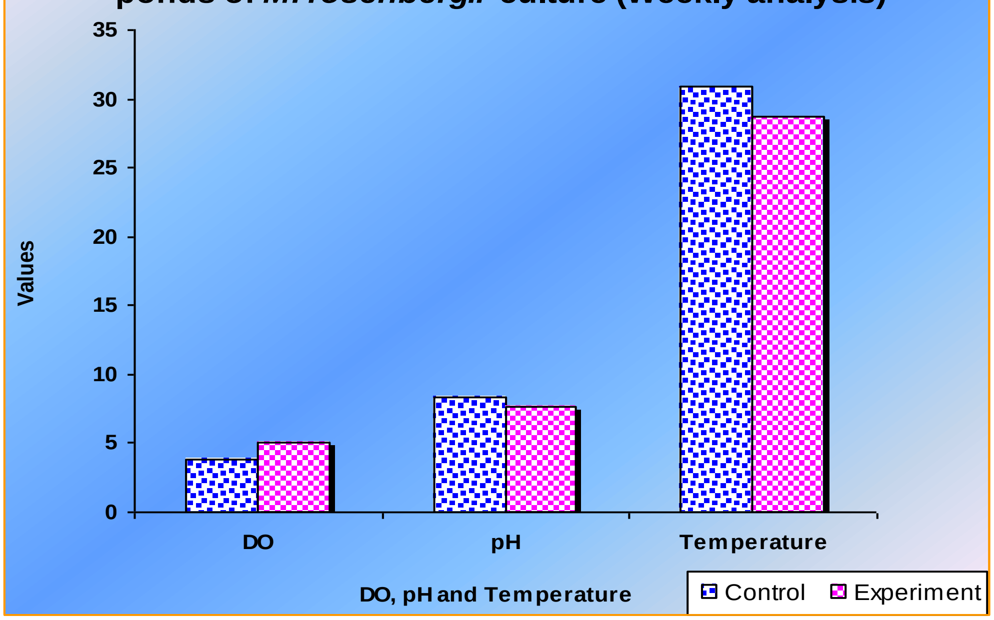

Fig. 28. Mean values of DO, pH and Temperature of water in control and probiotic experiment ponds of *M. rosenbergii* culture (Weekly analysis)

pond were presented in table. 2b**.** Manganese and Iron were showed negative correlation and this was found to statistically significant at P < 0.01 level.

2.4.3. Physico chemical parameters of pond water: (weekly analysis)

The weekly analysis of colour, odour, temperature, transparency, pH and DO of control and probiotic applied pond from 3rd February to 21st December 2008 was presented in table.3. Light green colour is appeared most of the months, except in October and earthy odour smell was observed in the beginning of the culture then no odour was observed except in September and October, in the experiment pond. The transparency levels ranged from 20 – 40 cm, mostly normal transparency level was recorded during the culture period. The temperature was varied between 26 – 34°C in probiotic experiment pond. The recorded dissolved oxygen ranges between 3.0 – 5.5 ppm during the culture period.

Control pond shows light green, thick green, greenish brown, golden yellow, dark green colours during the culture period. Earthy odours were smelled in the beginning of the culture, after that odourless and sandy odour was noticed. The't' test values, correlation and regression and ANOVA values for DO, pH and temperature of control and probiotic experiment pond were presented in table 3a,b,c and fig 28.

2.4.3.1. Monthly analysis:

Monthly recorded values of physical and chemical parameters of control and probiotic experiment pond were presented in table 4. The resulted values of physical and chemical parameters of control and probiotic experiment pond were found to be statistically significant at various levels. Normal pH ranges were appeared in both the ponds where as the alkalinity pH showed fluctuated. (fig. 29, 30). Higher alkalinity was recorded in the month of December in both the ponds. Total hardness of the water shows

Table-3: Physical and Chemical parameters of control and probiotic experiment pond of freshwater prawn *M. rosenbergii* culture (February – December 2008 weekly analysis)

Date	Colour		Odour		Temperature (°C)		Transparency (cm)		Dissolved oxygen (ppm)		pH	
	C	E	C	E	C	E	C	E	C	E	C	E
03.02.08	Light green	Light green	Earthy	Earthy	29	30	22-35	26-35	5.0	5	7.6	7.4
10.02.08	- do -	- do -	- do -	- do -	30	29	21-35	25-35	5.0	5	7.6	7.4
17.02.08	- do -	- do -	- do -	- do -	32	28	18-28	25-35	5.0	5	7.9	7.6
24.02.08	- do -	- do -	- do -	- do -	31	26	23-34	30-40	4.5	5	7.9	7.6
02.03.08	- do -	- do -	- do -	- do -	32	27	21-30	25-35	4.	5	8.2	8.2
09.03.08	- do -	- do -	- do -	- do -	31	28	18-27	25-37	4	5	8.2	7.4
16.03.08	- do -	- do -	- do -	- do -	29	26	18-23	25-36	4	4.5	8.5	7.6
23.03.08	- do -	- do -	- do -	- do -	31	27	21-30	27-35	4	4.5	8.5	7.9
30.03.08	- do -	- do -	- do -	- do -	32	30	22-35	20-33	4	4	8.5	7.4
06.04.08	- do -	- do -	Odurless	Odurless	30	28	21-30	25-37	4	5	8.5	7.4
13.04.08	- do -	- do -	- do -	- do -	30	32	19-33	25-40	3.5	5.5	8.5	7.6
20.04.08	- do -	- do -	- do -	- do -	31	30	18-32	21-33	3.5	5.5	8.5	7.9
27.04.08	- do -	- do -	- do -	- do -	31	29	21-30	25-37	3.5	5.5	8.8	7.4
04.05.08	- do -	- do -	- do -	- do -	32	28	24-32	24-38	3	5.5	8.8	7.6
11.05.08	- do -	- do -	- do -	- do -	31	26	18-32	20-32	3	5	8.8	7.6
18.05.08	- do -	- do -	- do -	- do -	33	27	18-28	21-34	3	5	8.5	7.6
25.05.08	- do -	- do -	- do -	- do -	33	30	20-33	21-28	3	5	8.5	8.2
01.06.08	- do -	- do -	- do -	- do -	33	36	20-34	23-37	4	4.5	8.2	7.4
08.06.08	- do -	- do -	- do -	- do -	33	32	21-34	25-35	4.5	4.5	8.5	7.6
15.06.08	- do -	- do -	- do -	- do -	33	34	22-35	24-35	4.5	4.5	8.5	7.9
22.06.08	- do -	- do -	- do -	- do -	32	30	22-30	25-35	4	4.5	8.8	7.6
29.06.08	- do -	- do -	- do -	- do -	33	29	24-37	24-32	4	4	8.5	8.2
06.07.08	- do -	- do -	- do -	- do -	32	28	21-35	25-38	4.5	4.5	8.2	8.2

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13.07.08	- do -	- do -	- do -	- do -	31	26	20-28	25-35	3.5	4.5	8.2	7.6
20.07.08	- do -	- do -	- do -	- do -	29	27	21-32	20-32	3.5	4.5	8.2	7.6
27.07.08	- do -	- do -	- do -	- do -	30	32	22-30	26-34	3	4.5	8.5	8.2
03.08.08	- do -	- do -	- do -	- do -	28	28	21-34	27-38	3	4.5	8.5	7.4
10.08.08	- do -	- do -	- do -	- do -	32	28	22-32	26-35	3	4.5	8.2	7.6
17.08.08	- do -	- do -	- do -	- do -	33	26	18-24	20-34	3	4.5	8.2	7.9
24.08.08	- do -	- do -	- do -	- do -	33	27	23-32	25-32	3	4.5	8.5	7.6
31.08.08	- do -	- do -	- do -	- do -	31	30	21-33	24-33	3	4.5	8.5	7.6
07.09.08	- do -	- do -	Sandy	Sandy	29	29	18-28	25-35	3	4.5	8.5	8.2
14.09.08	- do -	- do -	- do -	- do -	30	28	22-30	26-34	3	5.5	8.8	7.6
21.09.08	- do -	- do -	- do -	- do -	31	26	21-32	25-36	3	5.5	8.5	7.6
28.09.08	- do -	- do -	- do -	- do -	29	27	18-25	24-31	3	5.5	8.5	8.2
05.10.08	Thick green	Thick green	- do -	- do -	30	30	24-30	24-35	3.5	5.5	8.5	7.4
12.10.08	- do -	- do -	- do -	- do -	32	28	22-35	26-35	3.5	5.5	8.5	7.6
19.10.08	- do -	- do -	- do -	- do -	34	32	20-30	24-30	3.5	6.5	8.5	7.9
26.10.08	- do -	- do -	- do -	- do -	33	34	21-37	25-37	3.5	6.5	8.5	7.6
02.11.08	- do -	- do -	Odourless	Odourless	32	30	22-31	26-36	3.5	4.5	7.9	7.9
09.11.08	- do -	- do -	- do -	- do -	31	29	21-33	24-33	4.5	4.5	7.6	7.6
16.11.08	- do -	- do -	- do -	- do -	30	28	28-35	25-35	4.5	4.5	7.6	7.6
23.11.08	- do -	- do -	- do -	- do -	30	26	18-28	30-40	5	5	7.6	7.6
30.11.08	- do -	- do -	- do -	- do -	29	27	21-35	25-35	5	5	7.6	7.6
07.12.08	- do -	- do -	- do -	- do -	28	28	20-34	24-34	4.5	6	7.9	7.9
14.12.08	- do -	- do -	- do -	- do -	26	26	22-33	24-33	4.5	6.5	8	8
21.12.08	- do -	- do -	- do -	- do -	27	27	24-30	20-30	5	6.5	8	8

C- Control **E - Experiment**

Table- 3a: T-test values of DO, pH and temperature of control and Probiotic experiment pond of freshwater prawn *M. rosenbergii* culture (On the spot values)

Parameters	**Ponds**	**Mean ± SEM***	**T-test value**	**P-value**
DO	Control	3.808 ± 0.102	37.106	0.000
	Experimental	5.000± 9.375E-02	53.336	0.000
pH	Control	8.293 ± 5.254	157.854	0.000
	Experimental	7.717 ±3.867	202.289	0.000
Temperature	Control	30.893 ± 0.258	119.512	0.000
	Experimental	28.702 ± 0.342	83.767	0.000

* : Mean sample 47 Significance at the 5 % level ($P<0.05$)

Table.3b: Correlation (r- value) and ANOVA (F-value) of DO, pH and temperature of control and Probiotic experiment pond of freshwater prawn *M. rosenbergii* culture

Parameters	(r-value)	F-value	p-value
DO	0.048	2.737	0.032*
pH	0.069	0.520	0.722
Temperature	0.394	1.082	0.393

Table- 3c: Regression values of Dissolved oxygen, pH and temperature of control and Probiotic experiment pond of freshwater prawn *M. rosenbergii* culture

Parameters	(R-value)
DO	0.048
pH	0.069
Temperature	0.394

Table-4: Physical and Chemical parameters of control and probiotic experiment pond of freshwater prawn *M. rosenbergii* culture (February – December 2008)

Paraameters	Feb		Mar		Apr		May		June		July		Aug		Sep		Oct		Nov		Dec	
	C	**E**	**C**	**E**	**C**	**E**	**C**	**E**	**C**	**E**	**C**	**E**	**C**	**E**	**C**	**E**	**C**	**E**	**C**	**E**	**C**	**E**
pH	7.6	7.9	8.2	8.2	8.5	8.4	8.5	8.4	8.5	8.5	8.2	8.2	8.8	8.2	8.8	8.5	8.5	8.5	8.2	7.6	8.2	7.9
Alkalinity pH	12.0	12.0	16.0	16.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.0	12.00	16.00	16.00	16.00	16.00	12.00	12.00	16.00	16.00
Electrical conducti-vity	1650	1180	1680	1190	1750	1680	1940	2000	1885	2260	2030	2250	1950	2230	1860	2140	1855	1920	1850	1865	1780	1780
Total dissolved solids	1130	1130	1220	1125	1300	1150	1250	1420	1350	1530	1365	1615	1265	1565	1260	1460	1255	1355	1210	1260	1150	1280
Turbidity(cm)	34	24	35	25	25	45	28	42	30	60	38	45	35	50	30	35	15	35	18	45	20	40
Total Alkalinity (ppm)	45	50	50	65	70	65	75	70	80	70	85	70	90	80	110	100	110	100	120	110	125	120
Total hardness (ppm)	245	225	220	220	235	220	240	210	290	190	220	170	180	180	160	160	140	140	190	160	210	190
Calcium (ppm)	104	45	104	30	77	38	69	25	69	45	77	30	64	25	67	27	62	32	104	40	104	45
Magnesium (ppm)	43	38	48	42	32	45	28.	44	28.	55	32.	42	26.	36	29	33	27	37	43	42	48	48.
Sodium (ppm)	24	24	25	24	20	20	23	22	22	22	20	21	18	17	20	20	22	23	24	27	22	24
Potassium (ppm)	20	20	20	20	18	22	22	22	22	18	18	18	16.	16	18	18	20.	20	20	22	20	22

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Phosphorus (ppm)	0.29	0.28	0.36	0.34	1.08	1.05	0.30	0.25	0.98	1.08	1.08	1.08	1.58	1.48	2.02	2.00	2.12	2.02	0.29	0.27	0.36	0.35
Iron (ppm)	1.00	1.2	1.00	1.6	2.4	2.6	3.0	3.2	1.5	2.8	2.5	2.8	1.30	1.8	1.60	1.9	1.60	1.9	0.8	2.1	0.90	1.90
Fluoride (ppm)	1.20	0.15	1.40	0.25	1.50	0.15	0.70	0.15	1. 5	0.15	1.50	0.15	1.50	0.15	1.50	0.15	1.50	0.15	1.20	0..20	1.40	0.25
Chloride (ppm)	358	341	392	376	271	251	298	285	292	275	293	251	246	218	284	263	285	261	365	341	382	376
Free Ammonia (ppm)	0.24	0.20	0.32	0.21	1.04	0.80	0.56	0.44	0.10	0.09	1.07	1.02	1.05	0.90	1.01	0.70	1.03	0.80	0.24	0.12	0.32	0.25
Nitrate (ppm)	3	2	3	2	3	2	3	2	4	3	4	3	5	3	5	3	4	3	4	3	4	3
Sulphate (ppm)	40.	30	15	12	13	13	9	8	10	10.	13	11	13	12	17	14	18	15	40	35	15	18

C- Control **E - Experiment**

Fig. 29 pH of the water in control and probiotic experiment ponds of *M. rosenbergii* culture

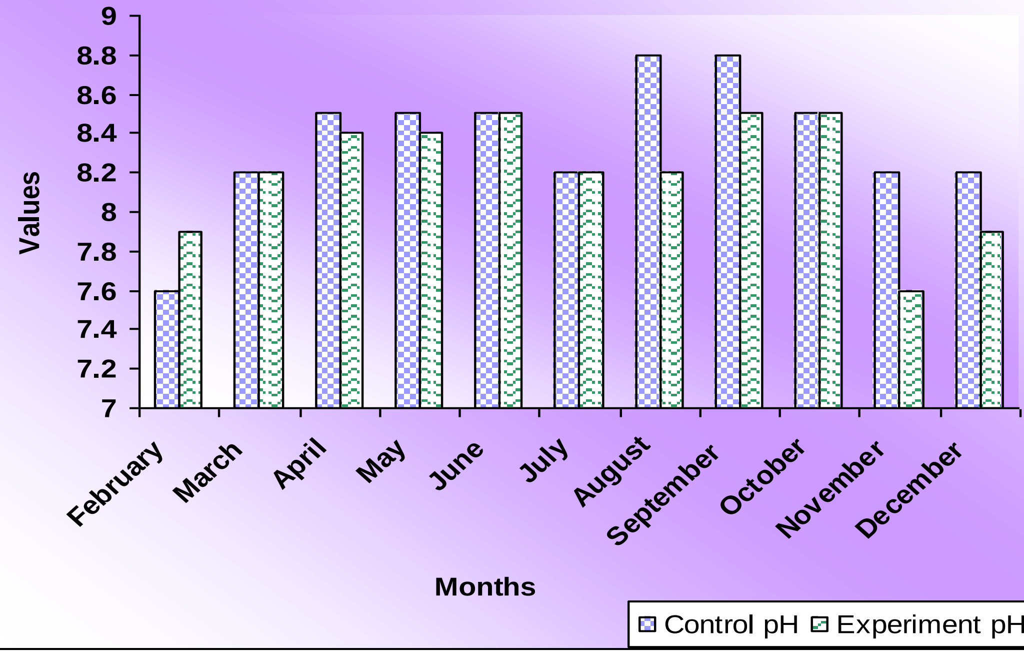


Fig. 30 Alkalinity pH of the water in control and probiotic experiment ponds of *M. rosenbergii* culture

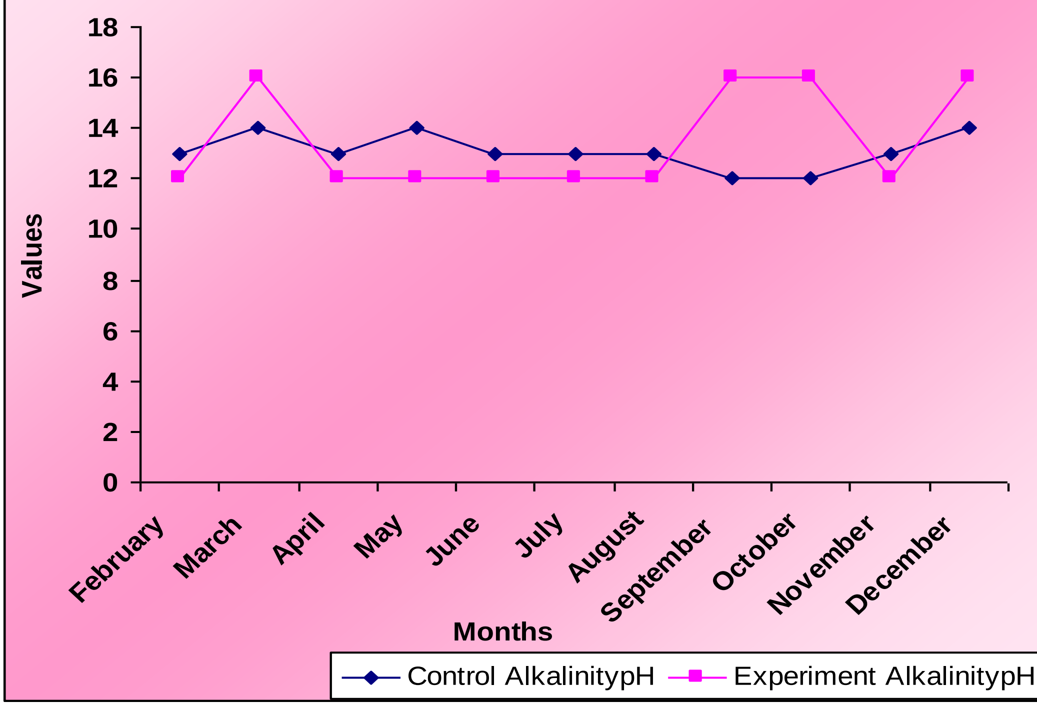


Fig.31 Total Alkalinity and total hardness of the water in control and probiotic experiment ponds of *M. rosenbergii* culture

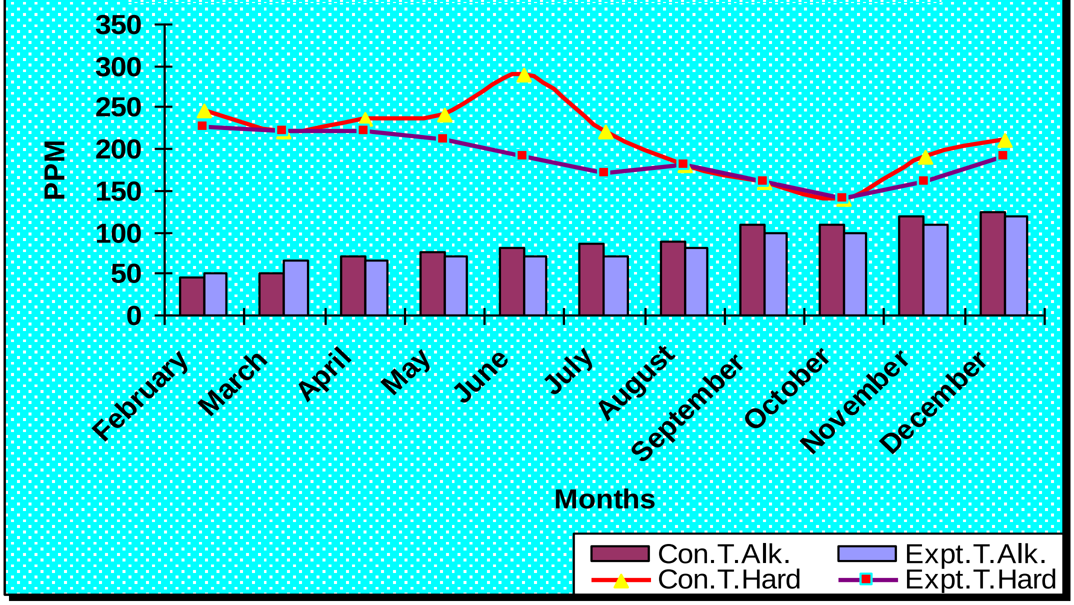


Fig.32 Chloride content of the water in control and probiotic experiment ponds of *M. rosenbergii* culture

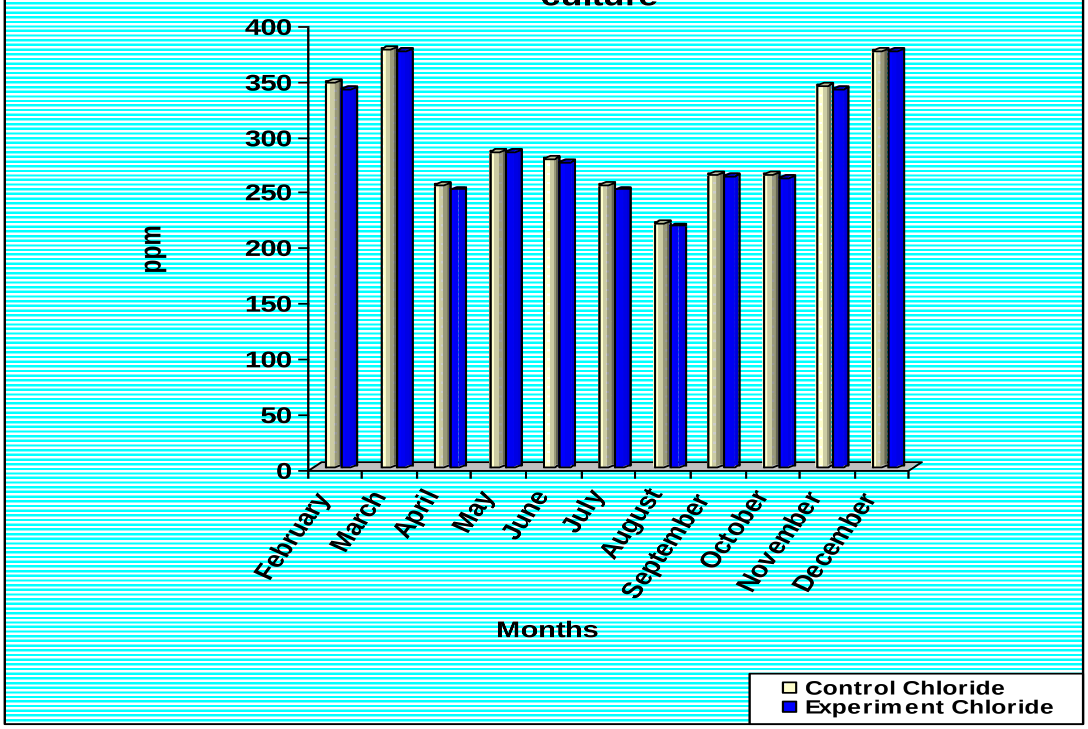


Table-4a: Levels of physical and chemical parameters of control and probiotic experiment pond (T-test analysis)

Parameters	Ponds	Mean ± SE •	t- values	p-value
pH	C	8.363 ± 0.102	81.960	0.000
	E	8.209 ± 8.990E-02	91.310	0.000
Alkalinity pH	C	13.090 ± 0.414	31.574	0.000
	E	13.454 ± 0.608	22.112	0.000
Electrical conductivity	C	1839.090 ± 34.946	52.625	0.000
	E	1863.181 ± 116.798	15.952	0.000
Total Dissolved solids	C	1250.454 ± 21.944	56.982	0.000
	E	1353.636 ± 53.746	25.186	0.000
Turbidity	C	28.000 ± 2.304	12.152	0.000
	E	40.545± 3.171	12.783	0.000
Total Alkalinity	C	81.818 ± 6.683	12.242	0.000
	E	87.272 ± 8.100	10.774	0.000
Total hardness	C	211.818 ± 12.796	16.552	0.000
	E	188.636 ± 9.047	20.849	0.000
Calcium	C	81.909 ± 5.454	15.016	0.000
	E	34.727 ± 2.442	14.219	0.000
Magnesium	C	34.909 ± 2.633	13.256	0.000
	E	42.000 ± 1.848	22.717	0.000
Sodium	C	21.818 ± 0.644	33.873	0.000
	E	22.181 ± 0.807	27.487	0.000
Potassium	C	19.454 ± 0.545	35.667	0.000
	E	19.818 ± 0.629	31.466	0.000
Phosphorus	C	0.950 ± 0.211	4.489	0.001
	E	0.927 ± 0.206	4.486	0.001
Iron	C	1.600 ± 0.220	7.248	0.000
	E	2.163 ± 0.182	11.853	0.000
Fluoride	C	1.354 ± 7.43 E- 02	18.230	0.000
	E	0.172 ± 1.236 E–02	13.969	0.000
Chloride	C	315.09 ± 14.962	21.058	0.000
	E	294.36 ± 16.467	17.875	0.000
Free ammonia	C	**0.634 ± 0.121**	**5.222**	**0.000**
	E	**0.502 ±0.104**	**4.807**	**0.001**
Nitrate	C	**3.818 ± 0.226**	**16.868**	**0.000**
	E	**2.636 ± 0.152**	**17.331**	**0.000**
Sulphate	C	18.454 ± 3.309	5.576	0.000
	E	16.181 ± 2.579	6.274	0.000

• : Mean of 11 samples Significant at 1% level (P<0.01)

Table-4b: Correlation co-efficient (r-value) of physical and chemical parameters of control and probiotic experiment pond

Parameters	Correlation (r-value)	p-value
pH	0.638	0.035•
Alkalinity pH	0.812	0.002†
Electrical conductivity	0.915	0.000†
Total dissolved solids	0.667	0.025•
Turbidity	- 0.063•	0.853
Total Alkalinity	0.958	0.000†
Total hardness	0.711	0.014•
Calcium	0.553	0.078
Magnesium	0.149	0.661
Sodium	0.881	0.000†
Potassium	0.500	0.117
Phosphorus	0.999	0.000†
Iron	0.805	0.003†
Fluoride	0.013	0.969
Chloride	0.989	0.000
Free Ammonia	0.980	0.000†
Nitrate	0.864	0.001†
Sulphate	0.973	0.000†

- Negative correlation • : Significance at the 0.05 level † : Significance at the 0.01 level

Fig.33 Free Ammonia content of the water in control and probiotic experiment ponds of *M. rosenbergii* culture

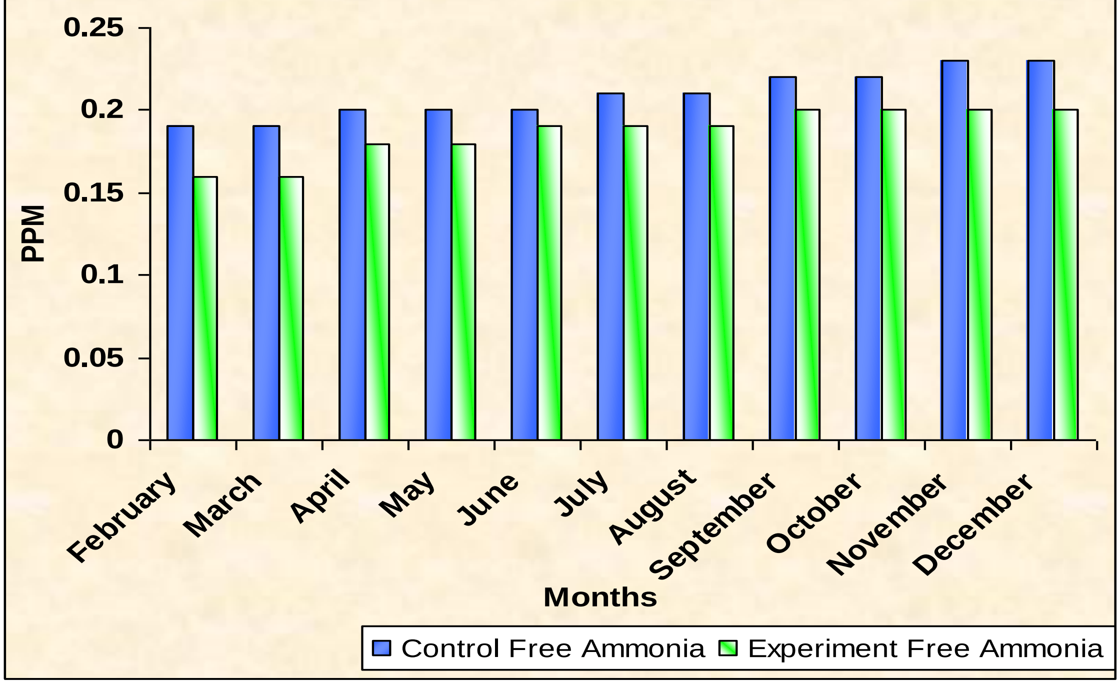
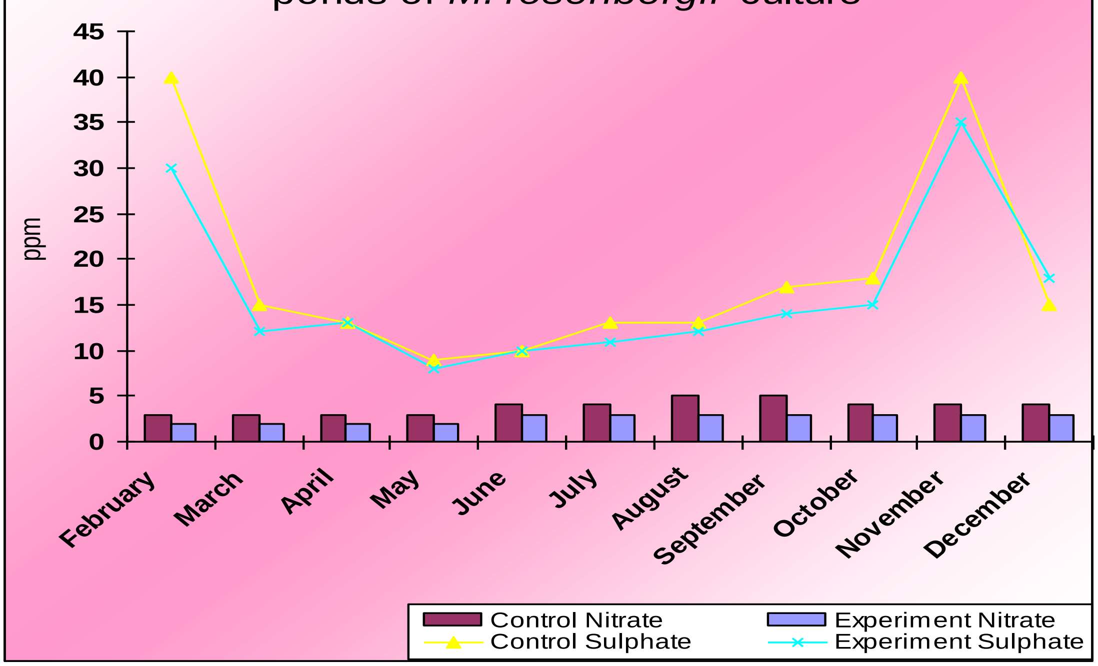


Fig.34 Nitrate and Sulphate content of the water in control and probiotic experiment ponds of *M. rosenbergii* culture



fluctuation during the study period (fig. 31). Nutrient such as calcium, magnesium, sodium, potassium, sulphates showed normal range. However, fluoride showed higher range in the month of April, June, July, August, September, October (1.5 ppm) in control pond. Phosphorous, iron and nitrate showed normal range in all the months. Chloride, Free ammonia, Nitrate and Sulphate content of the water shows fluctuation in control and probiotic experiment pond during the study period (fig. 32, 33 and 34).

The‘t’ test values of control and probiotic experiment pond were presented in table 4a. In the present experiment, very high mean difference values were recorded in total dissolved solids, calcium and fluoride showed significant and some values are found to be non significant. The positive and negative correlation co-efficient (r) values of physic-chemical parameters of control and experiment pond are presented in the table. 4b. Most of the parameters showed high correlation except fluoride (0.013) and magnesium (0.149)

2.4.4. Biological parameters:

2.4.4.1. Bacteria:

In the present study, the results of the soil bacterial analysis showed 7 and 15 genera in control and probiotic experiment pond (table 5 and 6) respectively from February to December 2008. *Actinobacter, Aeromonas, Pseudomonas*, *Bacillus*, *Enterococcus*, *Flavobacterium* and *Cornybacterim* were present in both the ponds. *Lactobacillus*, *Micrococcus*, *Rhodobacter*, *Enterobacter*, *Arthrobacter*, *Achromobacter*, *Acinetobacter* are present only in probiotic experiment pond. The bacterial load in the soil samples of control and experiment pond was presented in table 7. Higher bacterial load was recorded in August (5.3×10^4), July (7.1×10^5) months in control and probiotic experiment pond respectively, whereas very low bacterial load were recorded in control pond in the month of April (1.2 x 10^3) and in probiotic experiment pond in the month of October (3.5 x 10^3).

2.4.4.2. Fungi:

In the present study, the results of the fungal analysis showed 28 and 35 genera in control and probiotic experiment pond (table 8 and 9) respectively from February to December 2008. *Aspergillus* is the dominant genera have 10 species in control pond, next to this, *Pencillium* genera represents 3 species. The *Curvularia*, *Drechslara* and *Fusarium* genera represents 2 species. However, the other genera are represent only one species. Monthly analysis of mesophilic fungal species composition in control and probiotic experiment pond was presented in table 10, 11 and fig. 37. In probiotic experiment pond *Absidia*, *Cladosporium* and *Geotridum* and *Mucor* are additional genera recorded. *A. tamari*, *A. chavallari* and *A. ohraceus* were the additional species recorded in probiotic experiment pond. *A. terreus*, *A. fumigatus* and *A. flavipes* were the most commonly found mesophilic fungus both in control pond and probiotic experimental pond. *A. niger* is the higher mesophilic fungi observed in both ponds.

Further, mesophiic fungi total composition, percentage contribution and CFU of control and probiotic experiment pond were recorded in table. 12, fig. 35 and table. 13, fig.36 respectively. In the present study, 50.39% and 45.82% of *Aspergillus* genera contributed in control pond and probiotic experiment pond respectively. Among this, *A. nidulus*, contributed 6.93%, in control pond where as in probiotic experimental pond the *A. niger* (9.42%) contributed higher percentage. The sporulative and yeast colonies contributed 3.60% and 2.25% in control pond whereas 3.05% and 2.81% in probiotic experimental pond respectively during the study period.

Table-5: Analysis of soil Bacteria in the control pond of freshwater prawn *M. rosenbergii* culture (February - December 2008)

S.no	Bacteria	Months										
		February	**March**	**April**	**May**	**June**	**July**	**August**	**September**	**October**	**November**	**December**
1.	*Actinobacter*	+	-	+	+	-	-	+	+	-	+	+
2.	*Aeromonas*	+	+	+	_	+	+	-	-	+	+	-
3.	*Pseudomonas*	+	+	+	-	+	+	+	+	+	+	-
4.	*Bacillus*	+	+	+	+	+	+	+	-	-	+	+
5.	*Enterococcus*	+	+	-	-	+	+	+	-	-	+	-
6.	*Flavobacterium*	-	+	+	-	-	+	+	+	-	-	+
7.	*Cornybacterium*	-	-	+	+	+	+	-	-	+	+	+

+ : Present - : Absent

Table-6: Analysis of soil Bacteria in the Probiotic experiment pond of freshwater prawn *M. rosenbergii* culture (February - December 2008)

S.no	Bacteria	Months										
		Feb	Mar	April	May	June	July	Aug	Sep	Oct	Nov	Dec
1.	*Actinobacter*	+	+	+	-	-	+	+	+	+	-	-
2.	*Aeromonas*	+	+	-	-	+	+	+	-	+	+	+
3.	*Pseudomonas*	+	+	-	-	+	+	+	+	+	+	+
4.	*Bacillus*	+	+	+	+	-	-	+	-	-	+	+
5.	*Lactobacillus*	+	+	+	-	-	+	-	+	+	+	-
6.	*Flavobacterium*	+	+	-	+	+	+	+	-	-	+	+
7.	*Cornybacterium*	+	+	+	+	-	-	+	+	+	+	+
8.	*Enterococcus*	+	+	+	+	-	+	+	+	-	-	+
9.	*Micrococcus*	+	+	+	-	+	+	-	-	+	-	+
10.	*Rhodococcus*	+	+	-	-	+	+	+	+	-	+	+
11.	*Rhodobacter*	+	-	+	+	-	-	+	+	+	+	-
12.	*Enterobacter*	+	+	-	-	+	+	+	+	-	+	+
13.	*Arthrobacter*	-	+	+	+	+	-	-	+	+	-	+
14	*Achromobacter*	+	+	-	-	+	+	+	-	-	+	-
15	*Acinetobacter*	-	+	+	-	-	+	-	+	+	+	-

Table- 7: Bacterial load in control and probiotic experiment pond of freshwater prawn *M. rosenbergii* culture (February - December 2008)

Months	Control (cfu/gm)	Experiment (cfu/gm)
February	2.6×10^3	4.4×10^4
March	2.2×10^4	6.3×10^5
April	1.2×10^3	4.9×10^6
May	2.1×10^3	4.7×10^6
June	4.3×10^5	5.9×10^6
July	5.1×10^5	7.1×10^5
August	5.3×10^4	6.2×10^6
September	4.6×10^4	5.8×10^6
October	2.2×10^4	3.5×10^4
November	2.6×10^3	4.4×10^4
December	4.3×10^5	5.8×10^6

Cfu : Colony forming unit

Table 8: Identification of mesophilic fungal species composition in serial dilution method of control pond of freshwater prawn *M. rosenbergii* culture (CFU)

S.no	Fungus	No. of plates/colonies						Total	PC	CFU/gm soil
		Plate1	Plate2	Plate3	Plate4	Plate5	Plate6			
1	*Aspergillus niger*	12	10	11	10	12	10	65	17.01	6500
2	*A. terreus*	3	3	4	3	3	4	20	5.23	2000
3	*A .fumigatus*	4	3	5	3	3	4	22	5.75	2200
4	*A. flavipes*	2	3	3	3	3	3	17	4.45	1700
5	*A .nidulus*	5	5	5	4	5	4	28	7.32	2800
6	*A. versicolor*	2	2	3	2	3	2	14	3.66	1400
7	*A. glaucus*	3	3	3	2	3	2	16	4.18	1600
8	*A. ustus*	2	1	2	2	1	2	10	2.61	1000
9	*Curvularia lunata*	1	-	1	-	1	-	3	0.78	300
10	*C.tuberculata*	3	2	2	2	2	2	13	3.40	1300
11	*Pencillium Oxalium*	10	10	9	10	9	10	58	15.18	5800
12	*F. solani*	1	2	1	2	2	2	10	2.61	1000
13	*Acremonicim*	2	2	2	2	2	3	13	3.40	1300
14	*Humicola grisea*	3	3	3	3	2	3	17	4.45	1700
15	*Nigrospora sphaeriea*	2	1	2	2	1	2	10	2.61	1000
16	*Alternaria alternata*	3	3	3	4	3	4	20	5.23	2000
17	*Rhizopus stolonifer*	1	1	2	1	2	1	8	2.09	800
18	*Drechslera sp.*	2	2	1	2	1	2	10	2.61	1000
19	*D. halodas*	1	1	1	2	2	1	8	2.09	800
20	Non-sporulation	1	2	2	2	1	2	10	2.61	1000
21	Yeast colonies	2	2	1	1	2	2	10	2.61	1000

Cfu : Colony forming unit

Table 9: Identification of mesophilic fungal species composition in serial dilution method of Probiotic experiment pond of freshwater prawn *M. rosenbergii* culture (CFU)

S.no	Species	No. of plates/colonies						Total	PC	CFU/gm Soil
		Plate1	Plate2	Plate3	Plate4	Plate5	Plate6			
1	*Aspergillus niger*	7	7	6	7	7	6	40	9.85	4000
2	*A. terreus*	4	3	3	4	3	3	20	4.92	2000
3	*A. flavus*	2	2	3	2	3	2	14	3.44	1400
4	*A. fumigatus*	2	3	2	2	2	2	13	3.20	1300
5	*A. japonicus*	1	-	1	-	1	-	3	0.73	300
6	*A. flavipes*	3	2	3	2	3	2	15	3.69	1500
7	*A. tamarii*	3	3	3	3	3	3	18	4.43	1800
8	*A. cohraceus*	1	1	1	1	1	1	6	1.47	600
9	*A. chevalteri*	2	3	2	3	2	2	14	3.44	1400
10	*A. nidulus*	2	2	2	1	1	2	10	2.46	1000
11	*A. versicolor*	2	2	2	2	2	2	12	2.95	1200
12	*A. glaucus*	1	1	1	2	1	2	8	1.97	800
13	*A. ustus*	5	5	5	5	5	5	30	7.38	3000
14	*Pencillium Oxalium*	3	2	3	2	3	2	15	3.69	1500
15	*Curvularia lunata*	2	1	2	1	2	2	10	2.46	1000
16	*C.tuberculata*	1	1	-	1	-	-	3	0.73	300
17	*Scopularis brevicaulis*	1	1	2	1	1	1	7	1.72	700
18	*Trichoderma longibrachiatum*	2	2	2	1	1	2	10	2.46	1000
19	*Absidia corymbifera*	2	1	1	2	2	2	10	2.46	1000
20	*Fusarium oxysporium*	2	1	2	1	1	1	8	1.97	800
21	*Alternaria alternata*	2	2	3	2	2	2	13	3.20	1300
22	*Rhizopus stolonifer*	1	1	1	1	2	2	8	1.97	800
23	*Drechslera sp.*	3	3	3	4	3	3	19	4.67	1900
24	*D. halodas*	2	2	2	3	2	3	14	3.44	1400
25	*Acremonicim*	2	3	2	3	2	3	15	3.69	1500
26	*Humicola grisea*	2	2	2	2	2	2	12	2.95	1200
27	*Nigrospora sphaeriea*	2	3	2	3	2	3	15	3.69	1500
28	*Geotridum cardium*	1	1	1	1	1	2	7	1.72	700
29	*Cladosporium sphaerospermum*	3	2	2	2	2	2	13	3.20	1300
30	*Mucor racemosus*	2	1	2	1	2	1	9	2.21	900
31	Non-sporulation	1			1		1	3	0.73	300
32	Yeast colonies	2	2	2	2	2	2	12	2.95	1200
	Total	71	65	68	68	66	68	406		

Cfu : Colony forming unit

Fig.35 Percentage composition of Fungal species in probiotic control pond of *M. rosenbergii* culture

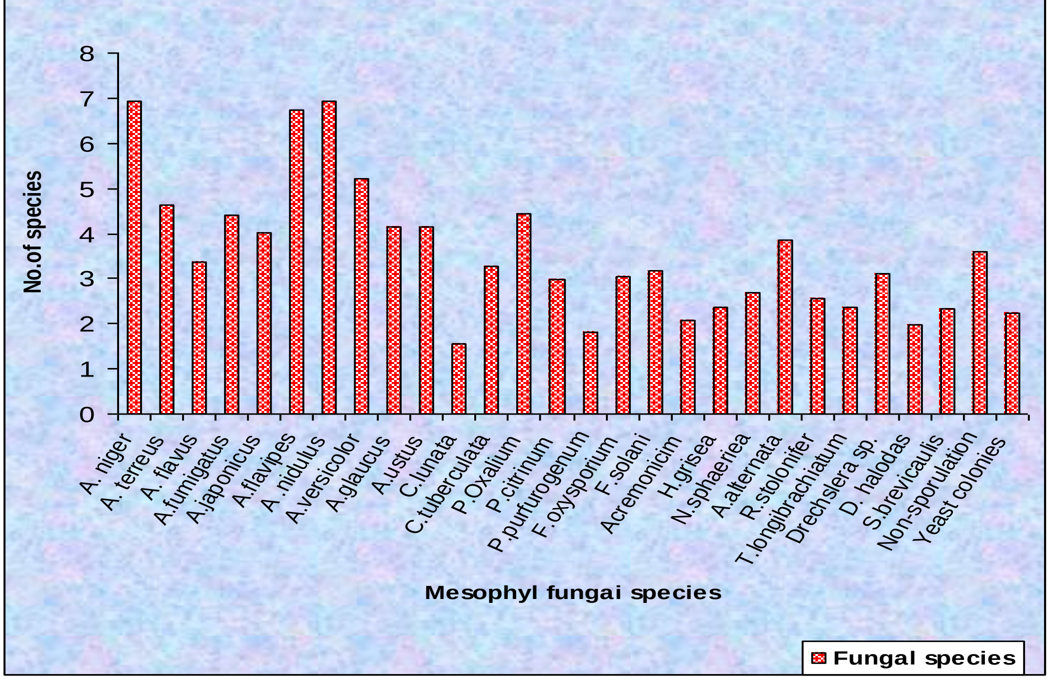


Fig.36 Percentage composition in Mesophyl fungal colonies in probiotic experiment pond of *M. rosenbergii* culture

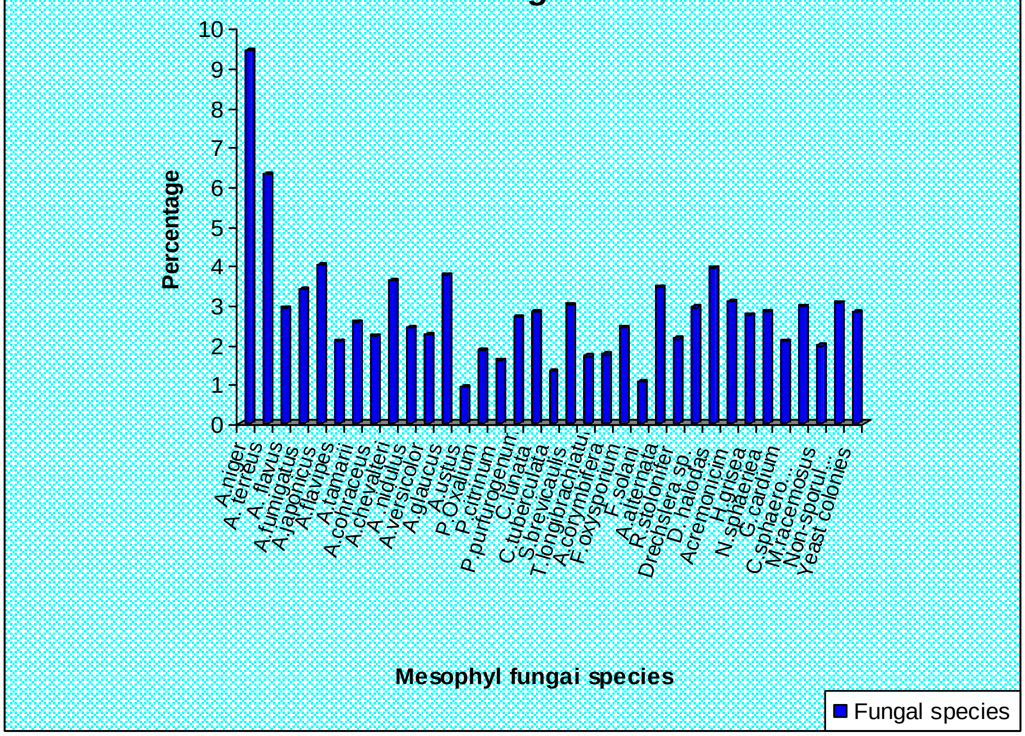


Table-10: Analysis of mesophilic soil fungal in the control pond of freshwater prawn *M. rosenbergii* culture (February - December 2008)

S.No.	Species	Feb	Mar	April	May	June	July	Aug	Sept	Oct	Nov	Dec
1.	*Aspergillus niger*	+	+	+	+	+	+	-	+	+	+	-
2.	*A. terreus*	+	+	+	+	+	+	+	+	+	+	+
3.	*A. flavus*	-	+	+	+	-	+	+	+	+	+	+
4.	*A. fumigatus*	+	+	+	+	+	+	+	+	+	+	+
5.	*A. japonicus*	-	+	+	+	-	+	+	+	+	+	+
6.	*A. flavipes*	+	+	+	+	+	+	+	+	+	+	+
7.	*A .nidulus*	+	+	+	+	+	-	+	+	+	-	+
8.	*A. versicolor*	+	+	+	+	+	+	-	+	+	+	+
9.	*A. glaucus*	+	+	+	-	+	+	+	-	+	+	+
10.	*A. ustus*	+	+	-	-	+	+	-	+	+	+	+
11.	*Curvularia lunata*	+	+	+	+	+	+	-	-	+	-	-
12.	*C. tuberculata*	+	+	+	+	+	-	+	+	+	-	+
13.	*Pencillium Oxalium*	+	+	+	+	-	-	+	+	-	+	+
14.	*P. citrinum*	-	+	+	+	+	+	-	-	+	+	-
15.	*P. purfurogenum*	-	+	-	+	+	+	+	-	+	+	-
16	*Fusarium oxysporium*	-	+	+	-	+	+	+	-	+	+	+
17	*F. solani*	+	+	-	-	+	+	-	+	+	+	+
18	*Acremonicim*	+	+	+	+	-	-	+	-	+	-	+
19	*Humicola grisea*	+	+	-	-	+	-	-	+	-	+	+
20	*Nigrospora sphaeriea*	+	+	+	+	-	+	+	+	-	+	+
21	*Alternaria alternata*	+	+	+	-	-	+	+	-	+	+	+
22	*Rhizopus stolonifer*	+	-	+	+	+	-	+	+	+	-	-
23	*Trichoderma longibrachiatum*	-	+	+	+	-	-	+	+	-	+	+
24	*Drechslera sp.*	+	+	+	+	+	-	-	+	+	-	+
25	*D. halodas*	+	-	-	+	+	-	-	+	-	-	+
26	*Scopularipis brevicaulis*	-	-	-	+	+	-	+	+	-	+	+
27	Non-sporulation	+	+	+	+	+	-	+	+	+	-	+
28	Yeast colonies	+	+	+	+	+	+	+	+	+	+	+

Table-11: Analysis of mesophilic soil fungal in the probiotic experiment pond of freshwater prawn *M. rosenbergii* culture (February - December 2008)

S.No.	Species	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec
1.	*Aspergillus niger*	+	+	+	+	+	+	+	+	+	+	+
2.	*A. terreus*	+	+	+	-	+	+	+	+	+	+	+
3.	*A. flavus*	+	+	+	+	+	+	-	-	+	+	-
4.	*A. fumigatus*	+	+	+	+	+	-	+	+	+	+	+
5.	*A. japonicus*	+	+	+	+	-	+	+	+	+	+	+
6.	*A. flavipes*	+	+	+	+	-	+	-	+	-	+	+
7.	*A. tamarii*	+	-	-	+	+	-	+	+	-	+	+
8.	*A. cohraceus*	+	-	+	+	+	+	-	-	+	+	-
9.	*A. chevalteri*	+	+	+	-	+	+	+	-	+	+	+
10.	*A .nidulus*	+	+	-	-	+	+	-	+	-	+	-
11.	*A. versicolor*	+	+	+	-	+	+	-	+	-	+	+
12.	*A. glaucus*	+	-	+	+	+	-	+	+	-	+	+
13.	*A. ustus*	+	-	+	+	-	-	-	-	-	-	-
14.	*Pencillium Oxalium*	+	+	-	-	+	+	-	-	+	+	-
15.	*P.citrinum*	-	+	-	+	+	+	+	-	+	+	-
16	*P.purfurogenum*	-	+	+	-	+	+	+	-	+	+	+
17	*Curvularia lunata*	+	+	-	-	+	+	-	+	+	+	+
18	*C.tuberculata*	+	+	-	-	+	-	+	-	+	+	-
19	*Scopularis brevicaulis*	+	+	-	+	+	+	-	+	+	+	+
20	*Trichoderma longibrachiatum*	+	+	+	+	-	-	+	+	-	-	+
21	*Absidia corymbifera*	+	+	+	-	-	+	-	-	+	-	-
22	*Fusarium oxysporium*	+	-	+	+	+	-	+	+	-	+	-
23	*F.solani*	--	+	-	+	-	+	-	+	-	-	-
24	*Alternaria alternata*	+	+	+	+	+	+	+	+	+	-	+
25	*Rhizopus stolonifer*	+	-	+	+	+	-	-	+	-	-	+
26	*Drechslera sp.*	+	+	-	+	+	-	+	+	-	+	+
27	*D. halodas*	+	+	+	+	-	+	+	-	+	+	+
28	*Acremonicim*	+	+	-	+	+	+	-	+	+	-	-

29	*Humicola grisea*	+	+	+	+	+	-	+	+	-	+	-
30	*Nigrospora sphaeriea*	+	+	-	+	+	+	-	-	+	-	+
31	*Geotridum cardium*	+	+	-	+	+	-	+	+	+	-	-
32	*Cladosporium sphaerospermum*	+	-	+	+	+	+	+	-	+	+	+
33	*Mucor racemosus*	+	+	-	+	+	-	+	-	-	-	-
34	Non-sporulation	+	+	+	+	+	-	+	+	+	-	+
35	Yeast colonies	+	+	+	+	+	+	+	+	+	+	+

Table- 12: Mesophile fungi total species composition and percentage contribution in the control pond of freshwater prawn *M. rosenbergii* culture (February - December 2008)

S.No.	Species	Feb	Mar	Apr	May	June	July	Aug	Sept	Oct	Nove	Dece	Tot	Pc	CFU
1.	*Aspergillus niger*	65	72	15	17	13	8	-	20	17	13	-	240	6.76	24000
2.	*A. terreus*	20	15	8	3	20	40	6	7	15	17	13	164	4.62	16400
3.	*A. flavus*	-	10	17	13	-	10	15	20	17	13	5	120	3.38	12000
4.	*A.fumigatus*	22	3	15	7	15	17	13	15	20	17	13	157	4.42	15700
5.	*A.japonicus*	-	25	17	13	-	10	15	20	17	13	13	143	4.03	14300
6.	*A.flavipes*	17	8	65	72	13	10	15	8	15	8	8	239	6.73	23900
7.	*A .nidulus*	28	75	55	17	13	-	10	15	20	-	13	246	6.93	24600
8.	*A.versicolor*	14	32	25	22	17	13	-	10	15	20	17	185	5.21	18500
9.	*A.glaucus*	16	40	8	-	20	17	13	-	10	15	8	147	4.14	14700
10.	*A.ustus*	10	5	-	-	15	20	-	22	20	25	30	147	4.14	14700
11.	*Curvularia lunata*	3	8	8	7	10	5	-	-	15	-	-	56	1.57	5600
12.	*C.tuberculata*	13	10	15	8	13	-	10	17	13	-	17	116	3.26	11600
13.	*Pencillium Oxalium*	58	42	10	5	-	-	15	20	-	3	5	158	4.45	15800
14.	*P.citrinum*	-	20	22	25	15	10	-	-	8	6	-	106	2.98	10600
15.	*P.purfurogenum*	-	2	-	4	5	20	15	-	10	8	-	64	1.80	6400
16	*Fusarium oxysporium*	-	5	8	-	20	17	13	-	10	15	20	108	3.04	10800
17	*F.solani*	10	5	-	-	15	20	-	23	10	17	13	113	3.18	11300
18	*Acremonicim*	13	10	15	8	-	-	10	-	8	-	10	74	2.08	7400
19	*Humicola grisea*	17	13	-	-	15	-	-	10	-	13	16	84	2.36	8400
20	*Nigrospora sphaeriea*	10	8	5	8	-	20	17	13	-	10	7	98	2.70	9800
21	*Alternaria alternata*	20	17	5	-	-	15	20	-	23	20	17	137	3.86	13700
22	*Rhizopus stolonifer*	8	-	20	17	13	-	10	15	8	-	-	91	2.56	9100
23	*Trichoderma longibrachiatum*	-	10	15	8	-	-	10	15	-	17	9	84	2.36	8400
24	*Drechslera sp.*	10	15	20	17	8	-	-	17	13	-	10	110	3.10	11000
25	*D. halodas*	8	-	-	17	13	-	-	17	-	-	15	70	1.97	7000
26	*Scopularipis brevicaulis*	-	-	-	15	12	-	10	15	-	17	14	83	2.33	8300
27	Non-sporulation	10	15	20	17	8	-	20	17	13	-	8	128	3.60	12800
28	Yeast colonies	10	9	8	11	4	2	3	8	10	8	7	80	2.25	8000

P.C. : Percentage contribution - : Absent

Table- 13 Mesophile fungi total species composition and percentage contribution in the probiotic experiment pond of freshwater prawn *M. rosenbergii* culture (February - December 2008)

S. No	Species	Feb	Mar	Apr	May	June	July	Aug	Sep	Oct	Nov	Dec	Total	Pc	CFU
1.	*Aspergillus niger*	40	65	72	13	10	15	32	25	55	20	32	379	9.42	37900
2.	*A. terreus*	20	15	85	-	20	17	13	45	10	15	13	253	6.29	25300
3.	*A. flavus*	14	38	8	7	10	5	-	-	15	20	-	117	2.91	11700
4.	*A. umigatus*	13	10	15	8	13	-	10	17	13	20	17	136	3.38	13600
5.	*A.japonicus*	3	25	17	13	-	10	15	20	17	13	28	161	4.00	16100
6.	*A.flavipes*	15	8	6	7	-	10	-	13	-	10	15	84	2.08	8400
7.	*A.tamarii*	18	-	-	17	13	-	10	15	-	17	13	103	2.56	10300
8.	*A.cohraceus*	6	-	15	17	13	8	-	-	17	13	-	89	2.21	8900
9.	*A.chevalteri*	14	40	8	-	20	17	13	-	10	15	8	145	3.60	14500
10.	*A .nidulus*	10	5	-	-	15	20	-	22	-	25	-	97	2.41	9700
11.	*A.versicolor*	12	17	13	-	10	13	-	10	-	8	7	90	2.23	9000
12.	*A.glaucus*	8	-	72	13	10	-	10	15	-	15	8	151	3.75	15100
13.	*A.ustus*	30	-	2	5	-	-	-	-	-	-	-	37	0.92	3700
14.	*Pencillium Oxalium*	15	20	-	-	15	10	-	-	8	6	-	74	1.84	7400
15.	*P.citrinum*	-	2	-	4	5	20	15	-	10	8	-	64	1.59	6400
16	*P.purfurogenum*	-	5	8	-	20	17	13	-	10	15	20	108	2.68	10800
17	*Curvularia lunata*	10	5	-	-	15	20	-	23	10	17	13	113	2.81	11300
18	*C.tuberculata*	3	2	-	-	13	-	10	-	8	17	-	53	1.31	5300
19	*Scopularis brevicaulis*	7	13	-	10	15	20	-	10	17	13	16	121	3.00	12100
20	*Trichoderma longibrachiatum*	10	8	5	8	-	-	17	13	-	-	7	68	1.69	6800
21	*Absidia corymbifera*	10	17	5	-	-	15	-	-	23	-	-	70	1.74	7000
22	*Fusarium oxysporium*	8	-	20	17	13	-	17	13	-	10	-	98	2.43	9800
23	*F.solani*	--	10	-	8	-	9	-	15	-	-	-	42	1.04	4200
24	*Alternaria alternata*	13	15	20	17	8	6	20	17	13	-	10	139	3.45	13900
25	*Rhizopus stolonifer*	8	-	20	17	13	-	-	13	-	-	15	86	2.13	8600
26	*Drechslera sp.*	19	13	-	10	15	-	10	15	-	17	19	118	2.93	11800
27	*D. halodas*	14	15	20	32	-	17	13	-	10	15	22	158	3.93	15800
28	*Acremonicim*	15	32	-	22	17	13	-	10	15	-	-	124	3.08	12400
29	*Humicola grisea*	12	20	18	17	13	-	10	15	-	5	-	110	2.73	11000
30	*Nigrospora sphaeriea*	15	13	-	10	15	22	-	-	25	-	14	114	2.83	11400
31	*Geotridum cardium*	7	13	-	10	15	-	17	17	5	-	-	84	2.08	8400

32	*Cladosporium sphaerospermum*	13	-	10	15	15	17	13	-	10	15	11	119	2.96	11900
33	*Mucor racemosus*	9	13	-	10	15	-	32	-	-	-	-	79	1.96	7900
34	Non-sporulation	3	15	20	17	8	-	20	17	13	-	10	123	3.05	12300
35	Yeast colonies	12	11	10	15	8	4	3	12	17	11	10	113	2.81	11300

P.C. : Percentage contribution - : Absent

Table- 13a: Mesophile fungal population in control and probiotic experiment (t-test analysis)

Parameters	Ponds	Mean ± SE *	t value	p-value
Monthwise total	Control	322.545 ± 21.054	15.247	0.000
	Experiment	365.454 ± 17.736	20.605	0.000
Species wise total	Control	126.714 ± 9.875	12.831	0.000
	Experiment	114.857 ± 11.158	11.158	0.000

Significance at the 5 % level (P<0.05)

Table-13b. Correlation values of Mesophile fungal population in control and probiotic experiment pond (r-values)

Mesophile fungal	Correlation (r-values)	Significance
Monthwise total population	0.891	0.000†

† : Significance at 0.01 level

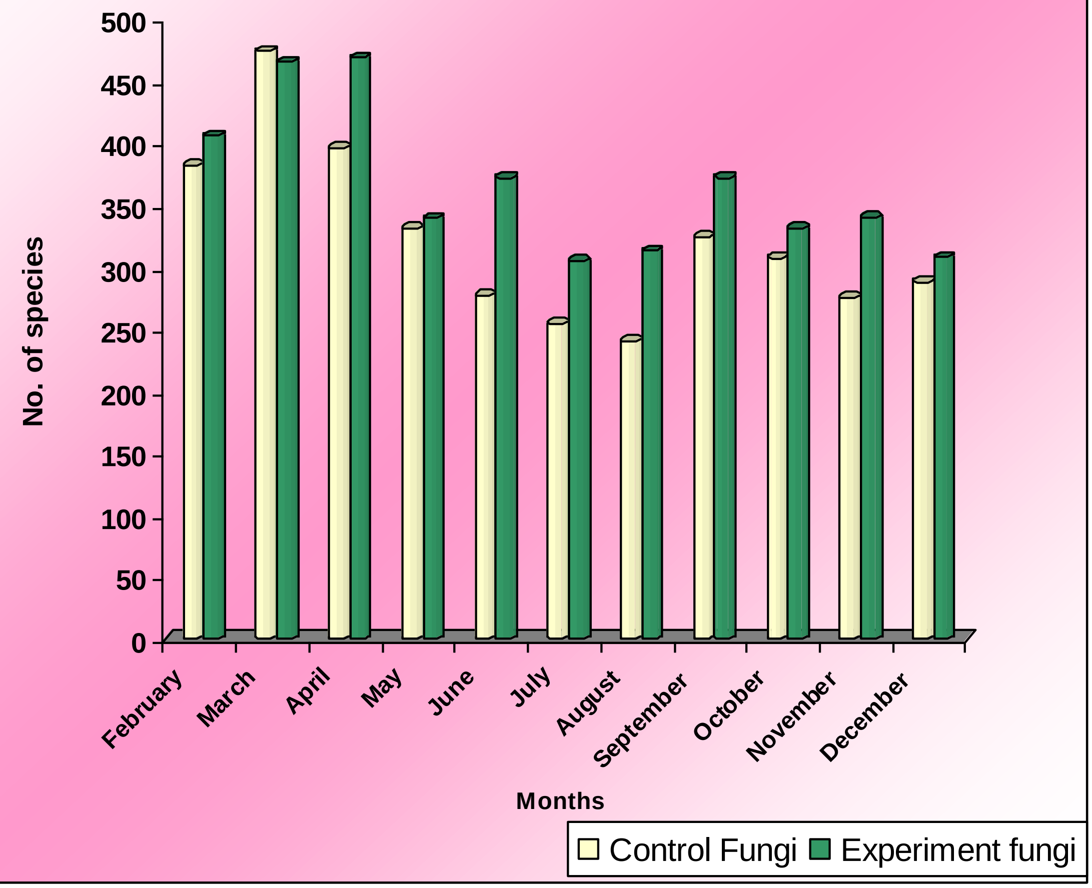


Fig.37 Monthly total fungal colonies of control and probiotic experiment ponds of *M. rosenbergii* culture

Higher CPU/g (colony forming unit) of soil was recorded with regard to *A. terreus*, *A. fumigatus* and *A. flavipes* than that of the other species of this genus throughout the culture period in control and experiment pond. In the present study, the monthwise mean population of probiotic experimental pond showed higher (365.45 ± 17.736) values while specieswise total mean value showed higher (126.71 ± 9.875) in control pond but the values showed statistically significant ($P<0.05$) in both the ponds with positive correlation co-efficient ($r=0.891$) (table 13a, b**)**.

2.4.4.3. Phytoplankton and Zooplankton:

In the present observations, analysis of phytoplankton samples showed the occurrence of 26 genera in control pond and 34 genera in probiotic experiment pond. 26 genera were occurred in both the ponds are same while 9 new genera found in experiment pond only (table. 14).

Qualitative analysis of zooplankton showed 10 species of rotifers, 11 species of cladocerans, 7 species of copepods and 2 species of ostracods in control pond (table. 15) and 11 species of rotifers, 12 species of cladocerans, 7 species of copepods and 2 species of ostracods in probiotic experiment pond (table.16, fig.39).

Total zooplankton population in probiotic experimental pond showed higher number when compared to control pond, but in monthwise higher percentage was in July (control pond) and May (probiotic experiment pond) month sample during the study period. In the present study, higher number of rotifers, cladocerans, copepods and ostracods were noticed in probiotic experimental pond in the month of July (427nos), July (458nos), May (325nos) and June (243nos) whereas in control pond during the month of July (351nos), July (415nos), May (235nos) and June (231nos) months respectively **(**table.17 fig.38, 39**).** The order of different groups of zooplankton contribution in control

Table- 14: Diversity of Phytoplankton in control and probiotic experiment pond of freshwater prawn *M. rosenbergii* culture

Control	Probiotic experiment
Anabaena sp.	Anabaena sp.
Chaetophora sp.	Ankistroclesmus sp.
Chlamydomonas	Capsosira sp
Chlorella sp.	Chaetophora sp.
Cladophora	Chamaesiphon sp.
Closterium sp.	Chlamydomonas
Cymbella sp.	Chlorella sp.
Desmidium sp.	Cladophora
Diatoma	Closterium sp.
Eugleana	Coelospherium sp.
Fragillaria	Cyclotella sp.
Gleocapsa sp.	Cymbella sp.
Microcystis sp.	Desmidium sp.
Microsteries sp.	Diatoma
Navicula sp.	Eugleana
Nitella sp.	Fragillaria
Nostoc sp.	Gleocapsa sp.
Oscillatoria sp.	Lyngbya sp.
Phormidium	Microcystis sp.
Pleodorina sp.	Microsteries sp.
Spirogyra sp.	Navicula sp.
Spirulina sp.	Nitella sp.
Tubellaria sp.	Nostoc sp.
Ulothrix sp.	Oscillatoria sp.
Volvox sp	Pecliostrum sp.
Zygnema sp.	Phormidium
	Pleodorina sp.

	Scenedesmus sp. Spirogyra sp. Spirulina sp. Tubellaria sp. *Ulothrix* sp. *Volvox* sp. *Zygnema* sp

Table- 15: Qualitative analysis of zooplankton in Control pond of freshwater prawn *M. rosenbergii* culture (February - December 2008)

Rotifers	Cladocerans	Copepods	Ostracods
Asplanchna sp. *Brachionus calyciflorus* *B. caudatus* *B. patulus* *B. quadrangularis* *B. rubens* *B. falcatus* *B. urcelaris* *Filinia sp.* *Keratella quadrata*	*Ceriodaphnia cornuta* *Ilyocryptus spinifer* *Diaphanosoma excisum* *D. sarsi* *Dunhevidia sp* *Leydigia sp.* *Monia macrocopa* *M. micrura* *Pleuroxus aduncus* *Simocephalus vetuloides* *Macrothrix sp.*	*Cryptocyclops bicolor* *Sinodiaptomus (Rhinediaptomus) Indicus* *Mesocyclops hyalinus* *M. aspericornis* *M. leukarti* *Heliodiaptomus viduus*	*Cypris sp.* *Stenocypris sp.*

Table- 16: Qualitative analysis of zooplankton in probiotic Experiment pond of freshwater prawn *M. rosenbergii* culture (February - December 2008)

Rotifers	Cladocerans	Copepods	Ostracods
Asplanchna sp. *Brachionus calyciflorus* *B. caudatus* *B. patulus* *B. quadrangularis* *B. rubens* *B. falcatus* *B. urcelaris* *B. forficula* *Filinia sp.* *Keratella quadrata*	*Ceriodaphnia cornuta* *Ilyocryptus spinifer* *Diaphanosoma excisum* *D. sarsi* *Dunhevidia sp* *Leydigia sp.* *Monia macrocopa* *M. micrura* *Pleuroxus aduncus* *Simocephalus vetuloides* *Daphnia carinata* *Macrothrix sp.*	*Cryptocyclops bicolor* *Sinodiaptomus (Rhinediaptomus) Indicus* *Mesocyclops hyalinus* *M. aspericornis* *M. leukarti* *Thermocyclops sp.* *Heliodiaptomus viduus*	*Cypris sp.* *Stenocypris sp.*

and probiotic experiment pond were cladocerans > rotifers> copepods > Ostracods > neonates > Copepodids & nauplii > eggs.

Mean ± S.E of total zooplankton population groups in different months of culture period in control and probiotic experiment pond are presented in table. 17a**.** Higher mean values were recorded in all the monthly samples in probiotic experiment pond except in October month in both the pond.

Monthwise zooplankton total population and their percentage contribution of control and experimental pond are presented in table.18 and fig 40, 41. Higher total percentage of 14.3, 12.1, 11.6 and 11.1 in the month of July, May, June and March in control pond whereas 14.1, 13.4, 11.3 and 10.7 percentage in the month of May, July, March and June in probiotic experiment pond respectively (fig.43). T test analysis of monthwise zooplankton of control and experiment pond was also tabulated in table 18a.

In the present experiment, the groupwise zooplankton population in control and probiotic experiment pond and their correlation co-efficient values are presented in table. 19, 19a and fig.42. The total copepods population and their percentage composition in control and probiotic experiment pond are presented in table.20 and fig.44. The high percentage of copepods was 13.82 and 15.80 in the month of May in control and probiotic experiment pond respectively. In our investigations higher numbers of copepods were noticed in the month of May in which *Crytocyclops bicolor* contributes higher species when compared to other species in control pond (table. 20). In the present study, the resulted copepods, mesocyclons genera contributed 31.05% and 35.89% in control pond and probiotic experiment pond, respectively (table. 20).

The noticed cladocerans population and their percentage contribution in control and experiment ponds were presented in table.21 and fig.45. Higher percentage

Table -17: Total zooplankton populations in the control and probiotic Experiment pond of freshwater prawn *M. rosenbergii* culture (February - December 2008)

	Feb		Mar		Apr		May		June		July		Aug		Sept		Oct		Nov		Dec	
Groups	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E	C	E
Copepods	79	168	198	218	183	145	235	325	198	251	168	183	162	162	125	156	112	147	124	145	116	156
Cladocerans	156	218	128	245	225	172	312	662	356	456	415	458	232	232	328	338	199	264	265	288	187	258
Rotifers	118	127	320	356	301	335	231	288	228	284	351	427	146	174	95	117	102	122	148	188	125	152
Ostracods	71	108	216	234	87	127	224	252	231	243	179	193	118	151	174	197	112	128	172	184	65	96
Eggs	112	128	126	182	68	77	0	15	75	-	78	88	195	215	112	125	121	128	165	188	114	182
Neonates	183	95	163	177	0	18	322	347	165	186	325	347	66	87	116	126	57	88	55	87	112	178
Copepodids &Nauplii	115	152	218	257	126	135	168	176	176	184	242	267	36	53	32	58	38	43	61	76	28	48

C- Control **E - Experiment**

Table 17a. Mean and SE values of total zooplankton population in control and probiotic experiment pond

Month	Ponds	Mean ± SE
February	Control	119.142 ± 14.988
	Experiment	142.285 ± 15.688
March	Control	195.571 ± 25.296
	Experiment	238.428 ± 22.680
April	Control	165.000 ± 36.255
	Experiment	144.142 ± 37.161
May	Control	248.666 ± 23.807
	Experiment	295.000 ± 74.442
June	Control	204.142 ± 32.142
	Experiment	267.33 ± 40.943
July	Control	251.429 ± 44.842
	Experiment	280.428 ± 51.591
August	Control	136.428 ± 26.138
	Experiment	153.428 ± 24.380
September	Control	140.285 ± 35.109
	Experiment	159.571 ± 33.701
October	Control	105.857 ± 19.87
	Experiment	131.428 ± 19.870
November	Control	141.428 ± 27.187
	Experiment	165.142 ± 21.147
December	Control	106.714 ± 18.824
	Experiment	152.857± 25.248

Number of samples 11

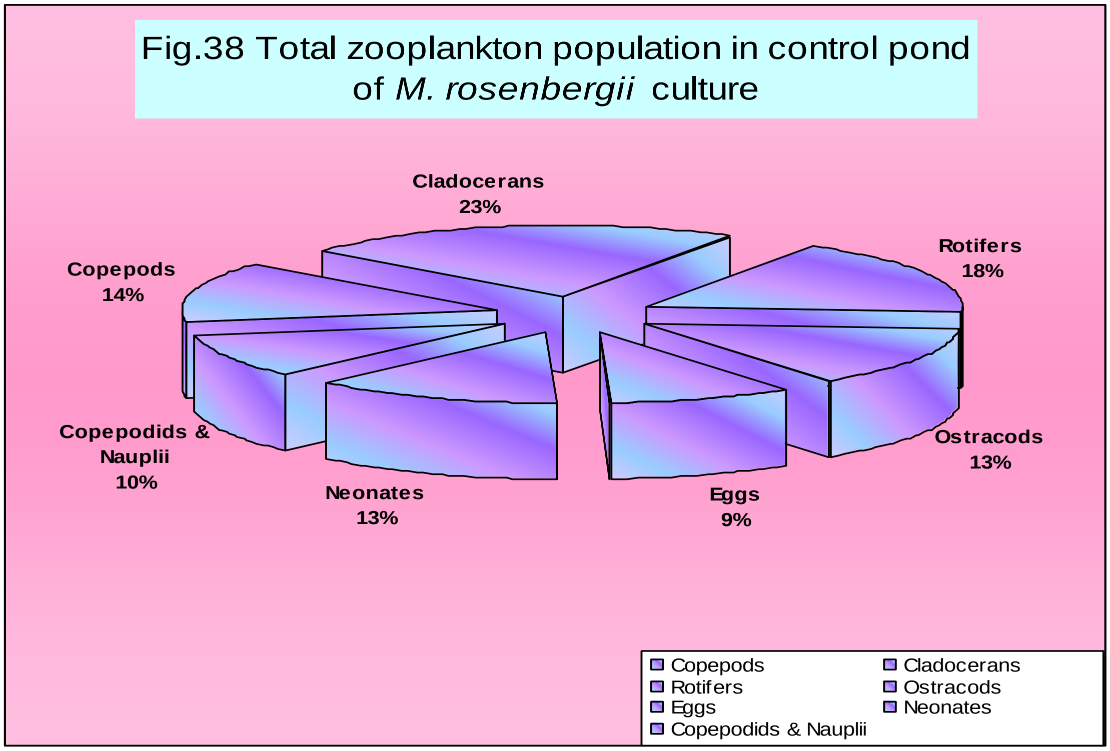


Fig.38 Total zooplankton population in control pond of M. rosenbergii culture
Cladocerans 23%
Rotifers 18%
Copepods 14%
Copepodids & Nauplii 10%
Neonates 13%
Eggs 9%
Ostracods 13%
Copepods
Cladocerans
Rotifers
Ostracods
Eggs
Neonates
Copepodids & Nauplii

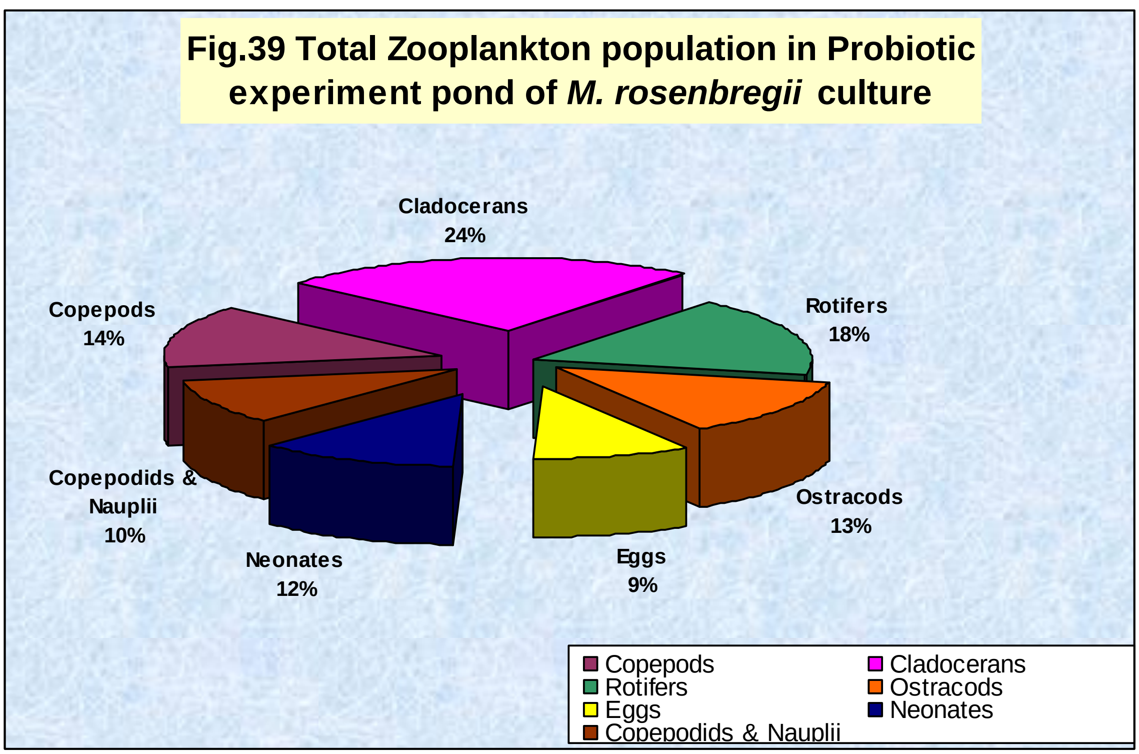


Fig.39 Total Zooplankton population in Probiotic experiment pond of M. rosenbregii culture
Cladocerans 24%
Copepods 14%
Rotifers 18%
Copepodids & Nauplii 10%
Ostracods 13%
Neonates 12%
Eggs 9%
Copepods
Cladocerans
Rotifers
Ostracods
Eggs
Neonates
Copepodids & Nauplii

Table- 18 Monthwise total zooplankton population and percentage in the control and probiotic experiment freshwater prawn *M. rosenbergii* culture pond

Months	Control		Probiotic Experiment	
	Total	percentage	Total	percentage
February	834	6.787	996	6.801
March	1369	11.141	1669	11.397
April	990	8.057	1009	6.870
May	1492	12.142	2065	14.102
June	1429	11.630	1604	10.734
July	1758	14.307	1963	13.405
August	955	7.772	1074	7.334
September	982	7.992	1117	7.607
October	741	6.030	920	6.282
November	990	8.057	1156	7.894
December	747	6.079	1070	7.307
	12287		14643	

Table-18a: Monthwise zooplankton in control and Probiotic experiment ponds (t-test analysis)

Groups	Ponds	Mean ± SE	T-test value	Significance
Copepods	Control	154.545 ± 14.175	10.902	0.000
	Experiment	186.909±17.096	10.932	0.000
Cladocerans	Control	254.818 ±26.9	9.473	0.000
	Experiment	326.454 ± 43.489	7.506	0.000
Rotifers	Control	196.818±28.225	6.973	0.000
	Experiment	173.909± 32.734	7.137	0.000
Ostracods	Control	149.909±18.634	8.045	0.000
	Experiment	233.636±16.713	10.405	0.000

Mean of 11 sample Significant at the 5% level (P<0.05)

Fig.40 Monthwise total zooplanktons percentage in control pond of *M. rosenbergii* culture

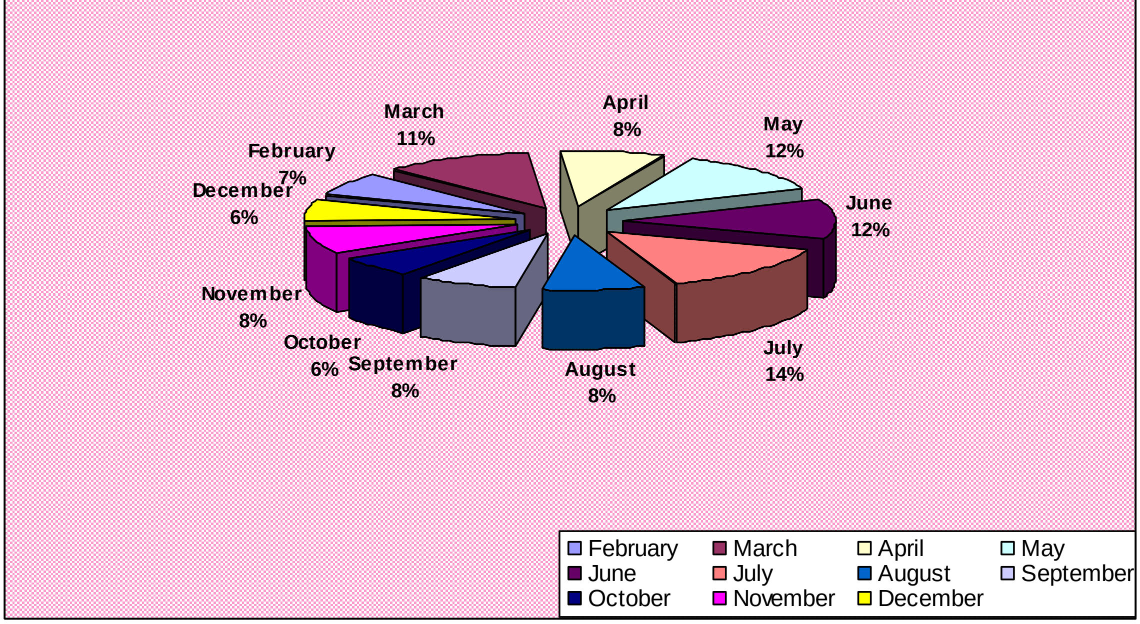


Fig.41 Monthwise total zooplankton percentage in Probiotic experiment pond of *M. rosenbergii* culture

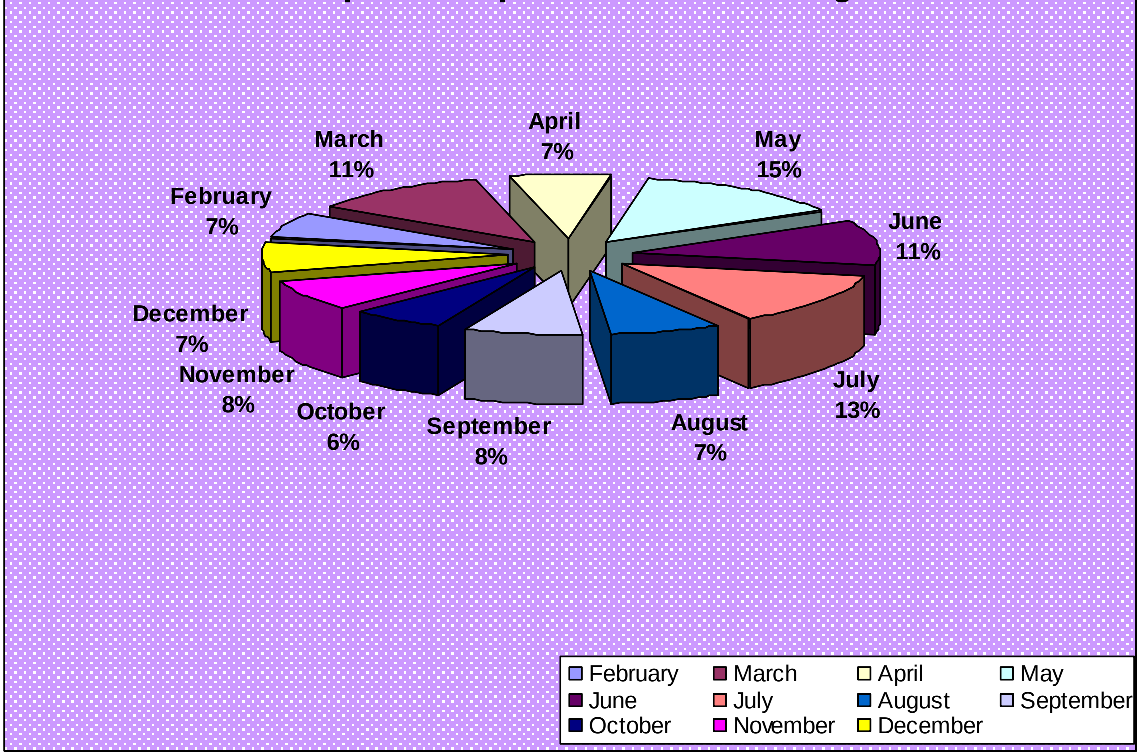


Fig.42 Groupwise zooplankton population in control and probiotic experiment ponds of *M. rosenbergii* culture

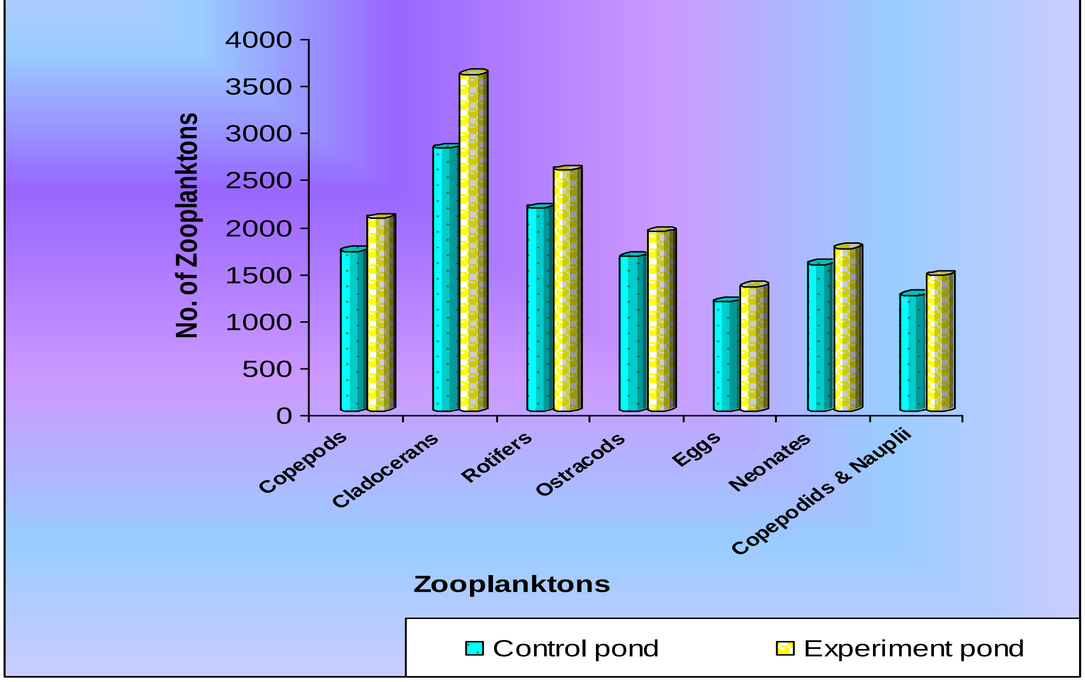


Fig.43 Monthwise zooplankton in control and probiotic experiment ponds of *M. rosenbergii* culture

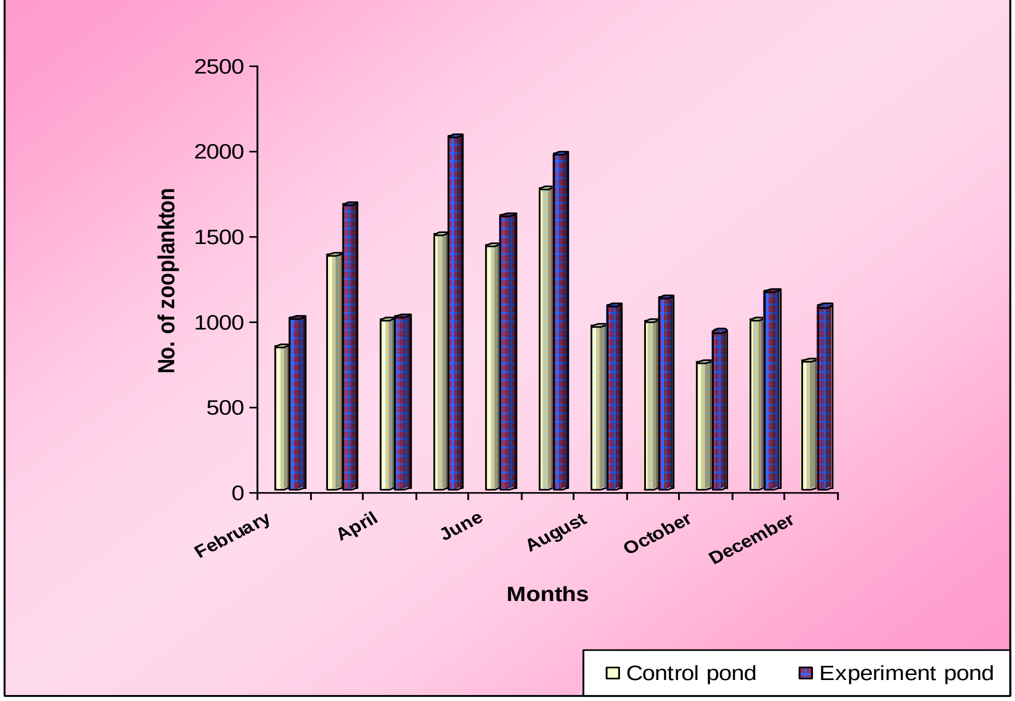


Table- 19: Groupwise zooplankton population and percentage in control and Probiotic Experiment pond

Months	Control		Probiotic Experiment	
	Total	**percentage**	**Total**	**percentage**
Copepods	1700	13.83	2056	14.04
Cladocerans	2803	22.81	3591	24.52
Rotifers	2165	17.62	2570	17.55
Ostracods	1649	13.42	1913	13.06
Eggs	1166	9.48	1328	9.06
Neonates	1564	12.72	1736	11.85
Copepodids & Nauplii	1240	10.09	1449	9.89
	12287		14643	

Table- 19a: Correlation coefficient (r-value) of total zooplankton species of control and probiotic experiment pond

Total Zooplankton species	Correlation (r-value)	Significance
Groupwise total	0.996	0.000
Monthwise total	1.896	0.000
Total Rotifer Population	0.992	0.000†
Total Copepods Population	0.762	0.006†
Total Cladoceran Population	0.697	0.017•
Total Ostracods Population	0.990	0.000†

- :Significance at 0.05 level † : Significance at 0.01 level

Table -20: Total copepods population and percentage in Control and Probiotic Experiment ponds

Months	Control		Probiotic Experiment	
	Total	percentage	Total	percentage
February	79	4.64	168	8.17
March	198	11.64	218	10.56
April	183	10.76	145	7.05
May	235	13.82	325	15.80
June	198	11.64	251	12.20
July	168	9.88	183	8.90
August	162	9.52	162	7.87
September	125	7.35	156	7.58
October	112	6.58	147	7.14
November	124	7.29	145	7.05
December	116	6.82	156	7.58
	1700		2056	

Table- 21: Cladocerans population and percentage in Control and Probiotic Experiment ponds

Months	Control		Probiotic Experiment	
	Total	percentage	Total	percentage
February	156	5.56	218	6.07
March	128	4.56	245	6.82
April	225	8.02	172	4.78
May	312	11.13	662	18.40
June	356	12.70	456	12.69
July	415	14.80	458	12.75
August	232	8.27	232	6.46
September	328	11.70	338	9.41
October	199	7.09	264	7.35
November	265	9.45	288	8.02
December	187	6.67	258	7.18
	2803		3591	

Table- 22: Total rotifer population and percentage in Control and Probiotic Experiment ponds

Months	Control		Probiotic Experiment	
	Total	percentage	Total	percentage
February	118	5.45	127	4.94
March	320	14.78	356	13.85
April	301	13.90	335	13.03
May	231	10.66	288	11.20
June	228	10.53	284	11.05
July	351	16.21	427	16.61
August	146	6.74	174	6.77
September	95	4.38	117	4.55
October	102	4.71	122	4.74
November	148	6.83	188	7.31
December	125	5.77	152	5.91
	2165		2570	

Table- 23: Total Ostracods population and percentage in control and probiotic Experiment ponds

Months	Control		Probiotic Experiment	
	Total	percentage	Total	percentage
February	71	4.30	108	5.64
March	216	13.09	234	12.23
April	87	5.27	127	6.63
May	224	13.58	252	13.17
June	231	14.00	243	12.70
July	179	10.85	193	10.08
August	118	7.15	151	7.89
September	174	10.55	197	10.29
October	112	6.79	128	6.69
November	172	10.43	184	9.61
December	65	3.94	96	5.01
	1649		1913	

Table- 24: Species wise total zooplankton in control and Probiotic experiment ponds (T-test analysis)

Class	Ponds	Mean ± SE	T-test value	Significance
Copepod sp.,	Control	238.571±49.723	4.798	0.003†
	Experiment	293.714 ± 44.132	6.655	0.000†
Cladoceran sp.,	Control	255.090±58.448	4.364	0.001†
	Experiment	299.333 ± 39.743	7.532	0.000†
Rotifers sp.,	Control	216.500±39.668	5.458	0.000†
	Experiment	233.636 ± 23.019	10.149	0.001†
Ostracods sp.,	Control	824.500±214.5	3.844	0.162
	Experiment	956.500 ±14.5	6.596	0.010•

Number of samples 11 • : Significance at 0.05 level † : Significance at 0.01 level

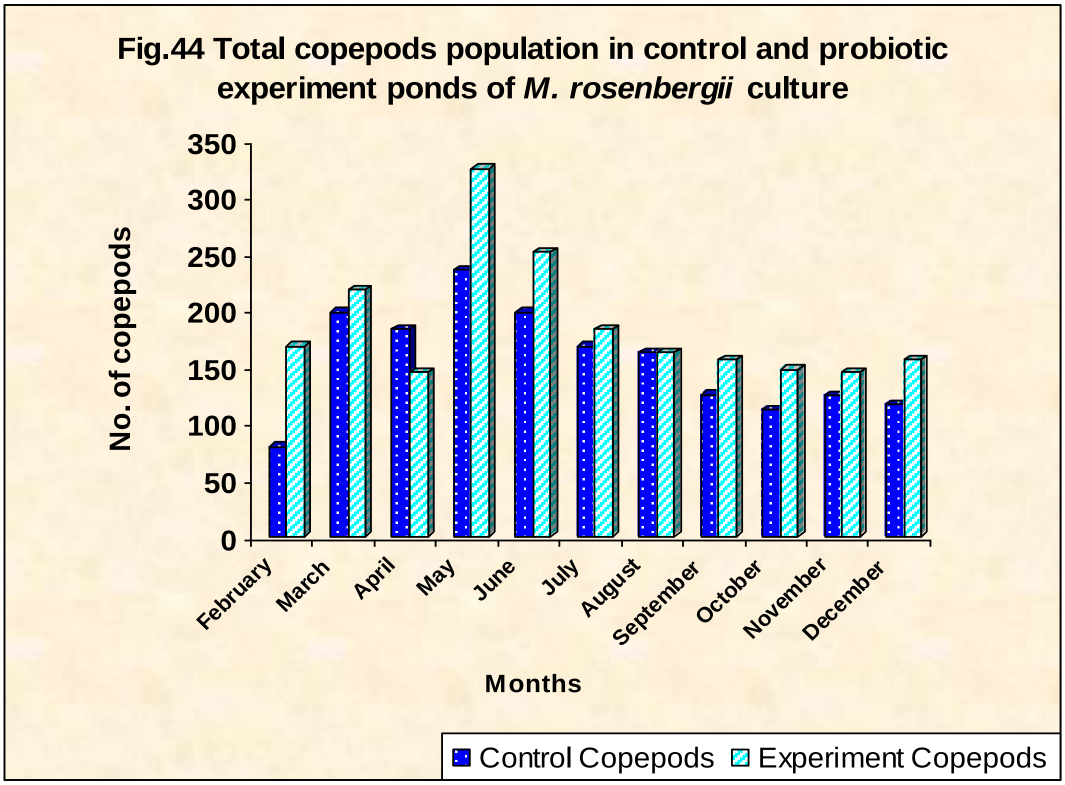


Fig.44 Total copepods population in control and probiotic experiment ponds of *M. rosenbergii* culture
No. of copepods
0
50
100
150
200
250
300
350
February
March
April
May
June
July
August
September
October
November
December
Months
Control Copepods
Experiment Copepods

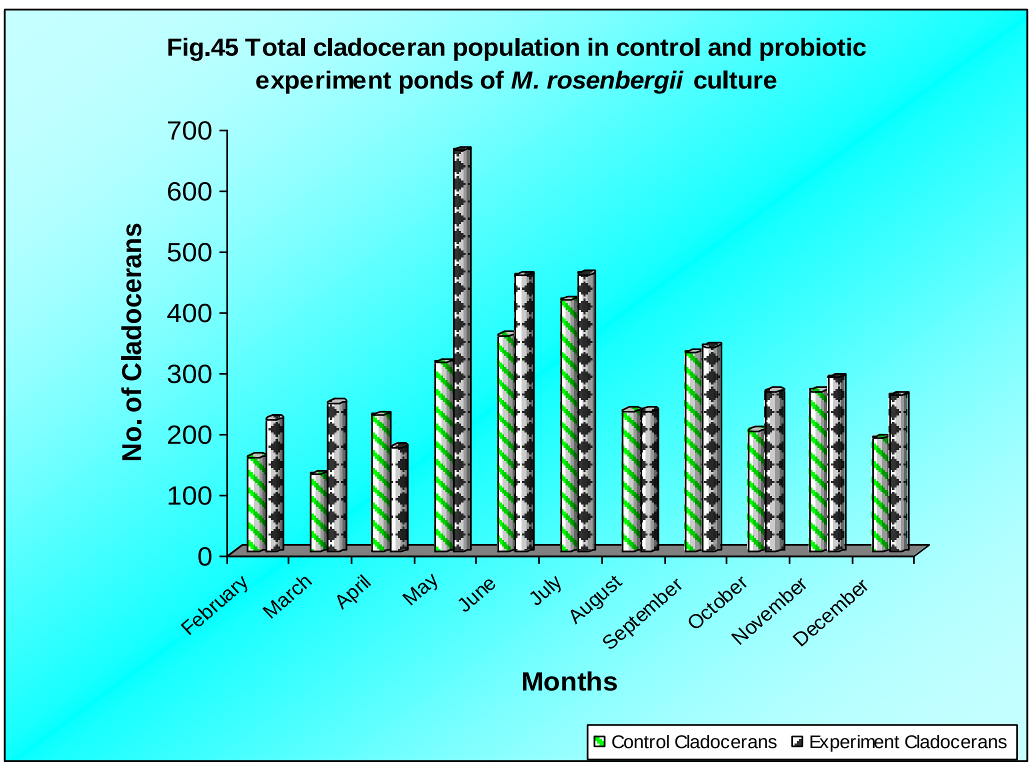


Fig.45 Total cladoceran population in control and probiotic experiment ponds of *M. rosenbergii* culture
No. of Cladocerans
0
100
200
300
400
500
600
700
February
March
April
May
June
July
August
September
October
November
December
Months
Control Cladocerans
Experiment Cladocerans

contributions presented in probiotic experimental pond during May month only when compared to control pond, whereas in control pond in the month of July (14.80%), June (12.70%), September (11.70%) and May (11.13%) represented higher numbers. In the present experiments, higher numbers of cladocerans were observed during July month in control pond and in this *Ceriodaphnia cornata* contribute maximum numbers. In addition to this, in the cladocerans, *Ceriodaphnia cornuta* contribute 25.33% and 19.66% in control and probiotic experiment pond, respectively (table. 21).

Higher number of rotifers were noticed in the month of July and its specieswise, *Asplancha* sp. contributes higher number. The recorded total rotifer population in the present study in control and probiotic experiment pond and their percentage contribution during culture period are presented in table 22 and fig.46. *Brachinous* constituted important genera of rotifer 7 and 8 species of these genera occurred in control and probiotic experiment pond respectively. 71% of *Brachionus* genera and 22% of *Asplancha* sp. contributed in rotifers of control ponds where as in probiotic experimental pond 70% and 11.28% respectively (table.22**)**.

In the present experiment, the Ostracods recorded higher number in experimental pond when compared to control pond (table.23 and fig.47). In the present study, higher percentage and numbers were observed in the month of May, March, September in control and June, March, July in probiotic experimental pond respectively. *Cypris* and *Stenocypris* are the two genera of Ostracods were recorded in both the ponds. *Cypris* sp. was (63%) in control and 50.75% in probiotic experiment pond occurred higher percentage in both the ponds than that of *stenocypris* sp. (table 23**).**

Among specieswise, *Asplanchna* sp. (rotifer), *Ceriodaphnia cornuta* (cladocerans), *Cryptocyclops bicolor* (copepods) and *Cypris* (ostracods) showed higher number in

Fig.46 Total Rotifer population in control and probiotic experiment ponds of *M. rosenbergii* culture

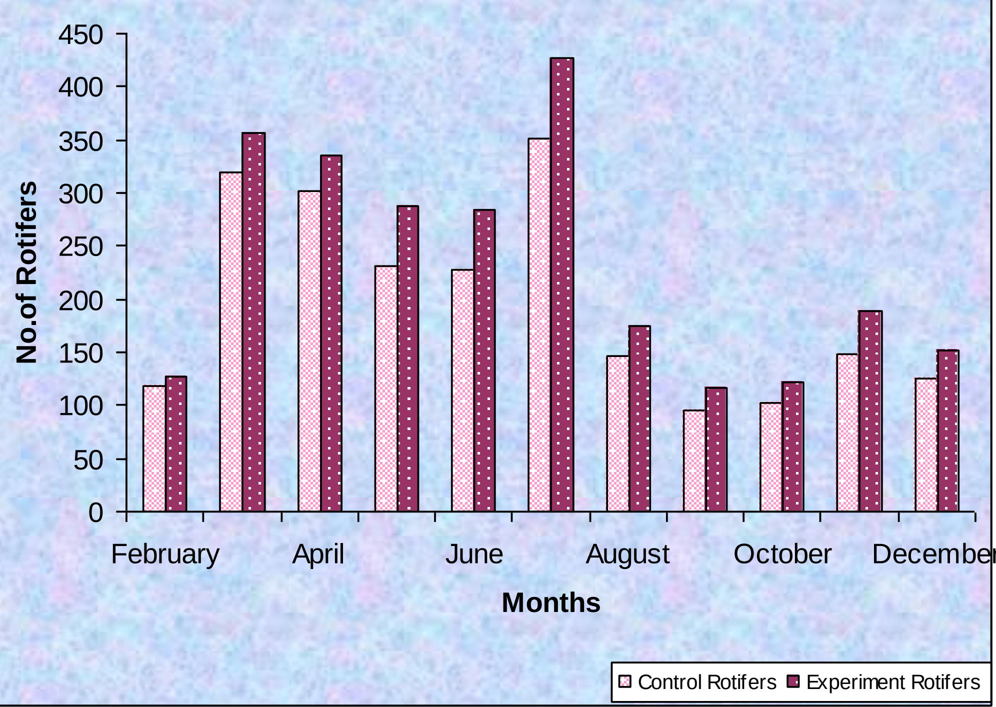
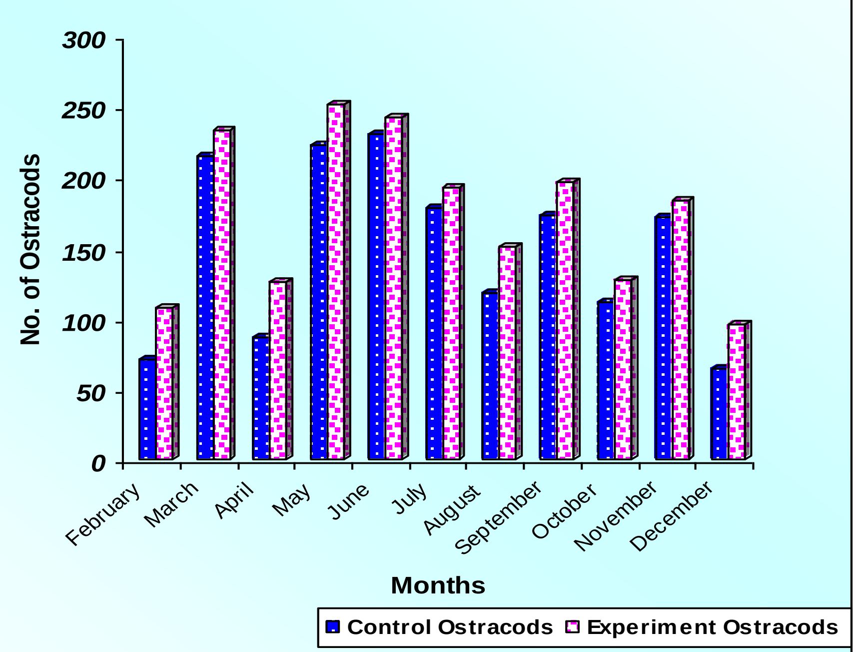


Fig.47 Total ostracods population in control and probiotic experiment ponds of *M. rosenbergii* culture



control pond whereas in probiotic experiment pond *Brachionus calyciflorus* (rotifer), *Ceriodaphnia cornuta* (cladocerans), *Cryptocyclops* bicolor (copepods) and *Cypris* (ostracods) showed higher numbers. The similar trend was recorded in month/groupwise total zooplankton population.

In t-test analysis, monthwise and specieswise total zooplankton showed significant (P<0.05) results (table.24). The total specieswise population showed higher degree of correlation in rotifer population (r= 0.992) and Ostracods (0.990) in between the control and experiment pond and the results were found to statistically significant at various levels (table.19a)

2.4.5. Length and weight of harvested prawn *M. rosenbergii*:

Totally nine trial netting were done in both the ponds during the culture period and their average body weight for control and probiotic experimental ponds were presented in table. 25. Higher average body weight was recorded in probiotic experiment pond compared to the control pond. The increased mean body weight were recorded in August (19.90 g) and September (54.22 g) but in October it was decreased and in November mean weight increased (49.2 g) and again decreased (32.46 g) condition was noticed in December month in the experimental pond. In this present study, same trend also noticed in length parameters.

The ranges of length, weight of the prawn *M. rosenbergii* of control and experimental pond were presented in table. 26**.** In general, length proportionately increased in August, September and October whereas it was stagnant in November and December month (fig.48). The mean and S.E values and t' test analysis of length and weight of control and probiotic experiment pond were recorded in table. 26a, b and fig.49.

Table- 25: Average animal body weight of control and Probiotic experiment pond of *M. rosenbergii* culture period.

S.No.	Days of Culture	Control Pond (gm)	Probiotic experiment pond (gm)
1	65th day	7.82	9.32
2	90th day	12.48	16.96
3	124th day	19.80	24.10
4	146th day	27.02	33.50
5	176th day	36.75	42.84
6	229th day	42.49	54.22
7	252nd day	46.75	49.21
8	284th day	38.10	40.00
9	304th day	31.32	32.46

Table- 26: The length and weight ranges of control and Probiotic experiment pond of *M. rosenbergii* culture

Month	Parameters	Control pond	Experimental pond
August	Length (cm)	5-15	11-15
	Weight (gm)	4-28	12-39
September	Length	10-17	7-21
	Weight	9-42	8-73
October	Length	5-16	7-21
	Weight	4-39	7-78
November	Length	9-17	8-21
	Weight	8-49	5-75
December	Length	8-17	7-21
	Weight	7-60	8-75

Number of samples 100

Table- 26a: The mean and S.E. values of length and weight of control and Probiotic experimental pond of *M. rosenbergii* culture

Month	Parameters	Control pond	Experimental pond
August	Length	9.705 ± 0.325	13.025 ± 0.219
	Weight	14.735 ± 0.903	19.900 ± 0.850
September	Length	12.875 ± 0.308	17.888 ± 0.426
	Weight	17.041 ± 1.181	54.222 ± 2.605
October	Length	14.022 ± 0.151	13.833 ± 0.663
	Weight	28.750 ± 0.713	32.277 ± 3.567
November	Length	14.400 ± 0.476	17.578 ± 0.399
	Weight	38.100 ± 2.062	49.210 ± 4.015
December	Length	14.720 ± 0.274	13.400 ± 0.289
	Weight	31.320 ± 1.804	32.466 ± 2.162

Table- 26b: T-test analysis values of length and weight of control and Probiotic experiment pond

Month	Parameters	Control pond	Experimental pond
August	Length	29.795	59.413
	Weight	16.303	23.386
September	Length	41.699	41.90
	Weight	14.420	20.812
October	Length	93.041	20.865
	Weight	40.314	9.049
November	Length	30.246	43.983
	Weight	18.472	12.257
December	Length	17.358	46.287
	Weight	53.726	15.016

Table- 26c: Correlation co-efficient (r-value) of control and probiotic experiment pond

Month	Control pond		Experimental pond	
	r-value	P-value •	r-value	P-value •
August	0.801	0.000	0.519	0.001
September	0.759	0.000	0.756	0.000
October	0.865	0.000	0.942	0.000
November	0.844	0.000	0.900	0.000
December	0.920	0.000	0.884	0.000

• : Significance at the 0.05 level

Table- 27: Correlation co-efficient values of length of control pond during the *M. rosenbergii* culture period

Month	r- Value	Significance
August. Vs September.	0.124	0.220
August Vs October	0.241	0.016•
August Vs. November	0.164	0.103
August Vs. December	0.232	0.020•
September Vs October	0.244	0.014•
September Vs. November	0.178	0.076
September Vs December	0.335	0.001†
October Vs. November	0.76	0.452
October Vs December	0.299	0.003†
November Vs December	0.505	0.000†

• : Significance at the 0.05 level †: Significance at the 0.01 leve

Table- 28: Correlation co-efficient values of length of experimental pond

Month	r-value	Significance
August. Vs September.	0.039	0.704
August Vs October	0.178	0.080
August Vs. November	0.105	0.303
August Vs. December	0.077	0.450
September Vs October	0.106	0.298
September Vs. November	0.769	0.000†
September Vs December	0.198	0.051•
October Vs. November	0.119	0.244
October Vs December	0.229	0.023•
November Vs December	0.281	0.005†

•: Significance at the 0.05 level † : Significance at the 0.01 level

Table- 29: Correlation co-efficient values of weight of control pond

Month	r- Value	Significance
August. Vs September.	0.092	0.364
August Vs October	0.097	0.337
August Vs. November	0.043	0.673
August Vs. December	0.008	0.934
September Vs October	1.000	0.00†
September Vs. November	0.480	0.00†
September Vs December	0.373	0.00†
October Vs. November	0.293	0.003†
October Vs December	0.412	0.000†
November Vs December	0.392	0.000†

† : Significance at the 0.01 level

Table- 30: Correlation co-efficient values of weight of experimental pond

Month	r- value	Significance
August. Vs September.	0.195	0.052•
August Vs October	0.168	0.095
August Vs. November	0.229	0.022•
August Vs. December	0.392	0.000†
September Vs October	0.099	0.329
September Vs. November	0.720	0.000†
September Vs December	0.080	0.426
October Vs. November	1.000	0.000†
October Vs December	0.134	0.182
November Vs December	0.159	0.114

• Correlation is significant at the 0.05 level (2 – tailed)
† Correlation is significant at the 0.01 level (2 – tailed)

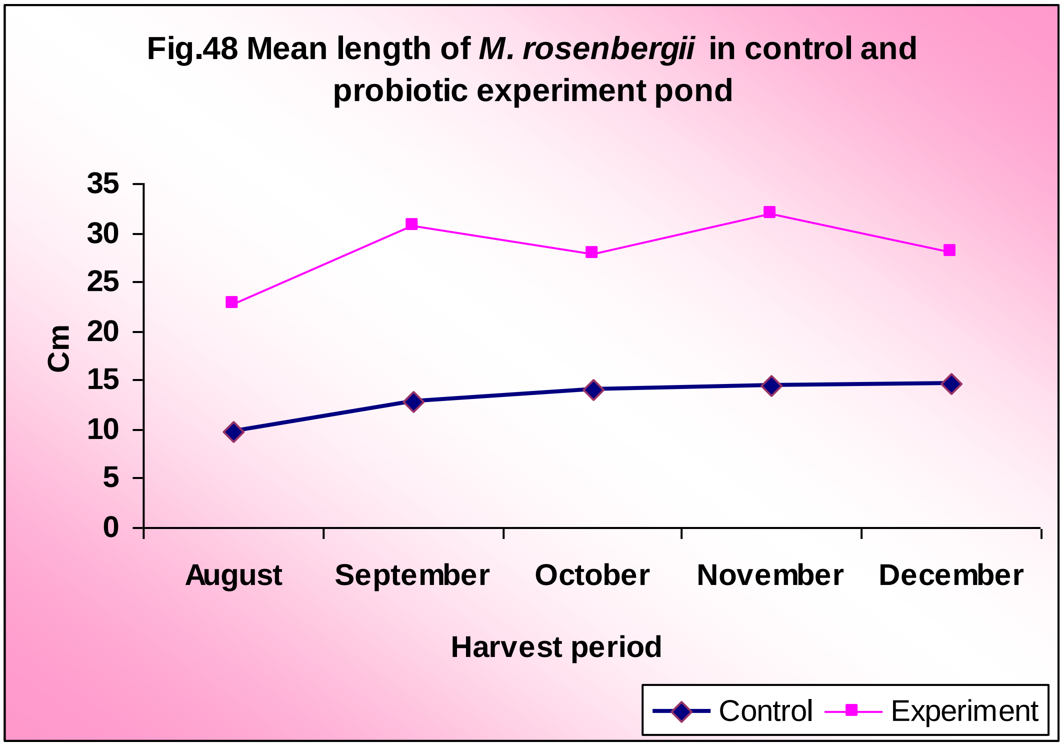


Fig.48 Mean length of *M. rosenbergii* in control and probiotic experiment pond
Cm
35
30
25
20
15
10
5
0
August
September
October
November
December
Harvest period
Control
Experiment

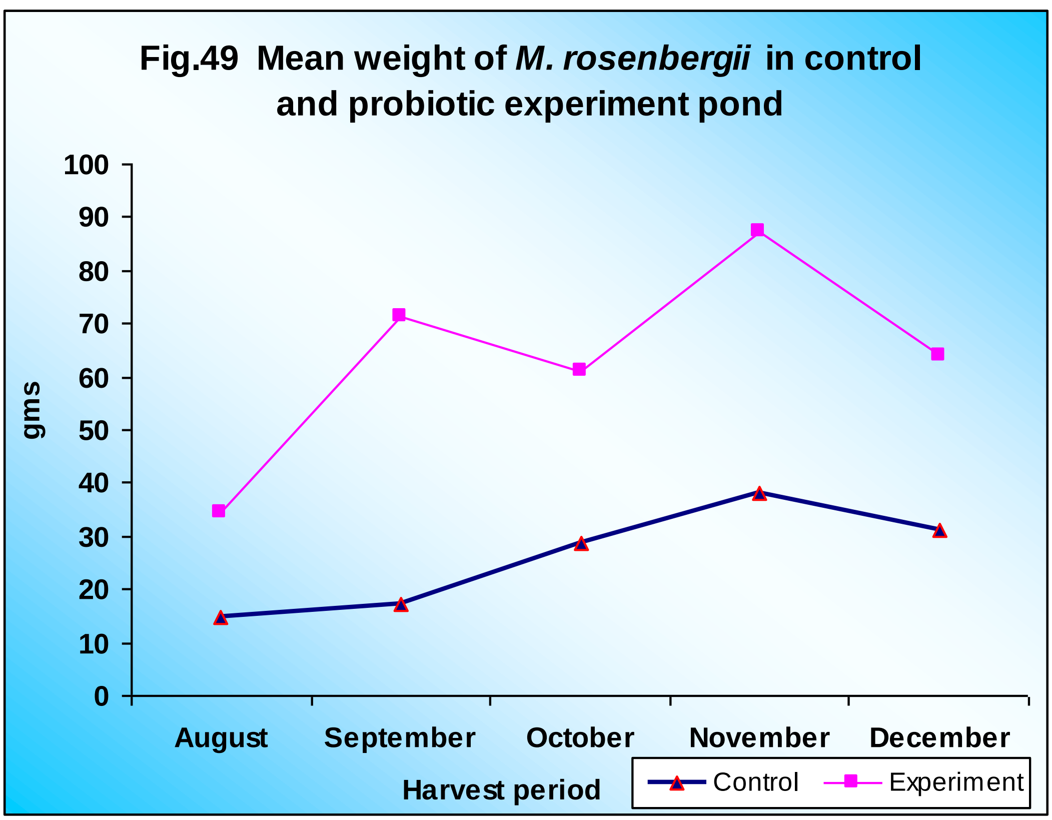


Fig.49 Mean weight of *M. rosenbergii* in control and probiotic experiment pond
gms
100
90
80
70
60
50
40
30
20
10
0
August
September
October
November
December
Harvest period
Control
Experiment

In the present study, the correlation co-efficient (r-value) of length and weight of *M. rosenbergii* in control and experimental pond culture showed significant (0.01 levels) results, (table. 26c). Correlation co-efficient values of length and weight of control and probiotic experiment pond between months during the culture period of *M. rosenbergii* showed statistically significant at various levels (table. 26 - 30).

2.4.6. Growth performance of freshwater prawn *M. rosenbergii*

The partial harvest details like numbers of count/kg, number of kilogram and export rate (Nellore market rate) were given for control and experiment pond in table. 31, 32. The highest production was recorded in the month of August in control and probiotic experiment ponds 237 kg and 372 kg respectively followed by 176 kg and 245 kg in the month of September in control and probiotic experiment ponds respectively. The lowest production was noticed in the month of November (95 kg) this was found to be significant between the control and probiotic experiment groups of prawns (table. 31, 32). The economic analysis of *M. rosenbergii* culture in control and experiment ponds was presented in table. 33, 34.

After 119 days the harvest was started from August upto December 2008, the final weight, weight gain, FCR and SGR in two types of pond culture were given in table. 35 and fig.50, 51, 52**)**. Statistical analysis of the production data revealed highly significant ($P<0.001$) differences among the two types of culture for all five parameters. The average weight of harvest prawn which determines the production was highest in probiotic applied culture pond (1178 kg) followed by control (866 kg**).** The survival performance of prawn was found that the best and highest survival rate was observed for group of prawn fed with probiotic diet than in the control pond.

Table- 31: Sale of prawns in Chennai commercial market value:

Control pond

Month	**Count**	**Kilograms**	**Nos. of animal**	**Rate (Rs.)**	**Amount (Rs.)**
July	F 35c	105	3675	90	9,450
August	29c	92	2668	710	65,320
	35c	103	3605	650	66,950
	38c	65	2470	620	40,300
	F 32c	12	384	110	1,322
		372	**9127**		**1,73,890**
September	26c	79	2054	740	58460
	36c	115	4140	640	73600
	39c	25	975	610	15250
	43c	26	1118	570	14820
		245	**8287**		**1,62,130**
October	27c	53	1431	630	33390
	34c	52	1768	560	29120
	43c	18	774	470	8460
	45c	46	2070	450	20700
	F 28c	17	476	120	2040
		186	**6519**		**93,710**
November	30c	41	1230	600	24600
	37c	32	1184	530	16960
	45c	19	855	450	8550
	48c	18	864	420	7560
	F 31c	32	992	120	3840
		142	**5125**		**61,510**
December	29c	21	609	690	14490
	38c	14	532	520	7280
	47c	38	1786	430	16340
	53c	12	636	370	4440
	70c	43	3010	200	8600
		128	**6573**		**51,150**

Table- 32: Sale of prawn in Chennai commercial market value:

Experimental pond

Month	Count	Kilograms	Nos. of animal	Rate (Rs.)	Amount (Rs.)
July	F 43c	60	2580	90	5,400
August	33c	63	2079	670	42,210
	39c	82	3198	610	50,020
	41c	63	2583	590	31,170
	F 28c	29	812	110	3,190
		297	**8672**		**1,26,590**
September	31c	52	1612	740	35880
	38c	71	2698	640	44020
	45c	35	1575	610	19250
	F34c	18	612	570	1980
		176	**6497**		**1,01,100**
October	30c	40	1200	600	24000
	34c	54	1836	560	30240
	40c	25	1000	500	12500
	F 36c	14	504	120	1680
		133	**4540**		**68,420**
November	28c	18	504	620	11160
	40c	34	1360	500	17000
	47c	33	1551	430	14190
	F 41c	10	410	120	1200
		95	**3825**		**43,550**
December	28c	13	364	620	8060
	50c	43	2150	400	17200
	72c	57	4104	180	10260
	F 80c	52	4160	120	6240
		165	**10778**		**41,760**

Table-33: Economic analysis of control pond of freshwater prawn *M. rosenbergii* culture

I. Capital cost	
Land lease @ 15000/ha	22,500
Pond reconstruction	8,000
PVC pipes, Plastic hose, Outlet wall, Trays	7,000
Plastic tubs, Nets	3,000
Electronic equipments	8,000
Miscellaneous	5,000
Total	**53,500**
II. Operational cost	
Seed 60,000 @Rs. 40 paisa	36,000
Pesticide	400
Bleaching powder 2 bag @ Rs. 430/ bag	860
Zeolite 100 kg@ Rs. 44/ kg	4,400
Shell lime 1200 kg @ Rs. 2/kg	2,400
Agrilime 400 kg @ Rs.2/kg	800
Dolamite 150 kg @ Rs. 1.50/kg	225
Groundnet oil cake 200 kg @ Rs.20/kg	4,000
Rice bran 3 bag @ Rs. 230/bag	690
Yeast 4 kg @Rs. 185/kg	740
C.P. Dissolved oxygen kit @ Rs. 1500/kit	1,950
C.P. pH kit @ Rs.1100/kit	1,100
Fish fingerlings 500 @ Rs. 2/fish	1,000
Total	**54,615**
Pond preparation	
Labour 2 days (4 person/day) @ Rs. 200/person	1,600
Tractor ploughing 2 hours @ Rs. 500/hours	1,000
C.P. Feed 1412 @ Rs. 35/kg	49,420
Power 11500 units @ Rs. 5.80/unit	66,700
Total	**1,18,720**
Labour (2 person) @ Rs. 2500/month	66,000

Harvest and Trial netting		10,000
Transport		10,000
Miscellaneous		5,000
	Total	**91,000**
	Total	**2, 64,335**

III. Fixed cost

Interest on capital cost @ 15.5%	8,292.50
Interest on operational cost @ 15.5%	40,971.92
	49,264.42

IV Total cost

Operational cost		2, 64,335
Interest		49,264
	Total	**3, 13,599**

V Gross income

Sale of prawn (866 kg)		3, 86,820
Sale of fishes (765 kg @ Rs.50)		38,250
	Total	**4, 25,070**

VI Income (V –minus IV) 1, 11,471

Net income (VI - I) 57,971

Table-34: Economic analysis of probiotic experiment pond of freshwater prawn *M. rosenbergii* culture

I. Capital cost	Rs. P
Land lease @ 15000/ha	22,500
Pond reconstruction	9,000
PVC pipes, Plastic hose, Outlet wall, Trays	7,000
Plastic tubs, Nets	3,000
Electronic equipments	8,000
Miscellaneous	5,000
Total	**54,500**
II. Operational cost	
Seed 60,000 @Rs. 0.60 paise/pl	36,000
Pesticide	450
Bleaching powder 2 bag @ Rs. 430/ bag	860
Zeolite 100 kg@ Rs. 44/ kg	4,400
Shell lime 1300 kg @ Rs. 2/kg	2,600
Agrilime 250 kg @ Rs.2/kg	500
Dolamite 200 kg @ Rs. 1.50/kg	300
Groundnet oil cake 200 kg @ Rs.20/kg	4,000
Rice bran 3 bag @ Rs. 230/bag	690
Yeast 4 kg @Rs. 185/kg	740
C.P. Dissolved oxygen kit @ Rs. 1950/kit	1,950
C.P. pH kit @ Rs.1100/kit	1,100
Fish fingerlings 500 @ Rs. 2/fish	1,000
Probiotic	12,720
C.P Mutagen (vitamin & mineral mix) 2kg@ Rs.1000	2,000
C.P Sodamix (Water minerals) 330 kg @ Rs. 13	4,290

	73,600

Pond preparation	
Labour 2 days (4 person/day) @ Rs. 200/person/day	1,600
Tractor ploughing 2 hours @ Rs. 500/hours	1,000
C.P. Feed 1412 kg @ Rs. 35/kg	49,420
Power 11500 units @ Rs. 5.80/unit	66,700

	Total	1,18,720

Labour (2 person) @ Rs. 3000/month		66,000
Harvest and Trial netting		10,000
Transport		10,000
Miscellaneous		5,000

	Total	**91,000**

	TOTAL	**2,83,320**

III. Fixed cost

Interest on capital cast @ 15.5%		8,447.50
Interest on operational cost @ 15.5%		43,914.60

	Total	**52,362**

IV Total cost

Operational cost		2,83,320
Interest		52,362

	Total	**3,32,682**

V Gross income

Sale of prawn 1178 kg	5,51,840
Sale of fishes 842 kg @ Rs. 50/kg	42,100
	5,93,940
VI Net income (V –minus IV)	**2,61,258**

Income
(VI - I) **2,06,758**

Table 35: Growth performance of the freshwater prawn *M. rosenbergii* in control and probiotic experiment pond.

Parameters	August		September		October		November		December	
	C	E	C	E	C	E	C	E	C	E
Initial mean weight (g)	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02	1.02
Final mean weight (g)	12.5	38	35	65	32	70	48	75	49	75
Weight gain (g)	11.48	36.98	33.98	63.98	30.98	68.98	46.98	73.98	47.98	73.98
SGR •	15.98	24.65	18.87	35.54	14.75	32.84	19.57	30.82	17.77	27.4
FCR •	7.40	5.26	5.95	2.21	3.60	3.18	2.66	2.06	3.27	3.14
PER •	6.82	7.12	8.25	9.27	7.94	9.01	8.93	10.2	9.21	10.8

- significant at $P < 0.05$ level

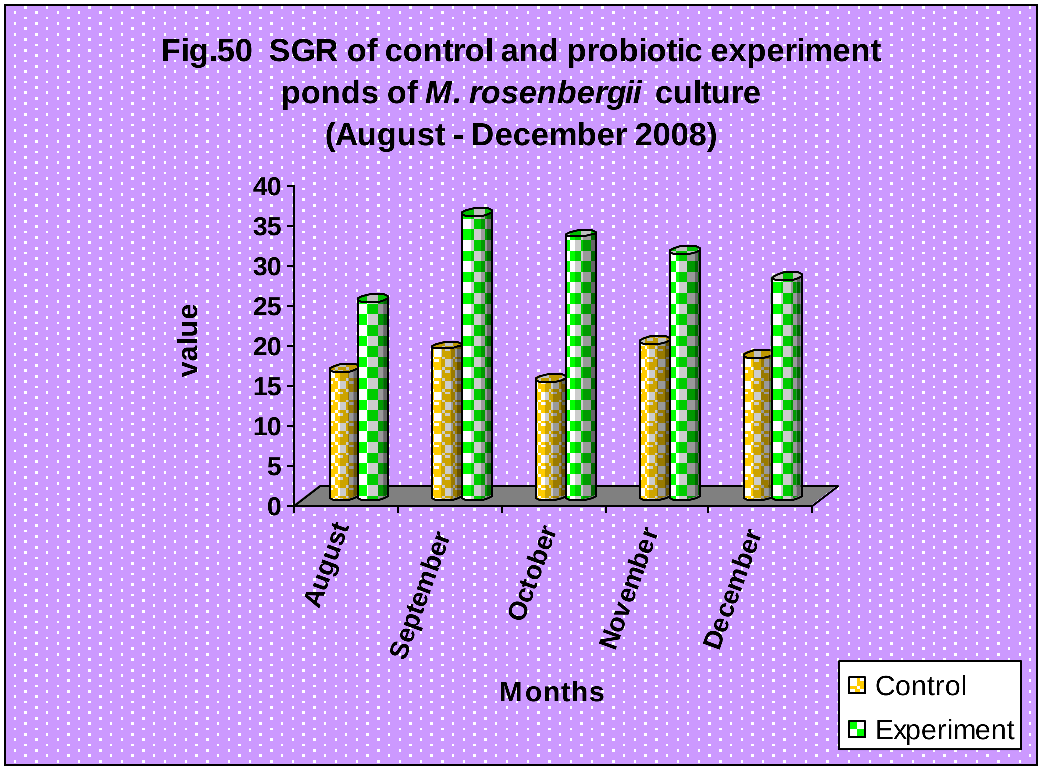


Fig.50 SGR of control and probiotic experiment ponds of *M. rosenbergii* culture (August - December 2008)
value
40
35
30
25
20
15
10
5
0
August
September
October
November
December
Months
Control
Experiment

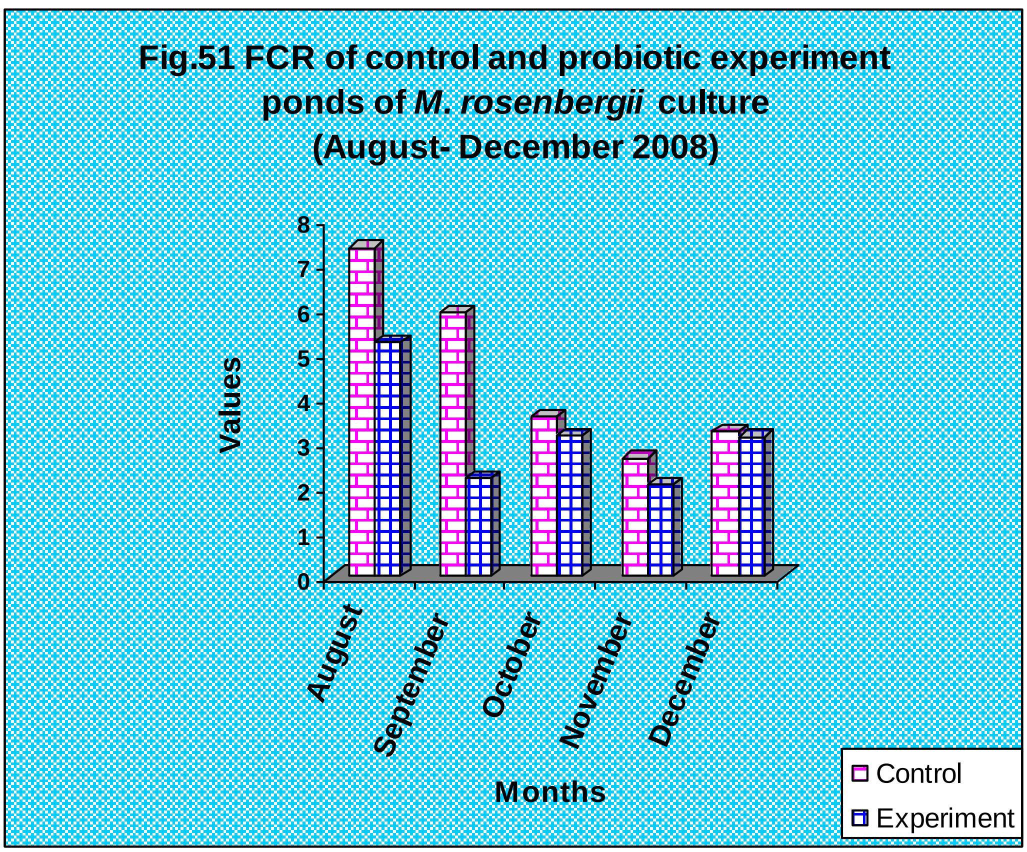


Fig.51 FCR of control and probiotic experiment ponds of *M. rosenbergii* culture (August- December 2008)
Values
8
7
6
5
4
3
2
1
0
August
September
October
November
December
Months
Control
Experiment

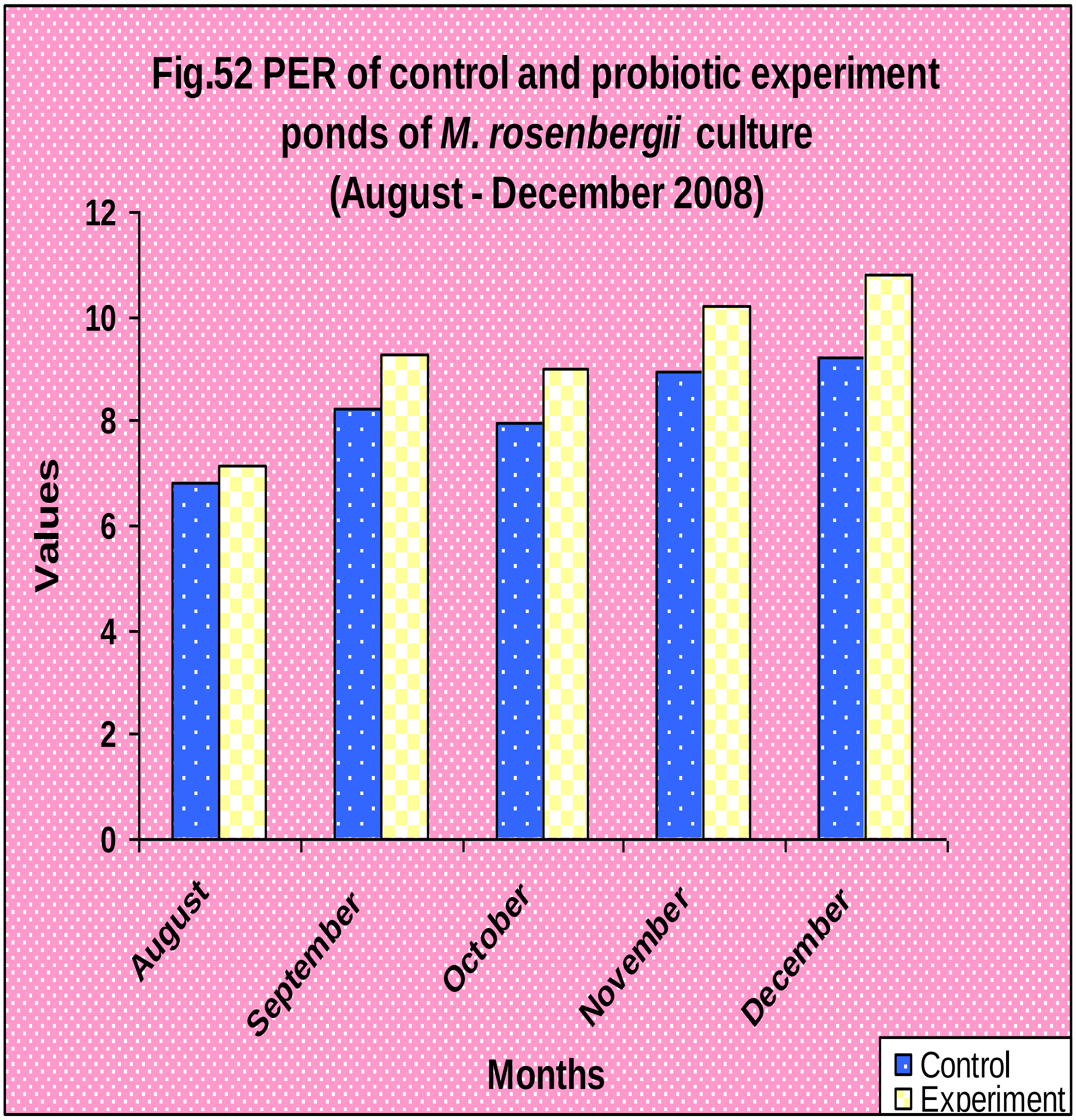


Fig.52 PER of control and probiotic experiment
ponds of M. rosenbergii culture
(August - December 2008)
12
10
8
6
4
2
0
Values
August
September
October
November
December
Months
Control
Experiment

Table. 36. Cost-return and partial budget analysis of *Macrobrachium rosenbergii* cultured in control and probiotic experiment ponds

Particulars	Control pond	Probiotic experiment pond
Total production (kg)	866	1178
Average weight at harvest (g)	43.66 (31.32)	39.46 (32.46)
Price kg_1 (Rs.)	446.67	468.45
Total cost (Rs.)	3, 13,599	3,32,682
Net revenue (Rs.)	57971	2,06,758
Productivity ha_1 (kg)	Rs.364	Rs.325
Productivity of feed (kg_1)	1412	1412
FE	0.75	1.03
FCR	1.63	1.19
Fish production (kg)	765	842
Productivity of labour (kg_1)	105.05	77.24
Employment generated (man days)	365	365
BCR	1.355	1.785

The highest SGR of 35.54 was observed for probiotic experiment prawn compared to control group (19.37) during the September and November months respectively. The FCR rate was maximum during the month of August of study period in the probiotic experiment and control pond respectively (table. 35). Results of harvest of *M. rosenbergii* showed the 90% survival in control pond and 100% in experimental pond. Though results indicate similar expenditure for fertilizers, probiotic feed, power, labour, trial netting and harvest for both the pond compared to control pond, experimental ponds realized good profit.

2.4.7. Cost-benefit analysis:

The cost - benefit analysis of control and probiotic experiment prawn *M. rosenbergii* was presented in the table.36. Significantly higher ($P<0.001$) gross earning as well as net profit with a benefit cost ratio was obtained. The benefit cost ratio (BCR – 1.785) was increased from probiotic experiment prawn than by control culture pond (BCR -1.355) (table.36) There was no difference was noticed in the total expenditure among the two ponds (Rs. 3,32,682 and Rs. 3,13,599) probiotic experimental and control respectively. The production cost worked out for one kilogram of prawn was Rs. 364.49 in control and Rs. 325.47 in experimental pond. Prawn seedlings were expensive inputs Rs.36, 000 of the total cost for the two feeds, probiotics and supplemental feed. However, Rs. 57,971 and 2, 06,758 of the total net income were obtained in control and probiotic culture pond respectively from prawn sale proceeds. Cost benefit analysis shows that when probiotics are used the cost of production increases by 0.9-15%, for an average production of 1.3 t/ha.

In the present study, the final harvest of fish was taken after 190 days culture period. There production of fishes in control and probiotic experiment pond was 765 kg and 842 kg respectively.

2.5. DISCUSSION

2.5.1. Physical and chemical parameters of the Soil

2.5.1.1. Soil texture

Probiotics used to supply beneficial bacterial strains to rearing water that will help to increase microbial species composition and to improve soil and water quality and maintain healthy environment for prawn culture.

In the present investigation, the soil texture of probiotic experiment pond showed higher mean values in slit and sand when compared to control pond, and the results were found to be statistically significant ($P<0.05$) of both ponds. In the present study, the percentage of clay, slit and sand were 20.018%, 35.090% and 29.590% in probiotic experiment pond respectively. The resulted percentages of soil textures were favourable for the growth of *M. rosenbergii* in probiotic pond than control.

Similar significant differences ($P<0.05$) was recorded by Mukhopadhyay *et al.* (1997) who reported 20.2% clay, 13.5% slit and 66.5% sand in low saline *M. rosenbergii* culture ponds. Further, the present result was coincided with the work of Reddy *et al.* (1988) who suggested 40% of sand, 30% of slit and 30% of clay as favourable range of soil texture for aquaculture. Further, the present study was supported by Correia *et al*. (2002) and Wudtisin and Boyd (2006). In the present study, the recorded increased weight and length of prawn may be due to higher percentage of clay (soil texture) in the experimental pond. This present study was concurrence with the work of Mohanty (2009) who reported that the proportion of sand, slit and clay were increased the yield of prawn/fish in the rice field.

The better soil texture by the application of probiotic to the pond may initiated by *Bacillus* sp. occurrence more frequently in sediments than in the water, at lower levels, Bacillus spores may account for upto 80% of the total heterotrophic flora (Paulraj, 2002) and therefore they were naturally ingested by prawn *M. rosenbergii* that feed in or on the sediments (Rengpipat *et al.,* 1998).

2.5.1.2. Organic carbon

Organic carbon of 0.5% and above was suggested to be favourable level for aquaculture (Boyd, 1995). In the present work, the level of organic carbon (0.149%) was found to be less than 0.5% in probiotic experiment pond. The reported results in the present study may be due to many factors such as plankton distribution, bacterial load, age of pond etc., that affect the concentration of organic matter in the pond soil. Further, the present study was confirmed with the work of Chien (1992) and Gately (1990) who reported that 35% of organic carbon in marine pond was due to concentration of organic matter in pond soil. In the present investigations, the organic component was increased in the month of September and November (0.17%) in control and in the month of December (0.17%) in probiotic experiment pond with significant level. The raised organic compound may be due to increased temperature in those months. Boyd and Zimmermann (2000), Wudtisin and Boyd (2006) and Mohanty (2009) who suggested that the decomposing of organic matter increases with increasing temperature found in the aquaculture ponds. Sahu *et al.* (2008) found that routine use of commercial probiotic in a shrimp farm resulted in reduced organic matter accumulation, improved water quality and enhanced environmental conditions.

2.5.1.3. pH

pH is one of the important factor for decomposition of organic matter which plays a role for the growth of organisms. In the present work, the observed values of pH in both the ponds were within this range 7.4 – 8.2 (table.2**)**. The soil pH was reported 7.5 – 8.5 to be ideal level of maximum decomposition of organic matter for soil microbes (Boyd, 1995). In the present study, pH was 7.4 noticed during October month in the both culture ponds. The observed higher organic matter in the October month may be due to low pH, which favours slow decomposition and accumulation of organic matter. Similar study was supported by Boyd and Pipoppinyo (1994). Sadek and Moreau (1996) suggested a pH range of 6.5 – 8.5 favourable for the prawn/fish culture. Further, the present study was correlated with the report of Mohanty (2009).

The body weight of *M. rosenbergii* was higher during the study period in both ponds except in the month of October due to low pH (7.4), however there is not much variation in body weight **(**table. 26**).** Further the present study was concordance with the work of Allan and Maguire (1992) who stated that growth reduction occurred at pH 5.5 and 4.9. The present report was consistence with the work of Chen and Chen (2003) who reported higher growth rate at pH 8.2 and also explained the favourable pH was 7.4 which stimulates growth rate. The present results are in agreement with the work of Cheng *et al.* (2003a) who reported that pH 7.27 or salinity at 5‰ however exhibited the greatest increased resistance to the *Lactococcus gravieae* infection. Higher growth was resulted at pH 7.7 in probiotic applied pond might be due to that *M. rosenbergii* exhibited increased phagocytic activity and clearance efficiency and greatest increased resistance to the pathogenic infection.

2.5.1.4. Nitrogen, Phosphorus and Potassium (NPK)

Nitrogen, Phosphorus and Potassium (NPK) are the widely used inorganic fertilizer which are prepared with varying proportion of nitrogen, phosphorus and potassium. Reddy *et al*. (1998) suggested that the favourable range of total NPK for aquaculture is 50:6:25 mg/100g of soil and above. Our data showed fluctuation in the concentrations of total nitrogen, total phosphorus and total potash (table.2) in experimental pond. In the present investigation, the noticed value of NPK was higher in experimental pond than control pond and found to be statistically significant. In the study period, the NPK content decreased subsequently in control pond, particularly in April and May month, whereas in probiotic experimental pond, the recorded values were gradually increasing (table. 2). The increased level of NPK can be attributed addition of probiotic, mutagen and sodamix to probiotic experiment pond. Similar study was also carried out by Rajyalakshmi *et al*. (1988) who reported lower nitrogen and phosphorus values in the brackish water ponds of Chilka lake fringe area. Reduced sediment nutrients level in the present study was in agreement with previous study, that nitrogen level in water was significantly decreased ($P<0.05$) after the probiotic application (Wang *et al.*, 2005).

In the present experiments, the addition of probiotic in experimental pond shows a significant improvement of the amount of total potash. In the present study, small quantity of fertilizer was added to initiate the plankton growth for both ponds. But, the increment nutrient (NPK) was higher in probiotic experimental pond. This may be due to that the application of probiotic can improve microbial growth in the soil, which helps to decompose the organic matter and thus converts into nutrients. Similar study was reported by Dhanahar *et al*. (2007) who studied the addition of liquid NPK along with dried distillery grain (4.50 kg/h) to initiate the plankton blooms and microorganisms. The present results was supported by Uddin *et al*. (2007) and Wahab *et al*. (2008) who also supplied superphosphate in the prawn culture pond.

2.5.1.5. Copper

Copper is commonly applied to aquaculture ponds to inhibit phytoplankton growth, kill organisms which produce odorous compounds responsible for off-flavour in fish/shrimp and control fish diseases (Boyd, 1990 and Tucker and Robinson, 1990). In our present study, the copper content ranged from 0.53 – 1.24 ppm and 0.50 – 1.24 ppm in control and probiotic experiment pond, respectively and was found to be significant between the control and probiotic culture pond. The recommended copper value for freshwater prawn culture pond soil was 0.15 to 0.40 ppm (Boyd and Zimmermann, 2000). But in the present study, exceeds level of copper did not pose any adverse effect or recognized, during the culture period in both ponds. In the present investigation, the resulted copper was highly significant correlation between copper with organic matter.

2.5.1.6. Manganese

Manganese concentration in natural surface water seldom reaches 1.0 mg/l and is usually less than 0.2 mg/l (Mc Neely *et al*., 1979). Manganese activates an essential part of enzyme systems that metabolizes protein and energy in all animals. In the present study, the noticed range of manganese was (4.27 – 6.35 ppm) in probiotic applied pond whereas (4.33 – 6.34 ppm) in control pond (table.2)**.** Manganese had a significant effect on prawn growth as the prawns grew faster in probiotic applied pond compared to control pond. This may be due that manganese has improved mean feed utilization ranged from 69.9 -76.7%.

Similar study was reported by Adhikari *et al*. (2007) who reported that the favourable concentration of manganese for higher food utilization and faster molting of

M. rosenbergii whereas the lesser feed utilization may be because of toxic effect of manganese or prawn by impaving normal physiological functions. Manganese may act as enzyme inhibition if it concentrations differ (more than 1.2 mg/l) from the actual physiological requirements which may lead to either toxic effect or an inhibition of growth (Bambang *et al*., 1995). The present study clearly demonstrated that the ranges of available manganese both in control and probiotic experiment ponds are favourable for the growth of *M. rosenbergii*.

2.5.1.7. Iron

Iron is an essential element that has a number of fundamental roles in cellular biochemistry and metabolism. In the present investigation, the resulted value of iron ranged from 3.31 – 5.34 ppm in control and 3.30 – 5.30 ppm in probiotic applied pond. The survival noticed in the probiotic applied pond than control were found to be significant ($P<0.05$) difference based upon the iron distribution in prawn growth between these two cultures. The present study was supported by Adhikari *et al*. (2007) who explained that the 0.32 mg/l of iron was ideal concentrations for the growth of *M. rosenbergii* in freshwater medium. In this study, the higher growth rate observed in the experiment than control pond may be improved feed utilization and increased molting frequency. Iron can also vary its redox state and can be rapidly oxidized from Fe^{2+} to Fe^{3+} (ferrous to ferric iron) in the presence of oxygen. This reaction generates the superoxide anion which through a series of redox reactions leads to the generation of toxic hydroxyl; radicals (the Haber – Weins reactions) (De Silva *et al*., 1996 and Aisen *et al*., 2001).

2.5.1.8. Electrical conductivity

In the present study, the electrical conductivity was ranged from 0.47 – 1.93 µs/m and 0.7 – 1.8µs/m in control and probiotic experiment pond respectively. Adhikari (2000) reported the range of 0.07 – 0.28µs/m electrical conductivity of freshwater ponds in Orissa, India. The present noticed EC are favourable for prawn culture. This resulted E.C was similar to the study of Wang *et al.* (2005) in *P. vannamei* ponds.

2.5.2. Physico- chemical parameters of pond water

2.5.2.1. Colour of the pond water

The observed colour in the present study may be (1) reddish brown, is caused by the blooming of diatoms and species such as *Chaetoceras*, *Navicula*, *Skeletonema, Cyclotella, Synedia, Achnathes Amphora* and *Euglena*, (2) light or bright green which is due to growth of green algae especially C*hlorella,* (3) dark green resulted when pond temperature goes high or accumulates fast organic deposits. In this pond blue green algae bloom faster than green algae, (4) dark brown caused due to rapid growth of dinoflagellates and brown algae resulted and (5) appearance of yellowish colour which is due to the growth of *Crystophyta* (table.3).

The present study was supported by Wang *et al*. (2005) who observed (combinations of *Bacillus*, *Saccharomyces cerevisiae*, *Nitrosomonas and Nitrobacter)* a brownish-green water color in commercial probiotic applied ponds that most shrimp farmers believed would increased *P. vannamei* growth and survival in China.

2.5.2.2. Transparency

In the present study, the recorded transparency ranges were between 18 – 40 cm in control pond and 20 – 40 cm in probiotic experimental pond which was low compared with standard values (25-30cm). Boyd and Zimmermann (2000) reported that the transparency of about 40 cm were ideal for *M. rosenbergii* culture. When water level is more than 1.2 m, the transparency levels are considerably low. In our present investigation, the optimum transparency (20–40 cm) observed in the probiotic experimental pond showed higher growth and productions of the algae. The present experiments was supported by different scientists reported different transparencies in *M. rosenbergii* mono and polyculture experiments in various places, (Sampaio and Valenti, 1996) 40 -75 cm; 44 – 59 cm (Sadek and Moreau, 2000); 12 – 38 cm (Ranjeet and Kurup, 2002); 15 -70 cm (Correia *et al*., 2003); 25 – 35 cm (Giap *et al*., 2005); 27 – 34 cm (Hossain and Kibria, 2006); 15 – 52 cm (Kunda *et al*., 2008) and 25 – 30 cm (Wahab *et al*., 2008).

Reddy *et al*. (1998) reported an ideal transparency of 22 – 35 cm for freshwater and 26 – 35 cm for brackish water aquaculture. Further, the present work was confirmed with work New (2002) who reported 25 – 40 cm was ideal for *M. rosenbergii* culture. Further, the higher transparency in the probiotic experiment pond water than control was found to be significant level in this study. The present study was supported by Wang *et al*. (2005) who reported higher transparency in commercial probiotic fed in white shrimp, *P. vannamei*.

2.5.2.3. Turbidity

Turbidity is the quantity of suspended material which interfere the light penetration, the suspended materials limit photosynthesis in the bottom layer of water column. Less than 30 cm is reported to be ideal turbidity for aquaculture. In the present study, the mean turbidity recorded 28.0 ± 2.304 cm and 40.545 ± 3.171 cm for control and probiotic experiment pond respectively (table. 4a). The observed low turbidity in the control pond culture of *M. rosenbergii*, was directly correlated to temperature variations in the pond, thus influences the production of prawn. It might be due to higher temperature, (26 – 28°C) given for optimum production. High turbidity raised the temperature and enhances the dissolved stratification in ponds (Tidwell *et al*., 1996). It is also reported to clog the gills of fish and prawns (Ramesha *et al*., 1999) which leads to stress or death of organisms.

2.5.2.4. Temperature

Temperature was one of the important ecological physical factors of pond water which determines the production of prawns. Ideal temperature range for many species of shrimps ares 25 - 30°C. In many countries, more than 35°C are described as lethal for shrimps culture (Vijaykumaran, 1998).

In the present study, the range of temperature fluctuation during the study period between 26 to 34°C and the noticed mean temperature for control was (30.893 ± 0.258°C) and for probiotic experimental pond (28.702 ± 0.342°C) (table.3a), which are favourable for the normal growth of prawn. The present observation was confirmed with the work of Zimmermann (1998) who explained that freshwater prawns cease to grow and may not survive for long period, when water temperatures are below 19°C or above 34°C. Similar results was noticed by New (2002) and Saxena (2003) who recommended 28 – 31°C and 29 -31°C for optimum growth, respectively.

In the present experiment, it was noted that there was comparatively higher yield of prawn in probiotic experiment than control pond based on temperature variations. This study was supported by Tidwell *et al*. (1994) who reported that prawns cultured in ponds with water temperature averaging 25°C had higher production (11.5 kg/ha/day) rates than those reported by D'Abramo (1998) for prawns cultured at 29°C (5.5 – 5.9 kg/ha/day). Further, the present observation related to temperature and yield in prawn pond was supported by various authors, (Azim *et al*., 2001; Cuvin-Araler *et al*., 2007; Wahab *et al*., 2008; Kunda *et al*., 2008; Mohanty, 2009 and Ramakrishna, 2010).

Further the present study was correlated with the study of Sadek and Moreau (2000) recorded 26 ± 2.9°C mean temperature in *M. rosenbergii*, *P. semisulcatus*, monoculture of bi-species culture, polyculture with Florida Red tilapia culture in commercial farm Egypt. Oanh *et al*. (2000) stated that the optimal temperature for post larvae development of *M. rosenbergii* from 26 - 30°C in probiotic applied tank. Das *et al*. (2006) who reported the temperature variation 27 - 31°C between the control and probiotic applied pond which are favourable for the growth of *M. rosenbergii*. Keysami *et al*. (2007) also studied the water temperature ranges between 27.1 – 29.5°C in *M. rosenbergii* culture pond applied with *B. subtilis* and noticed there were no significant effects of probiotic on the temperature variations in the treated and non treated groups. Deeseenthum *et al*. (2007) also reported temperature ranges between 21 - 35°C favourable for *M. rosenbergii* culture in probiotic mixed culture Bacillus KKUU 2 and KKUU3 applied pond and control pond.

2.5.2.5. Total solids

In the present observations, the higher levels of total solids were noticed in probiotic experiment pond (1353.636 ± 53.746) than that of control pond (1250.454 ± 21.944) which was found to be significant ($P<0.005$) (table.4a). According to Reddy *et al*. (1998), Boyd and Zimmermann (2000) and New (2002), less than 500 ppm total dissolved solids as normal for *M. rosenbergii* culture ponds. Similar studies of higher total suspended solids were reported by various authors in different stocking densities of *M. rosenbergii* monoculture and polyculture system, (Giap *et al*., 2005). Further, the present report of total solids was correlated with the study of Cuvin – Aralar *et al*. (2007) who reported 647 – 1020 mg/l of total dissolved solids in the cage culture system of *M. rosenbergii* with different stocking density in Eutrophic lake, Philippines. The present study was further supported by Mohanty (2009) who recorded 363 ppm total suspended solids in their *M. rosenbergii* with carps in phased harvested system in India.

2.5.2.6. pH

The pH of the water is a measurement of the level of hydrogen ion concentration (H^+) present in the water. It is directly related to alkalinity and hardness or the buffering capacity of water and should be maintained within tolerable limits of species. pH greater than 10 will be lethal to many species (Vijaykumaran, 1998). The optimum pH range for most of the prawn species is 7 - 9. In the present study, the resulted mean pH (8.363 ± 0.102 and 8.209 ± 8.990) was recorded in control and probiotic experimental pond respectively (table.4a). While discussing growth and mortality of shrimp in relation to pH, Boyd (1989) suggested that pH (4) is acid dead point, pH (4 – 6) slow growth, pH (6 – 9) best growth, pH (9 – 11) slow growth and pH (11) alkaline dead point.

The present investigation was supported by Sampaio and Valenti (1996) recorded pH 6.9 – 9.7 range and Sadek and Moreau (2000) found pH 8.2 ± 0.25 in *M. rosenbergii* culture pond. Kumar *et al*. (2000) also recorded almost the same level of pH values in *M. rosenbergii* (8.92 ± 0.59) and *M. malcomsonii* (8.82 ± 0.29). Further, the present study was confirmed with the observation of Ranjeet and Kurup (2002) recorded 5.4 – 8.1, average pH values in culture of *M. rosenbergii*. Correia *et al*. (2003) recorded a range of 6 – 8.4 pH values in different supplemental feeding experiment in Brazil. Danaher *et al*. (2007) and Wahab *et al*. (2008) recorded average pH of (7.89 ± 0.4) with different stocking density of *M. rosenbergii*.

The present noticed pH in culture pond of *M. rosenbergii* was concurrence with the study of many authors in polyculture pond. Azim *et al*. (2001) recorded (pH 7.0 – 7.89), Hossain and Kibria (2006) found (pH 6.8 – 8.1), Cuvin-Aralar *et al*. (2007) examined (pH

6.70 – 7.69 and 6.70 – 7.8), Kunda *et al*. (2008) recorded (pH 7 – 9), Wahab *et al*. (2008) examined (pH 7 – 9) and Asaduzamann *et al*. (2008, 2010) noticed (pH 6.11 – 7.6) for the best growth of prawn. Thus, commercial probiotic was helpful in maintaining the pH at desired level for the best growth of prawn *M. rosenbergii*. The present study was correlated by the investigation of Das *et al.* (2006) and Oanh *et al.* (2000) used streptomyces as probiotics and probiotic CP Bio-dream respectively in rearing the freshwater prawn *M. rosenbergii*.

2.5.2.7. Alkalinity pH

In the present study, the levels of alkalinity pH mean were 13.09 ± 0.41 and 13.45 ± 0.60 noticed in control and probiotic experiment pond respectively. The variation of total alkalinity pH range does not affect the growth of prawns in this study. The concentration of alkalinity of pond water did not vary significantly among the control and probiotic applied pond. The present study was supported by Preto *et al.* (2010) who reported the concentration of alkalinity similar to the present results.

2.5.2.8. Total Hardness and Alkalinity

Hardness of the water is determined by the concentration of divalent cations present in the water. In the present report, the recorded mean hardness was 211.81 ppm and 188.63 ppm in control and probiotic experimental pond respectively (table.4). However, Boyd and Zimmermann (2000) and New (2002) suggested a normal range of 40 – 150 ppm and Saxena (2003) reported 100-150 ppm of hardness for optimum growth in *M. rosenbergii* culture. The present study was supported by Sadek and Moreau (2000) reported 1250 –4115 mg/l higher hardness level but many authors reported that hardness no way enhanced the growth of *M. rosenbergii,* (Vasquez *et al*., 1989, Kumar *et al*., 2000, Giap *et al*., 2005 and Nair *et al*., 2006). Wudtisin and Boyd (2006) explained total hardness was consistently greater in concentration of total alkalinity and this is a common phenomenon in aquaculture ponds.

In freshwater aquaculture systems, alkalinity should be generally between 20 – 60 ppm (New, 2002). Saxena (2003) also suggested >50 ppm of alkalinity was ideal for *M. rosenbergii* culture. But, in the present observations, the resulted hardness was higher compared to New (2002). However, alkalinity above 200 ppm may also have an adverse effect on prawn production (Ramesha *et al.*, 1999). In the present study, the noticed level of mean alkalinities of 81.818 ± 6.683 ppm in control pond and 87.272 ± 8.100 ppm in probiotic experiment pond. Boyd and Zimmermann (2000) reported 20 – 60 mg/l was normal alkalinity range. Similar results were given by Quareshi *et al*. (2000) who also reported higher value of total alkalinity in their culture experiment. Ranjeet and Kurup (2002) recorded normal alkalinity level (40 – 87 ppm) in their *M. rosenbergii* monoculture experiments in coconut garden of Kuttanad, Kerala, India. Further, the present study was supported by observation of Wudtisin and Boyd (2006) reported 117 ± 58, 79 ± 23 and 104 ± 40 ppm of total alkalinity in the ponds. The different levels of total alkalinity were recorded by many authors, (Azim *et al*., 2004, Danaher *et al*., 2007, and Wahab *et al*., 2008) in different stocking density of *M. rosenbergii* culture.

2.5.2.9. Total dissolved Oxygen content

Dissolved oxygen in the culture medium is an important factor not only for the respiration of aquatic organisms but also to maintain a favorable and hygienic environment in the water body. The oxygen level in the studied period was higher in probiotic applied pond than the control group with significant level ($P<0.05$). New (2002) and Saxena (2003) recommended 4 ppm dissolved oxygen values are ideal for *M. rosenbergii* growth. In the present study, the mean value of the DO concentration was

3.80 mg/l, 5.00 mg/l in control and experimental pond respectively with recommended ranges for fresh water prawn culture (New, 2002 and Preto *et al.*, 2010).

Fresh water prawn become stressed at a DO level below 2mg/l and when it declines below 1mg/l, prawn become exhausted with serious physiological effects leading to suffocation (Boyd and Zimmermann, 2000, Pascual, 2006). The present observation was supported by Hossain and Islam (2006) who reported the minimum and maximum dissolved oxygen (20000 – 25000 PL/m^2) in control and experiment culture period. Further, the present study was corroborated to the work of Hossain and Paul (2007) who reported 5.1 – 8.2 mg/l in low cost diet on farm trial of *M. rosenbergii* culture. Many authors reported that there was a variation in the DO content in different culture pond of *M. rosenbergii*, by Lan *et al.* (2006), Nair *et al.* (2006) and Asaduzzaman *et al.* (2008, 2010) during their studied periods.

The addition of fishes in the culture ponds also increased the surface and bottom DO. In addition, fish's activity on the pond bottom and water column brings some oxygen to the bottom layers (Jiménez-Montealegre *et al*., 2002). In order to overcome the oxygen depletion, introduction of some fish sp., catla, and silver carp was introduced in the present culture, as these fishes heavily consumed the phyto and zooplankton which ultimately improved the oxygen content (Raman, 1992). In the present study, plankton feeding fish consumed excess phytoplankton, leading to reduced nocturnal respiration and thereby DO requirement, which inturns benefited prawn and other species in control pond and also even in probiotic pond (Ahmed *et al.,* 2008b). The present study was supported by Oanh *et al.* (2000) who studied the effects of probiotic on culture condition of freshwater prawn *M. rosenbergii* larvae.

2.5.2.10. Ammonia

Ammonia (NH_3) is one of the water quality parameter that causes major problems in fish, shell fish and prawn production. Toxicity of ammoniacal nitrogen is attributed primarily to the unionized forms which cause damage directly to gill epithelial tissue (Vijaykumaran, 1998). According to Adhikari and Saha (1999) and Ahmed *et al*., (2008b) prawns are very sensitive to unionzed ammonia and it should be below 0.02 and 0.015 ppm respectively in the pond water.

Levels of free ammonia observed in the present study are within the normal values in most of the monthly analysis but in mid of the culture period, the free ammonia content showed higher value (table.4) due to heavy phyto and zooplankton population and fast organic degradation. Similar reports were given by Reddy *et al*. (1998), Boyd and Zimmermann (2000), Kumar *et al*. (2000), Sadek and Moreau (2000), New (2002), Ranjeet and Kurup (2002), Saxena (2003), Nair *et al*. (2006), Danaher *et al*. (2007), Wahab *et al*. (2008), Kunda *et al*. (2008) and Mohanty (2009), recorded various level of ammonia on different culture method of *M. rosenbergii*.

However, there are few scientifically documented cases in which bacteria have assisted in bio-augmentation, with the notable exception of manipulating the $NH_3/NO_2/NO_3$ balance (Nikoskelainen *et al*., 2003) in which nitrifying bacteria are used to remove toxic NH_3 and NO_2. Fish expel nitrogen waste as NH_3 or NH_{4+} resulting in rapid buildup of ammonia compounds which are highly toxic to fish (Hagopian and Riley, 1998).

2.5.2.11. Nitrate

Ammonia is oxidized under aerobic conditions in two steps: oxidation of NH_3 to nitrite and oxidation of nitrite to nitrate. Several bacteria e.g. *Nitrosomonas,* convert ammonia to nitrite and other bacteria e.g. *Nitrobacter*, further mineralize nitrite to nitrate. Nitrifying bacteria excrete polymers (Hagopian and Riley, 1998) allowing them to associate with surfaces and form biofilms.

In the present study, the mean of total nitrate content was 3.818 ppm and 2.636 ppm in control and probiotic experiment pond, respectively. Similar study was reported by Ranjeet and Kurup (2002) and noticed.0.02 – 0.03mg/l of nitrite and 2.5 – 3.1mg/l of nitrate in mono bi-species and polyculture of *M. rosenbergii.* The present study was supported by Kumar *et al*. (2000), Sadek and Moreau (2000), Giap *et al*. (2005), Asaduzzamann *et al*. (2008) and Mohanty (2009) who were recorded different ranges in their culture ponds of *M. rosenbergii..*

2.5.2.12. Chloride

Chloride content of the water changes from season to season, region to region depending on geomorphological variations of the region. Ideal level of chlorides are suggested for freshwater aquaculture is very less (31 to 50 ppm) when compared to brackish and seawater aquaculture (>500 ppm). Boyd and Zimmermann (2000) suggested <250 ppm of chloride level for freshwater prawn culture. In the present study, higher levels of chlorides were recorded in control pond (mean 294.36 ppm) than probiotic experimental pond (mean 315.09 ppm) (table. 4a). Similar results reported by Quareshi *et al*. (2000) who recorded high chloride content (394.9 ppm) in *M. rosenbergii* culture pond**.** In the present experiments, the recorded chloride has no significant difference (P>0.05) between control and experimental pond and noticed chloride level of control pond was marginally higher than that of probiotic experimental pond.

2.5.2.13. Calcium

Freshwater prawn, like most crustaceans require high calcium concentrations for enzymatic processes involved in moulting and there is also a relationship between magnesium and neutral – muscular energy transmission. In the present study, the mean values of 81.909 and 34.727 ppm of calcium were recorded in control and probiotic experiment pond respectively (table. 4a). The concentration of the calcium studied in the present work is far less in the experimental prawn, *M. rosenbergii* culture pond compared to normal value (75 – 150 ppm) suggested by Reddy *et al.* (1998). According to Boyd and Zimmermann (2000) suggested 12 – 29 ppm range for calcium, <20 ppm for magnesium in freshwater culture ponds. New (2002) reported 0.01 – 18.6 ppm of calcium in prawn culture ponds in Brazil. Wudtisin and Boyd (2006) reported 55 ± 45, 39± 16 and 34.5 ± 16.1ppm of average values of calcium in 42 catfish, 40 freshwater prawn and 18 carp ponds in Thailand, respectively. Further, they suggested that calcium concentration was mostly above 20mg/l and averages exceed 30mg/l, compared to catfish, prawn and carp ponds, cat fish ponds had a higher average calcium concentration than other farms. These reported values are in agreement for the present results of noticed values of calcium in control and experimental pond, which are favourable for the growth of *M. rosenbergii*.

2.5.2.14. Magnesium

Calcium and magnesium on an average make up about 48% and 14% of the total cations present in the freshwater ecosystem. The mean of total magnesium concentrations recorded in the present study are 34.909 and 42 ppm in control and probiotic experiment pond respectively which are within the range (20 – 200 ppm). According to Wudstisin and Boyd (2006) the magnesium concentrations normally were above 5mg/l, with averages 11.6 – 15.0mg/l in catfish, prawn and carp ponds.

Magnesium is absolutely essential for chlorophyll bearing algae and plants. It is generally present in water as bicarbonate and in this form it resembles calcium bicarbonate in reaction with water.

2.5.2.15. Sodium, Potassium and Sulphate

In natural water, sodium occurs as halide (NaCl). Sodium is metabolised only by blue green algae but potassium is a necessary requirement for all algae. Under low potassium levels, growth and photosynthesis of algae are poor and the rate of respiration will be high (Jhingran, 1983). In the present study, the resulted mean value of sodium, potassium and sulphate were 22.81, 19.45 and 18.45 ppm recorded in control pond whereas 22.18, 19.81 and 16.18 ppm in probiotic experimental pond respectively as per the recommended amount. Boyd and Zimmermann (2000) suggested between 30mg/l of sodium, 300- 400 mg/l of potassium, <250 mg/l of sulphate for freshwater culture ponds. New (2002) also reported 0.26 – 30.0 ppm, 0.01 – 4.9 ppm and 0.1–2.60 ppm, sodium, potassium and sulphate ranges in *M. rosenbergii* culture respectively.

2.5.2.16. Phosphorus

In the present experimental study, the resulted total phosphorus mean values are 0.950 ± 0.211 ppm and 0.927 ± 0.206 ppm for control and probiotic experiment pond respectively. New (2002) reported 0.003 – 4.4 ppm of total phosphorus value in *M. rosenbergii* culture. The noticed value of the present results showed low value with very little variation among the months (table.4a). The present study was similar to the report of Hassan and Bandhopadhyay (1997) with combined cultivation of *M. rosenbergii* and *Ctenopharyngodon idella* culture pond. Findings of this study showed that the use of commercial probiotics in fresh water prawn, *M. rosenbergii* pond could improve the population density of various beneficial bacterial flora reduced concentration of nitrogen and phosphorus and increase the yield of prawn. This present study was supported by Wang *et al*. (2005) who also reported the reduction of nitrogen and phosphorus in commercial applied probiotic in shrimp culture.

2.5.2.17. Iron

Iron occurs in natural water either as bivalent ferrous or trivalent ferric form. Iron is necessary for the growth of microorganisms, and successful bacterial strains are able to compete successfully for iron in the highly iron-stressed gut environment (Verschuere *et al*., 2000a). Sideropheros are low-molecular-weight; ferric iron-specific chelating agents that can dissolve precipitated iron and make it available for microbial growth (Verschuere *et al*., 2000a)

In the present study, the mean of total iron content was recorded 1.60 and 2.16 ppm in control and probiotic experiment pond respectively. The values recorded in the probiotic experiment pond showed higher iron content compared to New (2002), <1 ppm value. Iron is needed by most bacteria for growth but is generally limited in the tissues and body fluids of animals and in the insoluble ferric Fe^{3+} form (Verschuere *et al*., 2000a). Adhikari *et al*. (2007) studied the impact of manganese and iron in water on survival, growth and feeding of juvenile *M. rosenbergii*.

2.5.2.18. Fluoride

In the present investigation, resulted high fluoride mean values in control pond (1.354 ppm) whereas in probiotic experiment pond showed low (0.172 ppm). This observed value was within the normal range (New and Zimmermann, 2000) for the freshwater prawn culture. Similar values were recorded in the study of Boyd and Zimmerman (2000) in freshwater prawn culture. In the present study, there may be some of slight variations in the results of chloride, nitrate, sodium, fluoride and this may be due to the progressive growth of the organisms, leading to a rapid increase in biomass, and water quality deteriorates, mainly as a result of the accumulation of metabolic waste of cultured organisms, decomposition of unutilized feed, and decay of biotic materials (Prabhu *et al*., 1999).

In the present experiments of DO, pH and temperature found to be statistically significant ($P<0.05$) in both the pond. Similar results were occurred in the one-way ANOVA analysis ($P<0.05$). The correlation co-efficient results also showed significant results (0.01 level) and obtained moderate degree of positive correlation for temperature and low degree of correlation recorded in pH and DO (table.3a,b,c). The eighteen studied water parameters values found to be statistically significant ($P<0.01$) in the both ponds of which the turbidity value (-0.063) showed negative correlation co-efficient (table.4b). Prabhu *et al*. (1999), Wang *et al.* (2005, 2007a) and Farzanfar (2006) used some micro-organisms on a shrimp farm to evaluate them as a factor for controlling the water quality. According to the results of this study, all factor of water quality parameters were at optimum level in the experimental pond compared with the control.

2.5.3. Bacteria

Bacteria are the most dominant group of microorganism and occur as *cocci, bacilli* or *spirilli* in soil. *Bacilli* are common, while *Spirilli* are reported to be very rare. In the present study, in addition to the three common genera viz., *Actinobacter*, *Aeromonas*, *Enterococcus* and *Cornybacterium* is also recorded in control pond where as in probiotic experimental pond addition to the six common genera *Actinobacter*, *Aeromonas*, *Lactobacillus*, *Cornybacterium*, *Enterococcus*, *Rhodococcus*, *Rhodobacter* and *Acinetobacter* were noticed. Of these 15 genera *Pseudomonas* and *Bacillus* occurred more common in both the ponds (table.5,6).

Our results with regard to the bacteria in *M. rosenbergii* culture are similar to those found by these authors. Lalitha and Surendran (2004) isolated 19 genera of bacteria in water and sediment from two farms located at Kottayam district in Kerala, India. Paulraj (2002) reported 40% and 60% of gram negative and gram positive bacteria from rearing water of *M. rosenbergii* culture, Chennai, India. Phatarpekar *et al*. (2002) reported altogether, 16 genera were identified from rearing water, egg, larvae and different organs of berried *M. rosenbergii* in larval rearing period, Goa, India. The generic composition of the bacterial flora isolated in *M. rosenbergii* hatchery system (Kennedy *et al*., 2006) in Chennai, India varied from 14 – 18 genera. Al-Harbi and Uddin (2004a), Lalitha and Surendran (2004) and Jeyasekaran *et al*. (2006) were examined different genera varied from 14 – 18 in *M. rosenbergii* hatcheries.

In the present study, higher distributions of bacteria in the probiotic experiment pond are directly proportional to survival rate and production of *M. rosenbergii* than control. Moriarty (1996, 1998) added *Bacillus* spp. as probiotic in the penaeid shrimp ponds; the result of this study shows increasing survival rate and decreasing of luminous *Vibrio* densities in the pond water.

Ahn *et al*. (1999) also reported 64% gram-negative and 36% of gram positive bacteria in Wang Song reservoir near Seoul. Phatarpekar *et al*. (2002) noticed gram negative comprising more than 75% of the total isolates strain in *M. rosenbergii* larval rearing. Al-Harpi and Uddin (2004a) examined gram-negative bacteria dominated the genera composition of bacteria from *M. rosenbergii* larva culture system although gram-positive bacteria still comprised a noticeable percentage. Lalitha and Surendran (2004) investigated 60 – 70% of bacteria in *M. rosenbergii* culture pond during their study. This present study was supported by Anderson *et al*. (1990), Joborn *et al*. (1997), Sugita *et al*.

(1998), Rengpipat *et al*. (1998), Moriarty (1998), Maeda (1999), Paulraj (2002), Hong *et al*. (2005), Kennedy *et al*. (2006) and Deeseenthum *et al*. (2007).

The resulted high bacterial load in the present study ($1.2 \times 10^3 – 5.3 \times 10^4$) in control pond and ($3.5 \times 10^4 – 7.1 \times 10^5$) in probiotic experiment pond probably due to sedimentation of organic matter and dissolved oxygen in the cultured pond (table.7). Similar results obtained by Phatarpekar *et al*., 2002 in a clear water system on day 10 ($1.3 \pm 0.9 \times 10^6$ CFU/ml) bacterial load in larval rearing of *M. rosenbergii*. The higher load of bacteria was attributed higher organic matter, (Otta *et al*., 1999 and Phatarpekar *et al*., 2002). Similar to the present study, Phatarpekar *et al*. (2002) observed a positive correlation between the level of total suspended solids and bacterial counts in control pond as in the present study. The total bacterial counts were significantly higher in intestines of the shrimp fed diets supplemented with probiotic B12 compared with the control groups reported by Robertson *et al.* (2000), and Zhang *et al.* (2008a).

2.5.4. Fungi

Water and oxygen are both absolutely necessary for growth of fungi and in addition, macroelements needed at much higher concentrations, (Onions *et al*., 1981). Okaemo and Olufemi (1997), Koilraj *et al*. (1999), Rao and Vasant (2000) and Surendran *et al*. (2000) reported that number of species occurred in different ponds differed based on the environmental conditions. Higher fungal diversity was also recorded by Girivasan *et al*. (1998) in peat soil. Okpokwasilli *et al.* (1998). Kumar and Sharma (1999) and Paulraj (2002) have isolated varied total number of fungus in different studied culture pond.

Totally 12 and 16 genera of fungi are contributed in control and probiotic experiment ponds in the present study respectively (table.8, 9). In this *Aspergillus* (13), *Pencillum* (3), *Fusarium* (2), *Prechslora* (2) and *Curvularia* (2) are contributed more than one species in both the ponds, whereas other fungai have only one species. Similar report was given by Koilraj *et al*. (1999). In the present study, *Aspergillus* (38.46%), *Pencillum* spp., (11.58%), and rest of them are only one species contributed 3.84% in control pond where as in probiotic experimental pond of *Aspergillus* spp. (39.39%), *Pencillum* spp. (9.09%), *Aspergillus* is a dominant genera in both the ponds, 10 species and 13 species of this genera occurred in probiotic control pond and probiotic experiment pond respectively. The present study was similar with the result of Girivasan *et al*. (1998) reported that *Aspergillus* constitutes nearly 60% of *Deuertomycetes* and was represented 10 species.

In the present experiments, 26 and 33 species of fungi were recorded in control and probiotic experiment pond respectively (table.12, 13). The present study was supported with the report made by Manoharachary and Ramarao (1983) who examined 47 fungal species, representing 32 genera from two freshwater mud ponds in Hyderabad, Further, the present work was resembled with the study of Okaeme and Olufemi (1997) reported about 18 species of fungi associated with pond water and soil. However, Okpokwasilli *et al*. (1998) reported 8 fungal species from a freshwater fish culture pond in Nigeria.

The application of supplemental feed in control and probiotic in experimental pond may modify the abundance of filamentous fungi. In the present study, among the filamentous fungi, the dominant genera observed (*Pencillium* and *Aspergillus*) in the probiotic experiment pond are produced antimicrobials and some toxins as well, which can inhibit the growth of a wide range of bacteria and other pathogenic organisms present in the aquatic environment which enhance the growth of the *M. rosenbergii* in experimental culture pond. In the present study, probiotic application does not only improve the soil and water quality but also enhances the proliferation of many beautiful microflora, including fungi. The change in environment microflora, would also influence the gut of the *M. rosenbergii* that reflect higher production in probiotic experimental culture pond compared to control pond.

Fungi and bacteria have different enzyme capabilities for break down compounds such as tannins, lignin and cellulose and their combined. So, the decomposition rates were higher in the experiment pond soil and indirectly the growth leads to higher in probiotic experiment pond. Bacteria and also fungi are used as food by widely differing animals, (Rheinheimer, 1985). Xianzhen *et al*. (1994) reported that heterotrophic productivity of aquatic bacteria is closely related to fish/ prawn yield.

2.5.5. Planktons

2.5.5.1. Phytoplanktons

Phytoplankton growth in ponds is stimulated by the addition of fertilizers and the waste products from shrimp (Burford, 1997) and provides food for assemblages of pond zooplankton and epibenthic fauna (Coman *et al*., 2003). In the present study 26 and 34 genera of phytoplankton were present in control and probiotic experimental pond respectively (table.14). Similar results were reported by Patnaik *et al*. (1988) who found 24 genera and 26 genera of phytoplankton in rearing pond and stocking pond, respectively Further , the present investigation was supported with the work of Danaher *et al*. (2007) estimated 82 genera of phytoplankton from six algal divisions were identified in Nile tilapia and *M. rosenbergii* polyculture. A total of 51 species were identified in 14 shrimp *Liptopenaeus vannamei* pond in Brazil by Case *et al*. (2008). The following authors were reported varied total number of phytoplankton of different genera in different culture of *M. rosenbergii*, Akpan and Okafor (1997), Azim *et al*. (2001), Aejaz *et al*. (2005), Uddin *et al*. (2007), Korai *et al*. (2008), Kunda *et al*. (2008) and Wahab *et al*. (2008).

In the present study, Cyanphyceae, Chlorophyceae and Bacillariophyaceae contributed 11, 19 and 4 species in control pond whereas 7, 16 and 3 species in probiotic experiment pond, respectively. Higher species diversity was occurred in Bacillariaphyceae in both the ponds. However, Chlorophyceae was dominant in various freshwater ponds are reported by Anand (1998), Azim *et al*. (2001), Aejaz *et al*. (2005), Danaher *et al*. (2007), Udddin *et al*. (2007), Korai *et al*. (2008) and Kunda *et al*. (2008).

Recent reports demonstrated that many bacterial strains may have a significant algicidal effect on many species of microalgae, particularly of red tide plankton (Fukami *et al*., 1997). Positive effects of bacteria on cultured microalgae have also been observed (Rico-Mora *et al*., 1998)**.** Probiotics could be specifically targeted for microalgae production; however, the subsequent effects of such bacteria towards the larvae must be established. The present study was supported with the work of Gomez-Gil *et al*. (2002), who found that the shrimp probiotic could be co-cultured with shrimp larvae food, *Chaetoceros muelleri*, without affecting the microalga.

Phytoplanktons are capable of producing substances toxic to other bacteria and could potentially act in a beneficial manner (Qi *et al*., 2009). In the present experiments, better algal growth was also observed and it could be associated with the maintenance of higher DO concentration compared with the control. The present study was similar to the work of Wang *et al*. (2005) in *P. vannamei* pond in Hai-Yan, China using *Bacillus* sp. The concentration of DO is associated with the density of phytoplankton and thus a greater deterioration of water quality induced was similar to the reports by Wang *et al.* (2005) in *P. vannamei* culture pond in China as in the present study.

In the present experiment, the total phytoplankton counts was decreased steadily during the last part of the trial in the control due to increased grazing pressure by the increased biomass of introduced fish in both the ponds. It is also reported that the filtration rate by fish for both green algae and Cyanobacteria increased linearly when water temperature increased (Turker *et al*., 2003). Wahab *et al.* (1999) and Azim *et al.* (2004) also found similar patterns as in the present study among the phytoplankton community and also stated that prawns were not seen grazing on periphyton. In ponds, freshwater prawn preferred to forage on animals such as trichopteran, chironomids, oligochaets. Nematodes, gastropods and zooplanktons (Coyle *et al.,* 1996; Tidwell *et al.,* 1997a and Uddin *et al.,* 2007) organisms associated with sediments.

2.5.5.2. Zooplanktons

Zooplankton assemblage comprises a significant component of the natural biota of shrimp/prawn culture (Martinez-Cordova *et al*., 1997; Coman *et al*., 2003, Preston *et al*., 2003 and Coman *et al*., 2006) farms. Anderson *et al*. (1987) reported that 53.77% of the nutrition of shrimp in pond comes from natural food. Castille and Lawrence (1988) suggested that natural food contributes more than 50% of the nutrition of *P. vannamei*. There have been numerous studies investigating the general zooplankton response to various sources of stress and subsequently, there use as a biological indicator has been well documented (Webber and Webber, 1998).

Analysis of zooplankton of the present study indicates the occurrence of copepods, cladocerans, rotifers and ostracods in both the ponds. Qualitative analysis of the zooplankton showed the occurrence of 30 and 32 species in control and experiment pond respectively (table. 15, 16). Similar to the present study, Wahab *et al*. (2008) also reported, 16 genera of zooplankton belonged to copepods (3 genera), cladocera (5 genera), rotifera (7 genera) and crustacean and nauplii in prawn-small fish culture practice in Bangladesh. Further, the present study was in accordance with the work of Uddin *et al*.

(2001) observed 17 genera of zooplankton were identified in prawn-tilapia stoked at a fixed 3:1 ratio, with and without substrate and periphyton development. (Kunda *et al*., 2008) found that rotifera (7 genera), cladocera 5 genera, copepod 3 genera and crustacean nauplii were reported in prawn – mola different stocking density.

Zooplankton showed variations with regard to their abundance during the different months of culture periods both in control and experiment pond. The initial zooplankton contents were low (115 and 152 numbers in control and experiment ponds, respectively) which increased tremendously in the month of March in both ponds, such a increased zooplankton density might have resulted from the microbial mixture and application of lime in both the ponds, whereas in higher numbers of zooplankton in the experimental pond indicates application of probiotic through feed, vitamin and mineral mix (mutagen) metal and minerals (Sodamix) and also due to the availability of phytoplankton on which zooplankton forage. Compared to the month of March, sudden decreased level of zooplankton was noticed in the month of April in both ponds which could have resulted due to the feeding of *M. rosenbergii* juveniles (table.17). Further, depletion in phytoplankton might also have caused lesser density of zooplankton

In the present observations, the zooplankton density again increased in the month of May and July in both the ponds **(**table.17a**).** During this month, higher density of zooplankton was recorded in probiotic experimental pond than in control pond. The abundance of zooplankton was also similar to that found by Azim *et al.* (2004) in a periphyton based carp culture in Bangladesh, suggesting that the zooplankton community were preferred by adult fish. However, in the present study the introduced fingerlings preferred the zooplankton as their food. Higher density of zooplankton during this month in the present work can be attributed to the availability of suitable phytoplankton. It is also proposed that the *M. rosenbergii* juveniles which were actively feed on the natural food might have now preferred pelletized feed than the natural food. In this case, the unconsumed pelletized feed which settles at the bottom of the pond might undergone decomposition and provides rich nutrients for the abundance of bacterial population. Availability of a high concentration of bacteria promotes growth of high density protozoans. Protozoans and bacteria inturn are effectively utilized by the zooplankton which increases the density to a very great extent.

The present study was similar to the study of Case *et al.* (2008) who reported the noticed value of high density of zooplankton in probiotic experimental pond in the present study might be due to application of probiotics to the pond when compared to control, which clearly indicated that probiotic applications enhance the microbial population without harm to cultivable freshwater prawn *M. rosenbergii*. The same trend was observed by Maeda (1999) who also reported production of high density of zooplankton. The present study was similar to the work of Preston *et al*. (2003) who reported variations in zooplankton abundance and composition of species. Further, after the first harvest water exchange was done more frequently (weekly once) because *M. rosenbergii* density was high in both the ponds, water was pumped upto 1.2m and above to avoid the dissolved oxygen problem. Due to this freshwater pumping, the plankton density was less after the first cull harvesting. During the production season, farm managers regularly exchange water. However, Coman *et al.* (2003) found no significant relationship between the volume of water exchanged and the change of zooplankton density.

The present report showed that zooplankton density of control and probiotic experiment pond was positively correlated with groupwise total and monthwise total zooplanktons. Total copepod population, rotifer, cladocerans and ostracod population of control and probiotic experimental pond was positively correlated and statistically significant at 0.01 level. Transparency reading were negatively correlated with zooplankton abundance, therefore zooplankton were higher when the algal biomass was higher (Coman *et al*., 2006). Many earlier investigations also recorded higher survival and weight gain in post larvae of *M. rosenbergii* when fed on zooplankton from a wild source than an artificial diet (Brown *et al.*, 1992; Collins, 1999 and Paulraj, 2002). Larger prawns (40g) have been found to benefit from increased feed quality late in the production season (Tidewell *et al*., 2004b).

2.5.6. Length and weight relationship

The range of length and weight of the control and probiotic experimental pond showed different growth pattern in the present study of *M. rosenbergii* culture. In control pond, animals weighed of about 4 – 60g from the first harvest to final harvest and the same trend also seen in probiotic experimental pond (5–75 g) (table.26), it indicates normal growth curves were occurred in both ponds simultaneously probiotic application pond showed higher growth rate compared to control pond (fig.48, 49). In the present study, the weight gain of *M. rosenbergii* among experiments was higher in the probiotic experiment pond (73.98 g) compared (47.98 g) in control pond (table.35). Similarly Siddiqui *et al.* (1999) who reported the weight gain in prawns ranging from 25.2 – 37.0g at a prawn density of 7500 ha -1. Further, the present study was coincidence with the work of Venkat *et al.* (2004) who reported the highest and lowest weight gain in *Lactobacillus sporogenes* (132.5%) and *Artemia* control (99.5%) respectively in *M. rosenbergii* post larvae. In the present experiment, the probiotic fed groups were significantly ($P < 0.01$) higher in length and weight which are highly positive correlated than control groups (table. 26a). The higher weight of prawn may be due to relative weight of hepatopancreas (observed in the present study) which plays a key role in food assimilation (Dhall and Moriarity, 1984) probably manifest the provision for energy utilization for growth and metabolism. This observation strongly corroborates with the earlier report (Kris *et al*., 1987 and Kurup *et al*., 1999).

In the present experiment, the mean ± SE values of weight showed higher values in probiotic experimental pond upto September month, (54.222 ± 2.605 g) (table.26a) after a decline was noticed (i.e. 230 days to 269 days), this may be due to low DO rate observed during the month of October and high temperature in the morning hours and this may influence the low harvest rate. So that the physiological function was low and may leads to less digesting capacity, this may inturn reduce the growth. In the present study also after the first partial harvest, to final harvest three to four types counts (marketable prawns) were caught and found that 68% of the prawn weighed more than 15 g; 14% weighed more than 30g, 10% weighed more than 40 g and 8% weighed more than 60g. This was supported by Garcia-Peerez *et al*. (2000). Danaher *et al*. (2007) who also suggested that even greater tilapia densities can have effect on prawn yield, survival and total pond production.

In the final harvest i.e., in the month of December, the average mean weight was 32.466 ± 2.162 g and 31.320 ± 1.804 g in probiotic experimental and control pond respectively (table.26a and fig.49). This was due to the over period of culture, normally the culture period extend upto 6 to 7 month in batch culture (Langer and Somalingam, 1993; Kumar *et al*., 2000; Sadek and Moreau, 2000; Lan *et al*., 2006 and Nair *et al*., 2006) in some culture condition it was extended upto 8 to 9 month (Sampaio and Valenti, 1996; Islam *et al*., 1999; Ranjeet and Kurup, 2002; Ahmed *et al*., 2008b; and Mohanty, 2009).

In the present study, it was 304 days in control pond and one day extra for probiotic experimental pond. In the present study, the length-weight relationship clearly indicates differential growth pattern. The end of the culture period, the growth was decreased. Several authors present separate length-weight and length- length relationships for males and females (Chow and Sandifer, 1991; Primavera *et al*., 1998 and Tzeng *et al*., 2001). However such separation of these morphometric relationships for males and females may not be necessary for periods at certain life history stage (Cheng and Chen, 1990; Dall *et al*., 1990 and Chu *et al*., 1995).

The present results was correlated with the work of Hui-Rong *et al*. (2001) who reported that probiotic fed animals was larger than that of unfed ones (13.3 cm and 12.7 cm respectively) and mean body weight between the treated and untreated shrimp (23.18 g and 20.6 g) respectively. In the present study, the reported variations in the length and weight of the animals in control and probiotic experiment pond may be due to environmental factors like pH, temperature etc. Further, the present study was supported by Indulkar and Belsare (2003) who reported higher percentage gain in weight of post-larvae was observed when fed the diet containing probiotic (GP@7.5g kg-1) diet compared to the control diet. Moreover, Garcia-Peerez *et al*., (2000), Das *et al*., (2007) and reported that the morphometric differences and variability in morphological characteristics were adaptive responses to the environment especially in crustacean populations.

The present study showed positive correlation in length and weight between months in control and probiotic experimental pond during the culture period (table.27 - 30). A difference in the average body weight of the prawn, in control and probiotic experimental pond during the trial netting in the present investigation reveals that higher body weight recorded in the probiotic experimental pond. Durairaj *et al*. (1992) and

Quareshi *et al*. (2000) reported similar average weight in all trial netting analysis. But last two trial netting analysis in the present work for both the pond (269th and 293rd days of final netting) showed decreasing trends, it indicates prawn growth is stopped in both the ponds and even though probiotic application was continuously broadcasted to the probiotic experimental pond. The enhanced growth and weight in *M. rosenbergii* probiotic application may be due to the degradation of organic matter thereby significantly reducing the sludge and slime formation. By improving total water quality and FCR, the overall health and immunity of the prawn will be improved, (Green and Green, 2003).

2.5.7. Growth and Survival

In the present study, the formulated probiotic have beneficial effects on water quality and disease control as well as survival rates (100 %) in the experiment ponds. In the present study, the mean average weight (32.46 g/0.6 ha/305 days) and the yield (1178 kg/0.6 ha) characteristic showed higher in probiotic experimental pond. The present result shows the average mean weight **(**31.32 g/0.6ha/304 days**)** of *M. rosenbergii* in control pond is mainly due to low survival rate compared to experimental pond. Similar study was recorded by Kurup *et al*. (1998a) and reported poor percentage of survival (16.59%) in the control pond and high average weight (97.178 g) by the application of probiotic feed. Further, the present study was supported by Ranjeet and Kurup (2000) and recorded high mean weight 43-83 g and 12-28% of survival in batch/size grade culture of *M. rosenberigii* in Kuttanad, Kerala, India.

Further, Similar results reported by Maeda and Liao (1992) on the beneficial effects of soil extract on the growth and survival of penaeid larva of *P. monodon*. The highest survival and production observed in the probiotic experimental pond in the present study may also be due to application of probiotic in the experimental pond. The present study was supported by Sadd *et al.* (1999) who demonstrated the positive effects of probiotic feed supplement, Biogen on the growth and survival Freshwater Prawn, *Macrobrachium rosenbergii*. Similar to the present study was reported by Oanh *et al.* (2000) who reported that the length of survival of larvae of *M. rosenbergii* was higher by the application daily usage of probiotic in this study. About 34 - 75% of survival of *M. rosenbergii* was reported by different authors (Ang, 1990; John *et al.,* 1995; Siddiqui *et al.*, 1996; Sadek and Moreau, 2000 and Quareshi *et al*., 2000)..

In the present study, higher growth in probiotic fed diet prawn *M. rosenbergii*, suggesting that the addition of probiotic enhance the growth performance and feed utilization. The present studied results are in agreement with work of Suralikar (1996) who reported better growth performance in *M. rosenbergii* post larvae fed on lactic acid bacteria *Bactobacillus lactis.* Growth response of white prawn, *Penaeus indicus*, to dietary L-carnitine reported by Jayaprakas and Sambhu (1996). All the probiotic-supplemented diets resulted in an increase of final weight, DWG and RGR, showing that the addition of probiotics increased the growth performance of shrimps, were reported by Swain *et al*. (1996) for Indian carp (Labeo rohita) and Wang *et al.* (2007) for shrimp *P. vannamei*. Noh *et al*. (1994) and Bogut *et al.* (1998) showed that a commercial probiotic preparation of *Streptococcus faecium* improved the growth and feed efficiency of Israeli carp (*C. carpio*). Himabindu (1998) has reported better growth performance in post - larvae of *M. rosenbergii* when fed with lactic acid bacteria *Lactobacillus sporogenes* (24 x 10^{7} cfu per 100g) than when fed with *L. acidophilus* (140 x 10^{11} cfu per 100g). Prabhu *et al*. (1999) studied the usefulness of a probiotic N.S. Series Super SPO TM in maintaining water quality and thereby enhancing growth rate and production in shrimp culture. These results have been reported also in the Indian white shrimp Fenneropenaeus indicus (Ziaei-Nejad *et al*., 2006) and in *P. vannamei* (Wang, 2007).

The present study was confirmed with the experiments of Ang (1990) on the monoculture of *M. rosenbergii* has produced 979.02 kg/ha/cycle, the average survival during this trial was 32.4% and the average weight recorded was 33.6gms, while Vasudevappa *et al*. (1998) reported 1,536 kg/ha and he recorded 80% survival and an average weight of 38.4 g at the end of 6 months culture.

Further, the present study was confirmed with the work of Ziaei-Nejad *et al*. (2006) who examined the effect of commercial *Bacillus* probiotic by three experiments on the digestive enzyme activity, survival and growth of *Fenneropenaeus indicus* at various ontogenetic stages. In the present work, the achievement of higher prawn growth rate in probiotic-treated groups was supported by several studies (Moriarty and Body, 1995; Moriarty, 1998; Rengpipat *et al*., 1998; Cima *et al*., 1999; Nikoskelainen *et al*., 2001; Meunpol *et al*., 2003; Das *et al.*, 2006 and Saad *et al.*, 2009). Survival rates, emergence time of post larvae, completion of post larvae development and water quality were found to be better in all trial with probiotic application in *M. rosenbergii* hatchery system in the present investigations.

According to the study of Wang and Xu (2006), who mixed probiotics (photosynthetic bacteria and Bacillus sp. isolated from carp ponds) and introduced with aquaculture pond which induced the best growth performance compared with individual probiotics, in growth performance in shrimp. This indicated that the quantity of probiotics is only one of the factors promoting the growth performance of shrimps. The enhanced growth performance in the present studied experimental pond than control of prawn might be due to increasing digestive enzyme activity induced by the probiotics. Furthermore, bacteria particularly members of the genus Bacillus secrete a wide range of exoenzymes (Moriarty, 1996, 1998) and that enzymes synthesized by the probiotics. The higher level of enzyme activity obtained with diets containing probiotics improved the digestion of protein, starch, fat and cellulose, which might in turn explain the better growth observed with the probiotic supplemented diets. Similar effects have been reported for fish and shrimp, in which digestion was shown to increase considerably in response to probiotics in the diet (Lara-Flores *et al*., 2003; Tovar-Ramírez *et al*., 2004; Ziaei-Nejad *et al*., 2006). Based on these results, use of a 10 g kg−1 (wet weight) supplement of probiotics (5 g kg−1 PSB and 5 g kg−1 BS) in shrimp *P. vannamei* diet was recommended to stimulate productive performance, (Wang, 2007).

Probiotic bacteria are a good candidate for improving the digestion of nutrients and growth than that with the control diets in aquatic organisms (Irianto and Austin, 2002; Lara-Flores *et al.*, 2003). Further the present study was correlated with the work of Devaraja *et al.* (2002) investigated shrimp (*P. monodon*) production showed better growth when ponds treated with two commercial mixed microbial probiotic products. Venkat *et al*. (2004) evaluated in their results that a significant growth was observed for larvae fed diets supplemented with probiotic in ***M. rosenbergii***. Moriarity *et al*. (2005) also achieved higher growth and weight of *M. rosenbergii* by the application of probiotics to the prawn culture pond than control groups. Farzanfer (2006) also reported higher growth rate by the use of probiotics in shrimp culture.

2.5.8. Feed Conversion Ratio

After complete harvest, the food conversion ratio (FCR) was recorded **(**1.31**)** and **(**0.96) in the present study of control and probiotic experiment pond respectively which was found to be statistically significant at P<0.05 level more or less similar to the values of (1.73 – 2.12) reported by Siddiqui *et al.* (1999). Abraham *et al.* (1995) observed a similar positive effect on addition of probiotic feed supplement 'Lactose' on the growth of shrimp with significant level ($P<0.05$) of FCR than the control group. Further, this was confirmed with the experiments of Tidwell *et al*. (1997a), with 0.04 ha stocking density 3.9 m^2 of *M. rosenbergii* with different organic fertilization and resulted 2.31 – 3.11 FCR values. The present study was in agreement with the work of Uma *et al.* (1999) who observed a significant improvement in FCR, FER and PER of shrimp larvae when fed with *L. plantarum* bio-encapsulated Artemia. Similar study was reported by Tidwell *et al.* (1996) and Quareshi *et al*. (2000) that 2.31±0.04 and 2.34±0.14 FCR in Kentucky state university and Mississippi state university (different latitude) respectively. Kumar *et al.* (2000) were recorded 2.3 ± 0.1, 2.2 ± 0.1, 2.8 ± 0.1 FCR in 61 day ungraded and 61 day graded and 133 day graded juveniles of *M. rosenbergii* culture respectively.Sadek and Moreau (2000) reported 1:4.1 ± 1 FCR in monoculture of *M. rosenbergii.* Tidwell *et al*. (2003) and Correia *et al*. (2003), reported 0.75 – 2.28 FCR in natural and supplementary feeding traits of *M. rosenbergii*. Nair *et al*. (2006) stated that the apparent FCR which has a direct effect on the cost of production, was the best for all – female (1.26 ± 0.02) followed by all-male (1.30 ± 0.05) and mixed cultures (1.62 ± 0.02), applied probiotic along with supplemental feed. Different authors reported less FCR in the experimental pond with different combinations, different food addition polculture, compared to control culture pond of prawn viz., Danaher *et al*. (2007), Gupta *et al*. (2007), Hossain and Paul (2007), Uddin *et al*. (2007), Tidwell and Coyle (2008) and Asaduzzaman *et al*. (2009).

Further, the FCR was calculated from the month of August to December 2008 (harvest period) and found maximum during the month of August in both ponds (table. 35). The FCR in the final harvest i.e in December month it was 3.27 and 3.14 for control and probiotic experimental pond respectively which showed significant at $P<0.05$ level. Similar to the present results, Siddiqui *et al*. (1997) reported higher FCR values of 3.7 (5 prawn/m^2) to 5.6 (20 prawns/m^2) for *M. rosenbergii* cultured in concrete tanks fed diet containing 34% protein. The present work was corroborated by the work of (Ziaei-Nejad *et al*., 2000) in Fenner *Penaeus indicus*, with improved feed conversion ratio (FCR) and specific growth rate (SGR) by the application of commercial *Bacillus* probiotics. The present investigation was concurrent with the test of Devaraja *et al.* (2002) investigated shrimp, *P. monodon* showed there were significant difference in FCR (feed conversion ratio) in pond treatment with a product containing *Bacillus* sp. and *Saccharaomyces* sp. compound with ponds treated with *Nitrosomonas* sp. and *Nitrobacter* sp. and untreated control pond. Similar study was made by Venkat *et al*. (2004) who observed a significant improvement in FCR, FER and PER of shrimp larvae when fed with *L. plantarum* bio-encapsulated in artemia.

The present study was related to Far *et al.* (2009) tested a commercial probiotic *B. subtilis* had the greatest FCR, SGR and growth performance by consistant application to the shrimp culture. SGR, FCR and PER are best in probiotic applied *M. rosenbergii* than control. Increased growth rate in probiotic culture may be due to the improved feed conversion via increased fatty acid oxidation and utilization of dietary energy (Moore *et al*., 2000). Further, the present study was strongly supported by Haroun *et al.* (2006) and Saad *et al.* (2009) by Biogen supplementation to diets resulted in reduced FCR and improved weight gain in *M. rosenbergii*.

Mortality was not found in the experimental groups in the present experiment indicating no adverse effect of probiotic on survival (one or two by cannabolism or aggressive behaviour).

Further, conversion ratio may differ with the nutritional quality of the food, although food conversion efficiency was reported to vary with environmental conditions. The factors which determine the quality of feed are its nutrient profile, particle size, texture, stability of nutrients, attractability, digestibility, anabolic efficiency and shelf life (Paulraj, 1999). Mariappan and Balasundaram (1999) opined that feed quality is an important criteria which directly influences the growth rate of shrimp/prawn and is a major factor responsible for a profit harvest from shrimp/prawn farms. The quality and content of protein in the feed might also play an important role in feed conversion. The present study also indicates high feed efficiency in probiotic experimental pond **(**FE - 1.03**)** than in control pond **(**FE - 0.75**) (**table. 36). The animal counts are considering an important revenue system.

2.5.9. Production

In the present study significantly higher ($P<0.05$) net production of *M. rosenbergii* noticed in probiotic experiment pond 1178 kg /0.06ha-1 and 866 kg /0.06ha-1 in control pond were calculated. The higher production in probiotic application may be due to increase in length and weight by probiont added its antagonism towards pathogenic bacteria, the growth enhancement factor may be due to the proteolytic enzyme produced by Bacillus sp. as Rengpipat *et al.* (1998). Further, the enzymatic activities are reported by Das *et al*. (2006).

Further, the present study was conformed with the work of Lan *et al.* (2006) who examined the effect of different stocking density 1, 2, 3 and 4 PL/m^2 in rotational rice – prawn system for 210 days culture of *M. rosenbergii* resulted 28.8 – 49.8% survival in 100m^2 plots and their yield ranged 194 – 373 kg. Further, the present results was coincided with the work of Kunda *et al.* (2008) also reported 49 – 57% survival for prawn culture with 45 – 58g of individual weight and the net production of freshwater prawn ranged 294 – 596 kg/ha respectively, during 4 month culture period depending on stocking density 10000 – 25000 PL/ha. Wahab *et al*. (2008) also stated similar reports that the mean harvesting weight (55.24g) and survival (48%) of prawn were significantly higher in the stocking density of 15,000 PL/m^2/ha and mola 2000/m^2/ha, then in treatments of 20000 and 25000 PL/m^2 stocking density. In the present investigation, the increased net profit was achieved by using the probiotics in the water and by incorporating the "Sanolife" probiotics along with feed, so that the intestinal tract of the prawn was colonized by probiotic bacteria successfully. This study was correlated with the work of Moriarty *et al*. (2005) also explained the same reason for the increased weight of the prawn.

The production from all male in the present study was higher than that of all female prawn. The net revenue was higher in male prawn in probiotic applied pond than control pond over Rs. 2, 06, 758 and Rs. 57,971 respectively. In the present study, the less production of female in both culture ponds, partially owing to the discount allowed for egg carrying female at marketing times. In the present study, females contribute 14.06% and 166 kg in probiotic experiment pond whereas in control pond was 27.06% and 131 kg, compared to other counts. Similar study was reported by Nair *et al.* (2006). The observed higher production (survival and weight) may also due to favourable water quality and biological parameters which are enhanced by the application of probiotic to the pond along with supplemental feed. Alam (1992) and Wahab *et al.* (2008) observed a prawn production of 220 kg ha-1 in rice fields from a 160 days culture period, close to the production found in this study.

The higher profit in probiotic applied pond might be due to better production of prawn compared to control. This present study was supported by Venkat *et al.* (2004) who have been reported highest and lowest body weight gain (production) were observed in probiotic fed group and control respectively. The present study was in concordance with the work of Aly *et al.* (2008) who also reported higher production in probiotic applied groups of prawn than control ones. Similar reports were also given by Padma kumar *et al.* (1992); Raja and Joshi (1992) and Durairaj *et al.* (1992).

In the present investigation, the included number of *M. rosenbergii* in each kg was 28 – 72 counts and 26 – 70c in control and probiotic experiments respectively. The number variation in the experiment culture pond may be due to size variation, heterogeneous individual growth, especially among males, forms a major obstacle in profitability in *M. rosenbergii* culture, (Ravishankar and Keshavanath, 1988). Below 20g animals contributed 10.79% in probiotic experiment pond and 17.27% in control pond. Similar study was carried out by Ranjeet and Kurup (2002) and have been reported that the larva first hatched contributed 25.63% of animal 50g class group conversely, the percentage of weight class >120g was high the later hatched group (63.4%) but the <50g weight class constituted only 16%. The trend followed more or less a similar pattern in size-graded post larval groups.

2.5.10. Fish Yield

In the present study, catla and carp yield showed 765/kg/0.6ha/210 days and 842 kg/0.6ha/211 days, in control and probiotic experiment ponds respectively. Both the pond showed 100 percent survival, the average mean body weight was 1.53 kg in control and 1.68 kg in probiotic experiment pond. In the present experiment, the fishes were introduced inorder to check plankton and their weight of *Catla catla* showed increased upto 4 kg which are more or less similar to the findings of Siddiqui *et al.* (1999) who reported the weight gain of 249.3 – 278.3 and 195.2 – 296.4 g for catla and rogu respectively in polyculture with *M. rosenbergii*.

Karplus *et al*. (1986b) reported 352 kg/ha of prawn of stocking density of 1/m^2 in polyculture for 110 day culture period. Brown *et al*. (1991) commented that polyculture considerably complicates the grow-out management of prawns. Jose *et al*. (1992) obtained 106 – 254 kg/ha in an experiment of polyculture in which prawn were stocked at a rate of 1/m^2. The present study was similar to Islam *et al.* (1995) reported a production of 172 kg/h/year where *M. rosenbergii* was stocked at 15000h-1 with silver carp, catla, rohu and mirgal. The present study was similar to Islam *et al.* (1999) reported a production of 172 kg/h/year where *M. rosenbergii* was stocked at 15000h-1 with silver carp, catla, rohu and mirgal**.** Uddin (2007) and Kunda *et al.* (2008) showed that in mixed culture the feeding niches of tilapia and prawn only partially overlap, and recommended this duo-culture as an alternative to polyculture of Chinese and Indian carps.

Cent percent survivals of fishes were recorded in both the ponds. But the observed similar survival (75 – 76%) of prawn with different tilapia densities revealed that addition of substrates might have minimized the territoriality and different water quality parameters fell in the favorable limits of *M. rosenbergii* due to maintaining a high C: N ratio in all treatments. A limited level of cannibalism during the molting is normal and may be responsible for a mortality of 4% monthly (AQUACOP, 1990).

2.5.11. Economic analysis:

In the present study, the seed and feed cost occupied 13.89% and 18.55% in control pond whereas 12.46% and 20.50% in probiotic experiment pond, respectively (table. 33, 34). Sandifer (1982) are of the view that the largest single item in the economics of shrimp farming was the seed cost representing on an average between 58.3% and 63.8% of the variable production cost. Ang (1990) reported that cost of postlarvae and feed are the two major expenditures in *M. rosenbergii* culture in Malaysia. Rhodes (2000) reported values of feed cost portions of the operating costs of the farms ranging between *5%* in the USA to 52% in Malaysia with 41% in Brazil. Quareshi *et al*. (2000) also reported that 65% of the total expenditure accounted for the feed used for raising prawn to marketable size. Schwantes *et al*. (2009) stated that feed and seed were necessary and were higher proportion of costs, averaging 56% and 17% respectively Prawn juveniles were expensive inputs (about 47% of the total cost) in all treatments followed by prawn feed (16.17%), Similar reports was given by Ramakrishna (2010).

Variable costs in prawn culture are cost of seed, feed, fertilizers, labour (family and hired), harvesting and marketing and miscellaneous. Muir (2003a) reported that prawn production cost comprise 28% of seed, 21% of feed and only 4% of labour. Ahmed *et al*. (2008) survey in Bangladesh showed, the average annual cost for human labour were calculated at US$ 112.61/ha/yr for extensive farms, in comparison to US$ 152.45/ha/yr for semi – intensive farms. In the present study, the labour cost was Rs.43.88/- (US$ 0.91) in control pond whereas Rs.30.61/- (US$ 0.63) in probiotic experiment pond. Nair *et al*. (2006) reported that the variable cost was Rs. 70,138 in all-male (the highest), Rs.45,720 in all female (the lowest) and Rs. 5,789 in mixed pond. Fixed cost included depreciation (water pump, net, feed machine etc), land use and interest on operating capital (Shang, 1990), within variable cost seed and feed dominated all other cost averaging 39% and 33% of the total cost (Ahmed, 2004).

In the present study, land lease cost (rental cost) was Rs.22,500/pond/yr/. In the present investigation, the capital cost of control pond were Rs. 51,500/- (US $ 1072.91) and in probiotic experimental pond Rs.52, 500/- (US $ 1093.73). Schwantes *et al*. (2009) stated that land rented cost was 5 to 75 dollars/ha/yr on a average 40 dollars in Thailand.

In the present observation**,** the cost of production of one kg of prawn in control pond is Rs.362.12**/- (**US$ 7.59) while in probiotic experiment pond it is Rs.282.41/**- (**US$ 6.78). The high price cost of one kg prawn in the probiotic experimental pond indicates application of probiotic and mineral combinations may enhance the microbial niche in the pond that improves the water quality and soil fertility. The highest average price of one kg of prawn was Rs. 295.07/kg in all-male ponds, followed by the mixed ponds Rs.288.71/ kg and the all- female ponds Rs.262.04/kg, (Nair *et al*., 2006).

In the present study, Rs. 4,25,070/- (US$ 1435.43), as gross income while the net income was Rs.57,971 /- (US$ 362.52) in control pond while in experimental pond gross and net income was Rs.5,93,940 /- (US$ 5877.08), Rs. 2,29,600/- (US$ 4783.33) respectively**.** In Southwest Bangladesh, the net income of farming is an average (US$ 1430/ha/yr), Muir (2003a). Hossain and Islam (2006) obtained highest net profit (TK 69006/ha) in stocking density of 10000 PL/m^2 and the lowest (TK. 28375/ha). Mohanty (2009) reported net-return from rice-fish/prawn culture ranged between Rs.49, 997/-ha to Rs. 74,533/-ha in various stocking density studies.

In the present study, the economic analysis of the data revealed that the net revenue realized was the highest in the control (Rs.57, 971) pond when compared to probiotic experimental pond (Rs. 2, 06, 758/-). The present study was supported by Moriarty *et al.* (2005) suggested the net profit was therefore greater which was achieved by using the respective probiotic in the water by incorporating the Sanolife probiotic in all the feed. Further the present study was similar to the study of Zhong and Guang (2008) who applied the probiotic bacteria, *Bacillus* sp. and EM at different stages of *P.vannamei* pond cultures, increasing the average per hectare upto 6400 Euro. Mohanty (2009) reported in rice-fish/prawn culture, when phased harvest is practiced, the net return was enhanced further by 49%. Average net profit US$ 3918/ha/yr were realized in Thailand *M. rosenbergii* culture by Schwantes *et al*. (2009). A number of authors worked out the cost and returns of monoculture and polyculture systems of *M. rosenbergii* (Ghaffer *et al.*, 1988; Law *et al.,* 1990; Prakash *et al*., 1990; Padma kumar *et al.*, 1992; Law *et al.,* 1993; Mathew, 1994; Sadek and Moreau, 1998; Quareshi *et al*., 2000; Nair *et al*., 2006; Uddin *et al*., 2007 and Kunda *et al*., 2008).

The Benefit-Cost ratio (BCR) of the semi – intensive system is 1.73 which is significantly higher than in the extensive system (1.57), in Bangladesh farming (Ahmed *et al*., 2008a). In the present study BCR of control and experiment pond was 1.35 and 1.78 respectively. Significant BCR was also reported by Nair *et al*. (2006) and Kunda *et al*. (2009). The higher BCR ratio was reported by many authors applying supplemental feed, probiotic application, stocking densities, mono-sex culture, selective harvest, monoculture and polyculture with availability of food, FCR rate and distribution of micro- organisms (Mohanty, 2009 and Ramakrishna, 2010). In the present study, there was a significant different ($P<0.05$) of gross income and net income between two farming system. The rate of income in control and probiotic experimental pond are depending upon the stocking rate, survival rate and growth rate, which are in turn affected by feeding, fertilization rate and environmental factors such as water quality are responsible for increasing farm productivity.