IMPACT OF FEED AND FEED INGREDIENTS ON THE ENVIRONMENT AND MICROFLORA OF FARMED SHRIMP

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November 2000


Dedicated to my beloved parents and brother

DECLARATION

I hereby declare that this thesis entitled '**Impact of feed and feed ingredients on the environment and microflora of farmed shrimp**' is a bonafide record of research work carried out by me and that it has not previously formed the basis for the award of any degree, diploma, associateship or other similar titles or recognition.

Cochin –14
8th November, 2000

S. Ranjit

CERTIFICATE

This is to certify that this thesis entitled "**Impact of feed and feed ingredients on the environment and microflora of farmed shrimp**" is a bonafide record of the research work done by Shri S. Ranjit under my supervision and guidance at Central Marine Fisheries Research Institute, Cochin during the tenure of his Ph.D. (Mariculture) programme (1995-1998). I further certify that this thesis has not previously formed the basis for the award of any degree, diploma or other similar titles or recognition.

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CONTENTS

1. INTRODUCTION

Shrimp constitute an important item of the epicurean diet and the most valuable commodity among seafoods in the international market. The insatiable demand of this resource for human consumption prompted its maximum exploitation from the natural resources with the result the production from capture fisheries has reached a stage of stagnation in most parts of the world. It is in this context, the technological success achieved in culturing shrimp with high production rates about two decades ago came up as a boon to the gloom of uncertainties about the future prospects of shrimp industry of the world. The years that followed witnessed a dramatic expansion of coastal shrimp aquaculture especially in the Asia-Pacific, which today accounts about 80% of the world's total shrimp production through farming. Although the global production of cultured shrimp has increased many-fold over the past two decades, the annual production trend suggests that the exponential growth period for shrimp culture is drawing to a close. According to available statistics, the world's shrimp farmers produced an estimated 0.81 million mt of whole shrimp during 1999, which is far behind the production level of 1.6 million mt predicted for the year 2000 by experts at the AQUATECH- '94 international conference on aquaculture organized by INFOFISH in August 1994 at Colombo, Sri Lanka.

A retrospect of the development history of shrimp farming industry in Asia would reveal that most of the major shrimp farming countries of the region, such as China, Taiwan, Indonesia, Thailand, Philippines and India, had suffered heavy production losses due to outbreak of diseases in their farms starting with the collapse of the Taiwanese shrimp industry in 1988. It is believed that the unplanned and uncontrolled development of the shrimp farming industry without giving any care for proper management of the culture systems was responsible for the disease outbreaks. The sustainable development of shrimp aquaculture therefore depends on environmentally sound farm management strategies. In aquaculture the production inputs, processes and quality of out-put can be controlled to varying extends, and ownership, care and environmental responsibility may be easily established and effectively maintained. Generally, however, a judicious control of the pond environment from the culturists' side rarely takes place and as a result the farming ends up with severe environmental consequences and crop failures.

Among the various environmental problems associated with high-density shrimp farming practices, water quality deterioration caused by the application of large amounts of artificial feed in excess of the requirement of the cultured stocks and the consequent negative impacts within the farms and on the external environment through farm effluents have become a matter of global concern. Excess application of compounded feed with high-protein content and an array of bioactive compounds including antibiotics is commonly practiced in intensive/semi-intensive shrimp farms in order to enhance growth and control or prevent diseases in shrimp. This makes the farm water overloaded with

nutrients and organic wastes derived from uneaten food and shrimp metabolism, which causes eutrophication and water quality deterioration. Feed wastes accumulated on pond bottom also stimulate multiplication of microorganisms and produce anaerobic condition. This creates a number of problems, because pond bottoms with accumulated anaerobic sediments have been extensively related to the onset of several diseases caused by opportunistic infectious organisms. Public health implications of the use of chemicals and the consumption of seafood grown in contaminated waters are problems of growing concern. Overuse of antibiotics has resulted in development of drug-resistant shrimp pathogens in Asia and Latin America, and there is concern that transfer of resistance to human pathogens could result in development of resistance in human pathogens. Adding antibiotics with shrimp diet has also the potential for contamination of shrimp tissue with antibiotic residues making shrimp unfit for human consumption.

India has a long tradition of shrimp aquaculture although scientific farming was started only in recent years. Large-scale shrimp farming adopting improved technologies began in the country during 1990-'91 and since then there was a boom for the shrimp culture industry in some states like Andhra Pradesh and Tamilnadu, with the result the annual production of cultured shrimp in the subcontinent shot up to a record level of 98000 tonnes in 1995. This success in high-tech shrimp farming, however, was short-lived and, like many other Asian countries, India also suffered heavy production losses due to disease outbreaks caused by improper farm management and environmental problems.

Environmental impact of shrimp culture has become a major issue in many tropical and subtropical countries where shrimp farming is practiced in intensive manner. The environmental problems that have arisen in different farming countries indicate the need for proper environmental impact assessments to rectify the shortcomings and adopt corrective farm management measures for sustainable aquaculture production and also at the same time reducing the negative impacts on the external environment.

During the FAO/NACA sponsored **Regional Study and Workshop on the Environmental Assessment and Management of Aquaculture Development** for the Asian region, with its final workshop held at Bangkok, Thailand in 1994, it was recognized that, for several shrimp farming countries of the region, the problems of increased hypernutrification and eutrophication, severe disease outbreaks, water quality mismanagement, and overcrowding of culture units, were generally related to lack of planning and management of aquaculture industry. During the workshop it was also apparent that the information available concerning the interaction between aquaculture and the environment was very inadequate, and whatever known of the environmental impacts caused by aquaculture was mostly anecdotal in nature and speculative. India, being one of the participating countries of this regional study, was no exception to this general situation. Therefore, generating scientific data on environmental impact of aquaculture has become a national priority in the country to plan/ensure an environmentally sustainable aquaculture industry.

Along the West Coast of India, Cochin is well known for its extensive shrimp farming activities. In recent years several of the traditional shrimp farms in the area have switched over to semi-intensive farming involving application of large amounts of supplementary feed and other modern farm management practices. These have the potential for environmental impact and shrimp quality problems. Outbreak of diseases and large-scale mortality of shrimp in semi-intensive farms have become a common feature in the area for the past 3-4 years. So far no attempt seems to have been made to investigate the environmental causes of the disease problems or the impact of the high-density monoculture practices newly introduced in the area on the internal and external environments of the culture systems. Therefore a study of the possible impact of the application of commercial feed in shrimp farming on the environment and microflora of farmed shrimp at Cochin was taken up and the results are presented in this thesis.

2. REVIEW OF LITERATURE

2.1 Shrimp aquaculture practices

Marine shrimp aquaculture, dates in origin to the brackishwater and marine ponds of Mediterranean area (Brown, 1983) and the South East Asian countries (Ling, 1977; Mc Vey, 1980) many centuries ago. It is now carried out extensively in the tropical waters by adopting different technologies evolved over the years (Fast, 1992; Liao *et al.*, 1992; Phillips *et al.*, 1993; Boyd and Clay, 1998). As a general rule, most of the cultivable shrimp species of the family Penaeidae, after completing metamorphosis in the sea, migrate to estuarine environment where they grow fast and attain robust sizes in 4-5 months before returning to the sea for further maturation and eventual breeding for propagation of species (Mistakidis, 1970; Motoh, 1984; Uno, 1984; Dall *et al.*, 1990). For the most part of the world especially in Asian countries, shrimp farmers take advantage of this biological attribute and raise the young shrimp in brackishwater pond system (Boyd and Clay, 1998).

Shrimp farming methods practised in different parts of the world have been reviewed in detail by many authors like Pillai (1990), Hopkins (1992), Lester (1992), Maguire and Allan (1992), Menasveta (1992) Primavera (1992), Quynh (1992), Shigueno (1992), Xin and Sheng (1992), Liao and Chao (1993), Alagarswami, (1995a). Based on operating characteristics and yield potentials, the currently practised shrimp farming is broadly classified as extensive, semi-intensive, intensive and super-intensive (Fast, 1992; Liao

and Chao, 1993; Phillips *et al.*, 1993; Rosenberry, 1996). Extensive shrimp farming is low-density farming in tide-fed impoundments located along bays, estuaries, backwaters and tidal rivers. The ponds are typically large and similar to natural ecosystems with respect to food availability, species diversity and environmental conditions. Water exchange is through tidal action. Stocking densities of about 0.1 – 1 postlarva or juvenile/m^2, which enters the pond along with fish and other undesirable species during water exchange are common. Young shrimp collected from the wild or hatcheries are also stocked in the farms sometimes to improve the yield. The shrimp feed on naturally available food items. Production of shrimp is very low ranging about 100-500 kg $ha^{-1}.year^{-1}$ (Phillips *et al.*, 1993). About 60-90% of the shrimp farms are of this type in Ecuador (Latin America), India, Bengladesh, Indonesia and Vietnam (Rosenberry, 1996). Semi-intensive, intensive and super-intensive methods are the modern, scientifically managed, farming practices aimed at raising greater number of selected species of shrimp per unit area of pond (medium to high-density farming) through natural plus supplementary or complete artificial feeding, extra aeration, increasing levels of daily water exchange by pumping etc. The ponds are relatively smaller in size. Shrimp production varies from 0.5 to 5 t. $ha^{-1}.year^{-1}$ in semi-intensive (Pillai, 1990) to 4 to 35 t. $ha^{-1}.year^{-1}$ in intensive (Liao and Chao, 1993) methods. Semi-intensive farming is practised in 50-90% of the total shrimp farms in Mexico, Peru, United States and Malaysia, while intensive farming predominates (70-80%) in Australia, Sri Lanka and Thailand (Rosenberry, 1996). According to New and Csavas (1993), the bulk of finfish

and crustacean aquaculture production in Asia is realized in semi-intensive pond farming systems.

In India, there are about 10,000 shrimp farms of which 60% carry out extensive farming, 35% semi-intensive and 5% intensive farming (Rosenberry, 1996). Extensive farming include the traditional culture practices such as the paddy-field prawn filtration process (*Chemmeenkettu*) of Kerala (Menon, 1954; Gopinath, 1956), the prawn and fish culture (*Bhasabhadha*) in the `Bheris' of West Bengal (Pillay, 1954) and the prawn filtration in `Khar' lands of Karnataka and `Khezan' lands of Goa (Alagarswami, 1995a) and the improved methods in the traditional culture (Pillai, 1990). Alagarswami (1995) reviewed comprehensively the shrimp farming practices currently in vogue in different maritime states of India.

Shrimp farming has been an age-old practice in coastal districts of Kerala, of which Ernakulam district alone accounts over 80% of the total shrimp culture area of the state. Almost the entire shrimp farms in this district are connected with the Cochin backwater system which forms the water source for culture operations. Menon (1954), Gopinath (1956), Raman and Menon (1963), George *et al.* (1968), George (1974) and George (1975) have described the traditional shrimp farming practices in the area, which are essentially extensive in nature. The system is of two types:

(i) Perennial fields where shrimp culture is practised throughout the year, and

(ii) Seasonal fields where paddy (a variety called "Pokkali") is cultivated during the monsoon months (June-September) and, the fields are inundated with saline water for shrimp culture for the rest of the year (November-April).

Vypeen Island near Cochin accounts for a very large concentration of both perennial and seasonal fields. In the recent years, interest is being shown to change the traditional systems into semi-intensive culture systems and improve shrimp production in the region through scientific farming of desired species like *Penaeus indicus* and *P. monodon* (Alagarswami, 1995a).

2.2 Physico-chemical and biological features of aquaculture systems

Several physico-chemical and biological factors like salinity, water temperature, dissolved oxygen, pH, nutrients, phytoplankton, epifauna, detritus, bottom sediments etc. influence the well-being of fish and shellfish in culture systems. Authors like Boyd (1982, 1989, 1997), Brune and Tomasso (1991), Boyd and Fast (1992) and Pillay (1990, 1992) have dealt with in detail the importance and interactive roles of the various environmental factors in aquaculture systems.

Salinity and its variations are of vital significance in mariculture systems as they profoundly influence the physiological processes of the animals under culture. Though many cultivable species of fish and shrimp tolerate wide range of salinities in brackishwater ponds, most favourable salinity regimes providing maximum survival and

growth are noted (Pillay, 1990 Boyd and Fast,1992). Temperature and dissolved oxygen are the other two major environmental variables which control the biotic and abiotic processes in culture systems. Temperature is the principal controlling factor of fish metabolism, while oxygen acts primarily as a limiting factor. The biochemical reactions controlled by temperature are subject to limitations by oxygen which is an essential reactant of aerobic metabolism. Boyd (1982) reports that dissolved oxygen concentration is greatest at 0 °c and it decreases with increasing temperature and so also its solubility in water with increasing salinity. Variations in water pH, an indicator of acidity and alkalinity conditions of water, have a marked effect on the performance of fish and shellfish. Extreme levels outside the range of pH 5 to 9 inhibit their growth and reproduction or cause mortality (Pillay, 1992). Acidity is known to reduce the rate of decomposition of organic matter and inhibit nitrogen fixation thereby affecting the overall productivity of aquaculture ponds (Pillay, 1990). Among nutrients, phosphorus and nitrogen (Primarily phosphate and nitrate) are the two major ones required by plants (Pillay, 1990). Phosphorus fertilizers are widely used in mariculture operations, and phosphorus originating from metabolic waste is an important factor in ponds that receive applications of feed (Boyd, 1982). Nitrogen is removed from water as nitrates by plants. Nitrogenous wastes are excreted by animals and nitrogenous compounds are released during bacteriological decomposition of plant and animal matter. They are eventually transformed into ammonia, which undergoes nitrification, the biological oxidation of ammonia to nitrate through nitrite by the action of aerobic bacteria, *Nitrosomonas* and *Nitrobacter* (Boyd, 1982; Pillay, 1990). Factors like pH, temperature, dissolved oxygen,

number of nitrifying bacteria etc. affect the nitrite conversion process. Abundance of plankton is an important factor in enhancing organic productivity of aquaculture systems. Phytoplankton plays a dominant role in the dissolved oxygen dynamics of fish ponds. Acting as the base of food web, it uses inorganic nutrients and sunlight to produce organic matter by photosynthesis. Nitrogen and phosphorus limit phytoplankton growth. In brackishwater with moderate or high salinity, diatoms are the dominant phytoplankton which requires larger amounts of nitrogen than phosphorus. When salinity is low, blue-green algae may become abundant in shrimp ponds. Many species of blue-green algae can fix elemental nitrogen, so they can grow well without a combined source of nitrogen (Nitrate or ammonia) provided phosphorus is plentiful (Boyd, 1982). Phytoplankton production in aquaculture ponds promotes growth of zooplankton and a variety of other organisms which constitute the natural live-food of many cultured species (Pillay, 1990). Detritus developed from dead organic matter decomposed by micro-organisms form the food of many benthic animals including shrimp in pond systems (Moriarty, 1987). It is also believed that detritus serves as an important carrier for attached micro-organisms and that these micro-organisms are the nutritional resources supporting detritivores. The various processes involved in detrital-based food chain in aquaculture environment have been reviewed at the conference on Detrital Systems for aquaculture convened by ICLARM in Italy in 1987 (Moriarty, 1987). Production of toxic metabolites such as carbon dioxide, ammonia and hydrogen-sulphide is a common feature in aquaculture systems, sometimes reaching in harmful concentration (Boyd, 1982). Ammonia reaches pond water as a by-product of metabolism by animals and by decomposition of organic

matter by bacteria. It constitutes more than half the nitrogenous waste excreted by crustaceans (Chen and Kou, 1993). The proportion of un-ionized ammonia (NH_3) to ionized ammonia (NH_4^+) in water increases with the increase in water temperature and pH, and with the decrease in salinity (Trussel, 1972; Whitefield, 1978). Toxicity of ammonical nitrogen is attributed primarily to its un-ionized form. High ammonia concentrations are most common in ponds with high feeding rates. Hydrogen sulphide is produced under anaerobic conditions of the sediment-water interface when organic input is high and bacteria use sulphate and other oxidized sulphur compounds in metabolism excreting sulphide. Un-ionized hydrogen sulphide is toxic to fish and shrimp. Pond soil and sediment play important roles in the phosphorus, nitrogen, sulphur and carbon cycles, mineralisation of organic bottom deposits, regulation of pH and many other ecological processes. As penetration of oxygen into sediment is very limited, the sediments are dominated by anaerobic processes (Blackburn, 1987; Boyd, 1989).

The environmental factors exhibit considerable variations in different types of systems because of different farm management principles adopted (Pillay, 1990). In scientifically managed shrimp farms, which are run under controlled environmental conditions , the water quality parameters are maintained within certain levels recommended to be the best suited for the cultured species(Boyd and Fast,1992; Villalon,1993) . Liao and Chao (1993), Moore and Brand (1993), Villalon (1993), Wyban and Sweeny (1993) and Yano (1993) have dealt with the ideal environmental conditions provided in semi-intensive, intensive and super-intensive shrimp farms of different parts

of the world. Extensive growout farms are repored to be like natural ecosystems with respect to nutrient inputs, nutrient cycling, oxygen dynamics and other environmental features (Fast, 1992).

Information on physico-chemical and biological features of shrimp farms of India are mainly due to the works of Pillai (1954), Datta *et al.* (1983), Banerjee and Pakrasi (1986) from the bheries of West Bengal, and those of George *et al.* (1968), Gopinathan *et al.* (1982), Sankaranarayanan *et al.* (1982), Gilbert and Pillai (1986), Nasser (1986), Gopalakrishnan *et al.* (1988), Nair *et al.* (1988), Devapiriyan (1990), and Joshi (1990) from the traditional prawn culture fields of Cochin in Kerala. Studies from intensive or semi-intensive shrimp farms of India are meagre and limited to a few general observations reported in recent years from Maharashtra, Andhra Pradesh and Tamilnadu (Gopalakrishnan *et al.*, 1997; Krishnan and Ramadhas, 1997; Kumaresan *et al.*, 1997; Padmavathi *et al.*, 1997;Raj *et al.*, 1998).

The Cochin backwater system, which forms the water source of a large number of shrimp farms of Ernakulam district, has also been studied extensively for hydrography (Cherian, 1967; Ramamirtham and Jayaraman, 1963; Wellershaus, 1971; Pillai *et al.*, 1973; Josanto, 1975; Lakshmanan *et al.*, 1982), tide cycle (Qasim and Gopinathan, 1969), primary production, plankton and nutrient distribution (George, 1958; Sankaranarayanan and Qasim, 1969; Menon *et al.*, 1971; Nair and Tranter, 1971; Gopinathan, 1972; Haridas *et al.*, 1973; Devassy and Bhattathiri, 1974; Gopinathan *et al.*, 1974; Kumaran and Rao,

1975; Pillai *et al.*, 1975; Madhupratap, 1978; Lakshmanan *et al.*, 1987) and sedimentology (Murty and Veerayya, 1972; Mallik and Suchindan, 1984).

2.3 Feed and feed application in shrimp aquaculture

Like any other organism, shrimp need nutrients or substances that will provide for growth, maintain life and give resistance to diseases (Pascual, 1983). These substances include proteins, fats, carbohydrates, vitamins and minerals. Extensive studies have been carried out on shrimp nutrition during the past two decades as may be seen from the reviews of New (1976, 1987), Kanazawa (1981, 1984, 1985), Pascual (1983), Akiyama and Dominy (1989), Akiyama (1992), Akiyama *et al.*, (1992), Wood *et al.* (1992), Liao and Sheen, 1993, Nandeesha (1993), New and Csavas (1993), Tacon (1993), Akiyama and Chwang (1995) and Ali (1997). In the natural environment, shrimp derive nutrients from a variety of food sources, both of plant and animal origin, like algae, diatoms, small crustaceans, molluscs, polychaetes and fishes that are found at the bottom of the aquatic systems (Gopalakrishnan, 1952; Dall, 1968; Pascual, 1983; Bailey-Brock and Moss, 1992). They also consume significant amounts of detrital aggregates (Chong and Sasekumar, 1981), which serve as important carrier for attached micro-organisms that constitute important components in shrimp diet. There is, however, considerable differences of opinion on the food value of bacteria which are ingested by shrimp along with detritus (Pillay, 1990, Akiyama *et al.*, 1992).

The traditional shrimp aquaculture practices are largely dependent on natural food resources which are produced within the culture systems. In semi-intensive or intensive culture ponds, where shrimp is stocked at high densities, the amount of natural food available is not sufficient to support good growth of the animal. Hence, it is necessary to increase production of natural food by fertilization, either with chemical or organic fertilizers, supplementing with artificial feed or a combination of both (Kompiang, 1990; Pillay, 1990). Parellel to intensification of shrimp farming industry, nutritionally balanced and high quality compounded diets were also developed and commercialized (New, 1976, 1987, 1990; Shigueno, 1984; Akiyama and Dominy, 1989; Tacon, 1993; Csavas, 1995; Ali, 1997). Today aquafeeds have emerged as one of the most critical components of successful shrimp farming, and the most expensive element in the operating costs of commercial shrimp culture (Jory, 1995 a; Tacon, 1998). About 50% of shrimp production through aquaculture in Asia is dependent on commercial feeds. The aquafeeds and feeding strategies of different Asian countries have been recently reviewed by Zaher and Mazid (1995) for Bengladesh, Nouv and Nandeesha (1995) for Combodia, Wang (1994) for China, Nandeesha (1995) for India, Djunaidah (1995) for Indonesia, Utama (1995) for Malaysia, Pantha (1995) for Nepal, Pascual (1995) for Philippines, Chou (1995) for Singapore, Somsueb (1995) for Thailand and Luu (1995) for Vietnam. Besides commercial aquafeeds, many farms manufacture their own feeds and reduce the cost of farming to a considerable extent. New and Csavas (1993) reviewed farm-made feeds and their use in Asia, and suggested that these feeds are practical in relatively smaller production systems.

Prawns and shrimp in their growth phase are bottom feeders subsisting mainly on plant food in juvenile stages and animal food during adulthood (Gopalakrishnan, 1952; Subramanyam, 1974; Suseelan, 1975; Dall *et al.*, 1990). Unlike fish that gulps or swallows its food instantly on capture, shrimp nibbles and consume the food slowly with the result considerable time is taken to complete the feeding process (Pascual, 1983; Jory, 1996). Due to this characteristic feeding behaviour, the compounded diet supplied to shrimp are generally in low-moist pelletised form ensuring the nutritional requirement of the targeted species, palatability, physical qualities like compactness, attractability, water-stability and long shelflife (Divakaran *et al.*, 1994; Anon., 1995; Jory, 1995 b, 1996) for profitable and pollution-free shrimp farming.

Feed that is provided to the shrimp contains major nutrients like proteins, fat, carbohydrate, vitamins and minerals in varying proportions as per the requirement of the cultured species. Of these, proteins are the most abundant and important constituent responsible for body maintenance, growth, formation of hormones, enzymes and also acting as a source of energy (Kompiang, 1990).

Recommended dietary protein levels in various species and sizes of marine shrimp vary from 30% to 57% (Pascual, 1983; Akiyama *et al.*, 1992; Liao and Sheen, 1993), higher level being required by younger stages (Anon., 1995). Fats assume significance due to their role in providing energy to carry out all the metabolic functions including protein synthesis and a source of essential fatty acids required by the shrimp. Fat in the

feed gives good texture and enhances feed palatability . Carbohydrates supplied in various forms like starch, glycogen, cellullose etc., considered as least expensive form of dietary energy for animals, are of limited use for the purposes of energy by penaeid shrimp. Starch, when used, gets gelatinized in feed under optimal processing conditions and gives a binding effect. Together with dietary lipid, starch provides the energy to shrimp and spare protein in feed for growth purposes. Fibre, mostly cellulose, in the feed is not digested in levels significant enough to be a factor in the nutrition of shrimp. Feed with high levels of fibre will increase faecal production and consequently pollute the water bodies creating an environmental hazard. Besides, they create binding problems during feed manufacture. Fibre is difficult to grind finely and the fibre strands may act as a conduit for water to enter the pellet. This creates fractures and lowers the water stability of the feed (Akiyama and Dominy, 1989). Vitamins are required by shrimp in minute amounts in feed. They are involved in specific metabolic reactions for the normal growth. Deficiency of vitamins in feeds results in reduced digestion and growth, stress conditions and reduces immune response in shrimp leading to attack by pathogens. Minerals in general are the constituent of the exoskeleton of shrimp and are involved in balancing osmotic pressure, structural homogeneity and also in transmission of nerve impulses and muscle contraction and relaxation. They act as catalysts in various reactions and are co-factors in metabolism.

Selection of proper feed ingredients is decisive to ensure nutritionally rich, cost effective and environment-friendly shrimp feeds. Raven and Walker (1980), New (1987),

Tacon (1993, 1998), Akiyama (1991), Raj (1992), and FDS (1994) have reviewed the conventional feed ingredients and noted fish meal, squid meal, shrimp meal, other crustacean meals, soybean meal, cereals and yeast as the ones extensively used in the manufacture of commercial shrimp feeds in Asian countries. Besides vitamins and minerals, a variety of feed additives like binders, pigments, attractants, enzymes, antibiotics etc., are added in small quantities to achieve water stability and other physical qualities of the pelletized feeds. The slow feeding habit of shrimp necessitates use of feeds having more water-stability in order to minimize leaching out of micro-nutrients (Akiyama *et al.*, 1992).

The best shrimp feed produced in the world could prove to be worthless if it is not managed properly. Feed management is a sequential process comprising feed selection, handling and storage, feeding regimes, and adjustments to feeding rates (New, 1990; Cruz, 1991; Tacon, 1993; Akiyama and Chwang, 1995; Jory, 1995 b). As aquafeeds are made up of highly perishable ingredients, it is important to handle them properly until use. Poor storage and handling of feeds result in product deterioration, reduced feed attractability and palatability, nutritional deficiencies and disease outbreaks (Jory, 1996). New (1990) and Tacon (1993) have indicated general guidelines for handling and storage of artificial shrimp feeds. The effects of ambient tropical temperatures on the chemical characteristics or nutritional value of shrimp feeds during long-term storage have been demonstrated by De la Cruz *et al.* (1989) and Divakaran *et al.* (1994). The various feed application methods including manual broadcasting, automatic feeders, high-pressure

blower type feeders, feed trays etc., feeding regimes and periodicity, and adjustments to feeding rates adopted in the intensive and semi-intensive shrimp farms of different parts of the world have been described/reviewed in detail by Jory (1996).

2.4 Environmental problems associated with shrimp aquaculture

The indiscriminate expansion of coastal aquaculture during the last 10-15 years have resulted in several environmental problems which evoked world-wide attention needing interaction between aquaculture and the environment (Rosenthal *et al.*, 1988; Apud *et al.*, 1989; Chua, 1992; Pillay, 1992; FAO/NACA, 1995; Wu, 1995; Boyd, 1997; Phillips *et al.*, 1997). A number of studies have been carried out in this direction from different parts of the world and they have contributed significantly to our understanding of the positive and negative effects of aquaculture on the internal and external environments (Chua *et al.*, 1989; Kissil *et al.*, 1992; Boyd *et al.*, 1998). Authors like Pullin (1989), Braaten and Hektoen (1991), Folke and Kautsky (1992), De Voc (1994), Piedrahita (1994), Phillips (1995), and Wu (1995) have elucidated the relationship between different forms of aquaculture and the environment, and the magnitude of environmental impacts caused by each of them. Environmental impact of aquaculture has also formed the focal theme of many conferences/meetings/workshops/studies at local, national and international levels in recent years, in which the various environmental issues connected with coastal aquaculture development in Asia and other regions of the world have been reviewed and measures recommended to properly manage the environmental impacts of aquaculture including those impacts of environment on aquaculture and the impacts of

aquaculture on the environment (GESAMP, 1991, 1997; Pullin *et al.*, 1993; Baird *et al.*, 1996; Phillips *et al.*, 1997).

Among the several environmental consequences associated with shrimp farming practices, water and sediment quality deterioration caused by the accumulation of nutrient and organic wastes derived from uneaten feed, faecal matters metabolic by-products, fertilizers and residues from biocides and chemicals, and the resultant effluents and their impact on the surrounding environments have become a major problem in many tropical and subtropical countries (Apud *et al.*, 1989; Phillips *et al.*, 1993; Primavera, 1993; Hopkins *et al.*, 1995 a&b; Bergheim and Asgard, 1996; Boyd and Clay, 1998). In semi-intensive/intensive shrimp farms where supplementary feeding is a major input, the amount of feed wasted plays an important role in the total waste loadings and 'self-pollution' (Beveridge *et al.*, 1991; Phillips *et al.*, 1993; Alagarswami, 1995 b). As feed settles directly on to the pond bottom, its wastage can have a significant effect on sediment quality and ultimately the health of the bottom-living shrimp (Boyd, 1989). The feed wastage is reported to change widely, and correlated with varying feed conversion ratios (FCR), higher the FCR larger will the quantity of uneaten or poorly converted feed (Phillips *et al.*, 1993). According to Boyd and Clay (1998), even in the best regulated feeding regimes, upto 30% of the feed is not consumed by shrimp, and consequently a considerable amount of waste accumulates in the pond. Various factors such as feed stuff quality, diet formulation, feed production technology, feeding practice

and feeding behaviour of the shrimp stock greatly influence the total feed wastage (Kongkeo, 1990; Beveridge *et al.*, 1991; Bergheim and Asgard, 1996).

In the total consumed feed, only 17-25% on dry weight basis is converted as shrimp meat and the rest is lost into pond water in the form of faeces, metabolic wastes like ammonia, urea, carbon dioxide, phosphorus etc. and moulted exuviae (Saclauso, 1989; Phillips *et al.*, 1993; Primavera, 1993; Boyd *et al.*, 1998). Faecal production is dependent on many factors like the principal feed constituents and their digestibility, water temperature, shrimp size, health, feeding rate and the synergistic/antagonistic effects of one dietary component on the digestibility of another (Beveridge *et al.*, 1991; Beveridge and Phillips, 1993). Beveridge *et al.* (1991) estimated the faecal dry matter production of shrimp in culture systems to be 26-27% of the ingested feed, based on the digestibility of the principal constituents in typical commercial diets. Studies have also shown that faecal production is comparatively higher for omnivorous/herbivorous species than carnivorous species due to the predominance of carbohydrate or other nonprotein dietary components in the feed (Primavera, 1993).

While the undigested fraction of feed is voided as faeces, the digested portion is absorbed across intestine and metabolized. Nutrients absorbed in excess to requirements may be excreted together with end-products (Boyd *et al.*, 1998) derived from catabolic breakdown of protein. Shrimp (and other crustaceans) excrete their metabolic wastes, predominantly nitrogen, as ammonia, urea, uric acid and other minor products in varying

amounts (Claybrook, 1983; Primavera, 1993). The rate of ammonia excretion has been observed to decrease with increasing body weight in *P. monodon* (Mohanty *et al.*, 1989) and *P. semisulcatus* (Wajsbrot *et al.*, 1989).

Fertilizers are widely used in semi-intensive culture systems to promote growth of shrimp food organisms, particularly for the early postlarval stages (Apud *et al.*, 1989; Boyd, 1989). Both organic and inorganic fertilizers are used in different dozes during the culture phase. The most commonly used organic fertilizers are live stock manures and inorganic fertilizers ammonium sulphate and calcium nitrate (Pillay, 1992; GESAMP, 1997). Inorganic and organic fertilization contributes to the nutrient and organic waste production in the pond system (Apud *et al.*, 1989; Pillay, 1992; Phillips *et al.*, 1993).

Besides fertilizers, a large variety of biocides and chemicals classified as therapeutants, feed additives, disinfectant, soil and water treatment compounds, algaecides and pesticides are applied in shrimp farms directly or through feed (Pillay, 1992; Phillips *et al.*, 1993; Primavera, 1993; GESAMP, 1997; Boyd *et al.*, 1998), which inadvertently affect the environment as well as the cultured and non-targeted organisms. Some of the chemicals used as pesticides, especially chlorinated hydrocarbons (DDT, endrin and aldrin) and organotins, are reported to be highly toxic and persistent compounds, which pose threat to shrimp health, product quality, human health and the wider environment (Baticados *et al.*, 1986; Apud *et al.*, 1989; Phillips *et al.*, 1993; Bergheim and Asgard, 1996). Antibiotics and antimicrobial drugs are widely used

through medicated feeds in hatcheries and grow out ponds in Asia and Latin America for the treatment of shrimp diseases (Phillips *et al.*, 1993; Primavera, 1993; Weston, 1996). Their use, however, has several serious consequences such as accumulation of antibiotic residues in shrimp for human consumption, development of antibiotic resistance in bacterial strains, etc., rendering the antibiotics ineffective (Pillay, 1992, Anon., 1994). A more detailed review on this subject matter is given elsewhere in this chapter.

The nutrient and organic wastes produced in intensive/semi-intensive shrimp culture systems consists of solid wastes formed mainly of uneaten feed, faeces and phytoplankton, and dissolved wastes contributed mainly by ammonia, urea and carbon dioxide derived from shrimp metabolism (Phillips *et al.*, 1993; Primavera, 1993). Solid wastes may be in suspended form or may accumulate on the sediment (Pillay, 1992). The solid organic wastes tend to settle on pond bottom where they are decomposed by micro-organisms and get converted into inorganic nutrients such as ammonia, phosphate and carbondioxide, adding to the dissolved waste production in the culture system (Fry, 1987; Phillips *et al.*, 1993; Primavera, 1993; Boyd *et al.*, 1998).

Accumulation of organic matter on pond bottom is associated with decreasing redox potentials, when release of harmful gases such as hydrogen sulphide and methane can occur causing stress and health risk to the cultured shrimp (Boyd, 1989). Heavy settlement of shrimp excreta and other organic wastes at bottom also results in the

formation of black, foul-smelling sludge which augments the anaerobic microbial activities and the consequent toxicity to the pond environment.

Environmental stress caused by water and sediment quality deterioration in shrimp farms has been implicated with severe disease problems and consequent production losses in many countries like Taiwan, Sri Lanka, Thailand, China, Philippines, India and Ecucador (Lin, 1989; Chen, 1990; Liao, 1990; Main and Fulks, 1990; Alagarswami, 1995 a; Jory, 1995 b; Boyd and Clay, 1998).

Although the potential environmental consequences of increased aquafeed application in high density shrimp production systems are well recognized, only limited work is on record in attempting to quantify the total loss to the environment of nitrogen and phosphorus against a given feed input, and its pollutive effect. Using data on diet digestibility, FCR and other parameters, Phillips *et al.* (1993) and Primavera (1993) estimated the nutrient waste production in intensive shrimp farms of Philippines and Paez-Osuma *et al.*, (1997) in semi-intensive shrimp farms of north western Mexico. These studies showed that 63-78% of Nitrogen and 76-86% of phosphorus fed to shrimps (*P. monodon*) in the Philippine ponds are lost to the environment, while in the Mexican ponds the environmental losses of nitrogen and phosphorus are 28.6 kg and 4.6 kg respectively per ton of shrimp (*P. vannamei*) produced. Analysis of comparative data from semi-intensive and intensive shrimp ponds of Thailand, Phillips (1995) showed nutrient wastage at the rates of 9.7 kg of nitrogen and 9 kg of phosphorus in semi-

intensive ponds and 53.1 kg of nitrogen and 15.7 kg of phosphores in intensive ponds per mt. of shrimp (species unspecified) harvested. These studies clearly demonstrated increased nutrient loads in shrimp farms with intensification of culture operations.

The impacts of shrimp aquaculture on the external environment have been reviewed in detail by many authors like Macintosh and Phillips (1992), Phillips *et al.* (1993), Primavera (1993, 1994), Hopkins *et al.* (1995), Alagarswami (1995 a), Phillips (1995), Paez-Osuma *et al.* (1997) and Boyd and Clay (1998). Discharge of effluent low in dissolved oxygen and the biological breakdown of dissolved and particulate organic matter and other waste material (BOD and COD) can reduce dissolved oxygen levels in the receiving waters. The discharge of high proportion of nutrients would result in hypernutrification (nutrient enrichment) and eutrophication, with increased primary productivity and potential risks of algal blooms. Increased sedimentation due to loading of organic matter may lead to changes in productivity and benthic community structure and also possible siltation (Chua *et al.*, 1989; Phillips *et al.*, 1993; Phillips, 1995).

Specific instances of environmental impact assessment for shrimp aquaculture, especially addressing issues connected with inadequately managed aquafeeds, are very few and limited to the studies of Primavera (1993) from Philippines, Satanpornvanit (1993) from Thailand and Hopkins *et al.* (1995 b) from the United States among notable ones. In India, some of the recent survey reports (Raj *et al.*, 1998; Joseph *et al.*, 1999) and experimental studies (Padmavathi, *et al.*, 1997) have furnished general information

on effluent quality of brackishwater shrimp farms of the east coast. Alagarswami (1995 a, 1995 b) briefly reviewed the interactions between shrimp aquaculture and the environment with particular reference to Indian conditions.

2.5 Bacterial flora of shrimp culture systems and farmed shrimp

Among the water borne microbial communites, bacteria are the most dominant and diverse group which play important roles in the biogeochemical processes and productivity of aquatic environment (Moriarty, 1987). They are ubiquitous in natural water bodies (Sindermann, 1990) where they may be free-living in the water, attached to sediment particles or specifically associated with the internal and external surfaces of higher organisms. Comprehensive accounts dealing with the quantitative and qualitative nature of microorganisms in general and more particularly of bacterial flora, and their influence on the aquatic environment and associated animals have been published by several authors like Shewan (1961), Skinner and Shewan (1977), Sieburth (1979), Liston (1980), Rheinheimer (1985), Austin and Austin (1987) and Austin (1988) to mention a few important ones.

Interests on bacteriology of aquaculture systems and cultured animals grew rapidly in recent years with the recognition of

(1) The significant roles played by bacteria in the eco-regulatory and balancing processes,

(2) Their potential for optimisation of production efficiency through appropriate biotechnological manipulations, and

(3) The health risks associated with pathogenic organisms developing from improperly managed farming practices (Moriarty, 1997; Ayyappan, 1998).

In the aquaculture environment as in other aquatic habitats, bacteria perform a wide variety of functional roles in recycling of organic matter. They are responsible for many of the key steps in the cycling of most of the major elements like carbon,nitrogen, phosphorus and sulphur through detrital food chains and sustaining pond productivity. (Fenchel and Blackburn, 1979; Krumbein, 1983; Anderson, 1987; Blackburn, 1987; Fry, 1987; Kirchman and Ducklow, 1987; Moriarty, 1987). Fry (1987) described in detail the various steps in nutrient cycles that are predominantly controlled by bacteria and the major groups of bacteria involved in each process. Since heterotrophic bacteria can convert organic detritus to proteins they also constitute an important food source for other organisms in the ponds (Moriarty, 1985).

Although considerable information is available on the bacteriology of captured shrimp and shrimp products from different parts of the world (Liston, 1980), studies on the bacteriology of cultured shrimp and the environment are limited. A few investigations on this aspect have been carried out in the United States (Vanderzant *et al.*, 1971; Christopher *et al.*, 1978), Japan (Yasuda and Kitao, 1980), South east Asian countries (Sarnianto *et al.*, 1985; Llobrerra *et al.*, 1990; Putro *et al.*, 1990; Reilly and Twiddy, 1992;) and Sri Lanka (Fonseka, 1990), besides in India. These studies demonstrated

maximum bacterial loads in freshly harvested pond reared shrimp ranging from 9 x 10^5/g in Sri Lanka (Fonseka, 1990) to 5 x 10^6/g in Texas Gulf coast (Vanderzant, *et al.*, 1971). Peranginangin *et al.* (1992), reviewing the microbiological quality of cultured tiger prawns of West Java, reported that the initial bacterial loads were generally high in cultured prawns due to high bacterial load of pond environment. Qualitatively, coryneform bacteria and to a lesser extent Vibrio in shrimp body and *Flavobacterium*, *Moraxella* and *Bacillus* in pond water, predominated the microbial flora of *P. aztecus* (Vanderzant *et al.*, 1971), and *Aeromonas*, *Pseudomonas* and *Vibrio* predominated that of other penaeids cultured in the Texas Gulf coast. In the tropical region, over 70% of the bacterial population of cultured shrimp was constitued by Micrococcus and Corynebacterium in Thailand and Micrococcus, Corynebacterium, *Vibrio* and *Bacillus* in Sri Lanka (Fonseka, 1990).

In India, Palaniappan (1982), and Singh (1986) made the earliest attempts to study the bacteriology of cultured shrimps and demonstrated that *Vibrio* spp. dominated in the microflora of almost all the life stages of *P. indicus* grown in confined environment. Later, investigating in greater detail along the southwest and southeast coasts, Nayyarahamed *et al.* (1995), Surendran *et al.* (1995), Sharmila *et al.* (1996) and Singh *et al.* (1998) added more to our understanding of the bacteriological profile of cultured shrimps and their environment, and furnished baseline data on the quantitative and qualitative nature of the native flora. A few studies have also been made on the microbial ecology of traditional shrimp culture systems of Kerala, which threw light on the general

heterotrophic bacterial activities for maintenance of pond fertility (Santhi Thirumani and Chandrika, 1995), bacterial role in hydrogen sulphide production (Ravi and Chandrika, 1993) and the seasonal variations in the abundance of bacterial groups involved in nitrogen cycle (Ninawe and Raj, 1993). Recently Dalmin *et al.* (1997) studied the quantitative abundance of beneficial and harmful bacterial populations present in water and sediment of a modified extensive shrimp farm while Krishnan and Ramadhas (1997) elucidated the relative importance of autotrophic and heterotrophic bacterial genera in the nitrification process in a semi-intensive shrimp farm on the Tamilnadu coast.

2.6 Bacterial diseases of shrimp and human health problems

Increasing occurrence of shrimp diseases in aquaculture systems and the consequent economic losses and public health risks have become a serious problem in the shrimp farming countries of the world (Jory, 1996; Karunasagar *et al.*, 1998). Over the past two decades, extensive investigations have been carried out on shrimp pathology, including that of cultured species, the world over, and the results documented have been reviewed from time to time by several authors like Johnson (1975, 1989), Lightner (1977, 1985, 1993), Couch (1978), Sindermann and Lightner (1988), Bell (1991), Fulks and Main (1992), Lightner *et al.* (1992), Shariff and Subasinghe (1992), Lavilla-Pitogo (1995), Karunasagar *et al* (1998) and Otta *et al* (1998). Nearly 30 diseases and disease syndromes of cultured penaeids, with both infectious and noninfectious etiologies, have been described (Sindermann and Lightner, 1988). The most important pathogens commonly associated with cultured shrimps are viruses, bacteria, fungi and parasites.

Among these, viruses and bacteria are the most important causative agents for significant mortality and morbidity of both larval and grow-out shrimp (Fulks and Main, 1992). Generally, bacterial disease in a culture system is found in conjunction with other diseases or as a reflection of a breakdown in the ecological balance within the culture system (Lavilla-Pitogo, 1995; Otta *et al.*, 1998). Gram-negative bacteria are predominant bacteria in marine environments and usually constitute the majority of bacteria present in the normal microflora of cultured and wild penaeid shrimp (Lightner, 1993). Most of the reported species of bacterial pathogens of penaeid shrimps have also been reported to be part of their normal microflora and opportunistic pathogens (Lightner, 1985, 1993; Shariff and Subasinghe, 1992; Otta *et al.*, 1998).

Though a number of bacterial genera have been found to constitute the normal microflora of cultured and wild shrimps, members of only about ten genera, namely, *Aeromonas, Alkaligenes, Alteromonas, Flavobacterium, Flexibacter, Leucothrix, Moraxella, Mycobacterium, Pseudomonas* and *Vibrio* have been so far reported to be pathogenic in penaeid shrimps (Otta *et al.*, 1998; Fulks and Main, 1992; Shariff and Subasinghe, 1992; Lightner, 1993). Among these species, vibrios are numerically the most abundant and commonly met with bacterial pathogens in cultured shrimp (Karunasagar and Indrani, 1995; Otta *et al.*, 1998). Since a large number of *Vibrio* species are normally present in the shrimp's microflora, the pathogenic vibrios are mostly opportunistic pathogns which establish lethal infections following primary infections with other pathogens, acute environmental stress, nutritional imbalance and/or

predisposing lesions (Shariff and Subasinghe, 1992; Lightner, 1993). Of more than a dozen pathogenic vibrios associated with penaeid shrimps (Otta *et al.*, 1998; Takahashi *et al.*, 1998), species such as *V. parahaemolyticus, V. vulnificus, V. alginolyticus, V. damsela, V. harveyi, V. splendidus* and *V. penaeicida* are the most important ones responsible for serious disease out-breaks in cultured shrimp populations (Sano and Fukuda, 1987; Anderson *et al.*, 1988; Liao, 1989; Liao *et al.*, 1992; Lightner, 1993; Lavilla-Pitogo and Pena, 1998; Otta *et al.*, 1998; Takahashi *et al.*, 1985). Bacterial infections in shrimps take various forms like appendage discolouration (Chen, 1992; Liu, 1989), gill disease (Shigueno, 1975; Lightner, 1985, 1993; Liao *et al.*, 1992), tail rot (Lightner, 1985; Liao *et al.*, 1992); Lavilla-Pitogo and Pena, 1998), shell disease (Alapide-Tendencia and Dureza, 1997) and septicemias (Anderson *et al.*, 1988; Liu, 1989; Liao *et al.*, 1992; Lightner, 1993; Karunasagar *et al.*, 1998; Lavilla-Pitogo and Pena, 1998).

Vibrios cause diseases in hatcheries as well as grow-out ponds. Significant mortalities associated with luminescent vibriosis, caused by *V. harveyi* and *V. splendidus*, were reported from *P. monodon* and *P. merguiensis* hatcheries in Indonesia (Sunaryanto and Mariam, 1986), Philippines (Lavillo-Pitogo *et al.*, 1990; Baticados *et al.*, 1991), Thailand (Tansutapanit and Ruangpan, 1987) and India (Karunasagar *et al.*, 1994). *V. harveyi* has also been reported as a pathogen of adult (growout) shrimps, seriously affecting the hepatopancreas of the host (Chen *et al.*, 1992; Lavilla-Pitogo and Pena, 1998; Otta *et al.*, 1998).

Serious epizootics in postlarval and juvenile *P. japonicus* was reported by Takahashi *et al.* (1985) from Japan, the causative agent of which has been recently identified as a new species, *V. penaeicida* (Ishimaru *et al.*, 1995; Takahashi *et al.*, 1998). In Malaysia, heavy mortalities in market-size *P. monodon*, suspected to be due to infection of *V. parahaemolyticus, V. alginolyticus* and *Pseudomonas* sp., causing multifocal necrosis, hemolytic inflammation and nodule formation in the lymphoid organ, heart, gills, hepatopancreas, antennal gland, cuticular epidermis and subcutis and other connective tissues. Ecuador and Texas witnessed heavy mortalities of shrimp (*P. vannamei*) in nursery and growout stages due to a massive vibriosis epizootic disease syndrome called "Sea gull syndrome" (SGS) during 1989-90 and it was hypothesized that relatively higher nutrient content (especially urea and nitrate) and salinities in the shrimp farms had resulted in "blooms" of the *Vibrio* spp. responsible for the disease (Lightner, 1993).

Leucothrix mucor and similar filamentous bacterial fouling organisms have been reported to cause mortalities in all stages of shrimp under poor water conditions (Shariff and Subsinghe, 1992). Infestation of these ectocommensals on respiratory surfaces of shrimp, especially when coupled with critically low dissolved oxygen levels in water, has led to considerable losses in pond culture of *P. vannamei* in the United States (Wyban and Sweeny, 1991). Mortality in such cases occurs due to hypoxia and impairment of molting process (Baticados, 1988).

Disease problems affecting shrimp culture industry of India have been recently reviewed by Karunasagar *et al.* (1998). Both viral and bacterial diseases have been found to cause mass mortalities in penaeid shrimp hatcheries and farming systems. Studies so far conducted have shown that bacteria, particularly *Vibrio* species, are the major problem in hatcheries, the important species associated with mortalities being *V. alginolyticus* and *V. harveyi* for *P. monodon* (Karunasagar *et al.*, 1994, 1998) and *V. campbellii* - like bacterium for *P. indicus* (Hameed and Rao, 1994). In growout system, severe mortalities of *P. monodon* and *P. indicus* occurred almost regularly on both east and west coasts since 1994 (Mohan and Shankar, 1995; Vijayan *et al.*, 1995; Indrani Karunasagar *et al.*, 1997) due to white spot baculovirus (WSBV) infection ("white spot syndrome")' the origin of which in India is still debated (Karunasagar *et al.*, 1998). Bacterial diseases have been reported to cause problems in certain locations. No epizootics have been reported so far. Shell disease due to luminous bacteria and *V. alginolyticus* has been documented in *P. monodon* and *P. indicus* (Hameed, 1994; Abraham and Manley, 1995; Otta, 1997). From Cochin region no study has yet been reported on bacterial diseases in farmed shrimp.

Human health hazards associated with consumption/handling of fish and shellfish contaminated with bacterial and other pathogenic microorganisms are well recognized (Shewan, 1961; Liston, 1980; Austin and Austin, 1987; Inglis *et al.*,1993 a & b). Many outbreaks of human diseases have been associated with marine fishery products (Reilly, 1998) especially those from wild stocks, and similar problems can result from

aquaculture due to poor management (GESAMP, 1991). Pathogenic bacteria may cause disease by `infection', that is by invasion of the body by live bacteria or by `intoxication', where toxic substances have been produced in the food during a period of growth by the pathogenic organism (Huss, 1991). A number of pathogenic or potentially pathogenic bacteria such as *V. parahaemolyticus, V. vulnificus, V. cholerae, Salmonella* sp., *Staphylococcus aureus* and *Streptococcus* sp., which are often implicated with food poisoning or outbreak of diseases like gasteroenterites, typhoid, cholera etc. in human beings, have been encountered in significant levels in cultured shrimps and their environment (Fonseka, 1990, 1995; Llobrerra *et al.*, 1990; Karunasagar, *et al.*, 1991; Reilly and Twiddy, 1992; Nayyarahamed *et al.*, 1995; Sunarya *et al.*, 1995; Surendran *et al.*, 1995;). Consumption of raw or partly cooked shrimp from affected areas is likely to cause diseases due to pathogens or toxins (Shuval, 1986). Consumption of raw shrimps was identified as the main mode of transmission in the explosive outbreak of cholera witnessed by the Philippines in 1961 (GESAMP, 1991) and in many other countries in subsequent years (Reilly, 1998). Karunasagar *et al.* (1990) reports that *V. parahaemolyticus* is the commonest among potentially pathogenic vibrios encountered in Indian seafoods. Though ingestion of a small number of *V. parahaemolyticus* is apparently harmless, in as little as two hours they can multiply resulting in a population capable of causing food poisoning. Food poisoning due to *V. parahaemolytius* has been reported from many parts of the world (Aoki *et al.*, 1967; Qadri and Zuberi, 1977). *V. vulnificus* has been associated with septicaemia with high mortality rate in

immunocompromised persons or those with underlying hepatic disorders (Colwell, 1984).

2.7 Application of antibacterial agents in shrimp aquaculture

In the context of increasing disease outbreaks in shrimp aquaculture and the severe economic losses that result, prevention and treatment of infectious diseases caused by bacteria and other microorganisms in hatcheries and production systems have become inevitable. The shrimp farmers tackle this problem by using antibacterial agents to cure diseases that have occurred or as a prophylactic measure (Pillay, 1992; Lavilla-Pitogo, 1995). A large variety of antibacterials, comprising both the antibiotics (produced by microorganisms) and antimicrobials (of synthetic origin) are applied in aquaculture (Alderman, 1988; Weston, 1996) of which oxytetracycline, chloramphenicol and oxolinic acid are the most commonly employed ones in shrimp farms (ADB/NACA, 1991; Primavera, 1993; Saitanu *et al.*, 1994; Pranee Srisomboon and Achara Poomchatra, 1995; Weston, 1996). They are administered through medicated feeds or added directly to the pond water.

In most of the developing countries, including India (ADB/NACA, 1991) where strict regulatory measures are not in force in the usage of aquachemicals (Chua, 1992; Bang and Lavilla-Pitogo, 1996; Weston, 1996) the antibacterials have been used indiscriminatly, which has led to concern over safety of the environment, cultured stocks and consumer health (Saclauso, 1989; De Voc, 1994; Lavilla-Pitogo, 1995; GESAMP,

1997). Accumulation of antibacterial compounds in pond sediments has the potential for reduction in microbial density and subsequent inhibition of microbial decomposition of organic matter and recycling of associated nutrients (Hansen *et al.*, 1992; De Voc, 1994; Weston, 1996; GESAMP, 1997).

Bacteria are well known for their ability to develop resistance to antibacterial drugs rapidly (Brown, 1989; Pillay, 1992). Significant levels of antibiotic-resistant bacteria that are human pathogens can occur in aquaculture ponds where antibiotics are routinely incorporated into animal diets (Twiddy and Reilly, 1995; Boyd *et al.*, 1998; Surendran and Nirmala Thampuran, 1999). Widespread use of oxytetracycline in South-East Asian shrimp ponds is reported to have resulted in the development of resistant strains of pathogenic vibrios, which has caused major problems in the treatment of *Vibrio* infections (Pillay, 1992). The use of one antibacterial agent can increase levels of resistance not only to that specific drug but to many others (Wood *et al.*, 1986) due to the production of plasmids encoding resistance to multiple antibacterials (Brazil *et al.*, 1986; Ramteke, 1997).

A matter of grave concern, relating to excessive application of antibacterials in aquaculture, is the accumulation of residues of antibiotics and antimicrobials in the tissue of farmed animal intended for human consumption (Primavera, 1993; Boyd *et al.*, 1998) since many antibacterials are not thermally degraded under normal cooking conditions (Moats, 1988). Unintended ingestion of antibacterials can have a number of adverse

consequences like toxicity, hypersensitivity, microbial resistance and other health hazards (Saitanu *et al.*, 1994; Weston, 1996).

Presence of residues of antimicrobial drugs in farmed shrimp has been increasingly reported in recent years (ADB/NACA, 1991; Chua, 1992; Saitanu *et al.*, 1994; Pranee Srisomboon and Achara Poomchatra, 1995; Surendran and Nirmala Thampuram, 1999). In Thailand, of several samples of cultured shrimp examined within the country, 8.4% showed positive for residues of oxolinic acid and oxytetracycline, and the level of the residues was above the accepted level (Saitanu *et al.*, 1994). The problem of drug residues in cultured shrimp in the Far East has had a profound effect on the seafood trade. Japan, the major importer of frozen shrimp from Southeast Asia, initiated a compulsory inspection programme for Southeast Asian shrimp, after detecting unacceptable levels of oxytetracycline and oxolinic acid in farm-raised shrimp from Thailand in 1990 (Primavera, 1993; Pranee Srisomboon and Achara Poomchatra, 1995). Similar restrictions were also adopted by the United States on import of shrimp products containing measurable amounts of chloramphenicol (Weston, 1996).

2.8 Plasmids in human pathogens from aquaculture systems

Bacterial pathogens acquire resistance to antibacterial drugs by the acquisition of foreign DNA or by modification of chromosomal DNA. The DNA then may be transferred between bacteria by a variety of routes including plasmids, conjugative transposons and bacteriophages as well as by free DNA (Chopra, 1985; Neu, 1992).

Plasmids are relatively small, circular DNA molecules which can autonomously replicate and are stably inherited (Prescott *et al.*, 1990). They are genetic elements that are independent of the chromosome and are able to exist either in a chromosomal state or in a plasmid state. In the plasmid state they can mediate resistance transfer at high frequency within and between bacterial species and may result in the transfer of resistance to many antebacterials simultaneously (GESAMP, 1997).

Plasmids perform a variety of duties in the bacteria, and they are classified on the basis of their mode of existence, spread and functions. Those plasmids which confer drug resistance to the bacteria are called 'R-Factors' or R-plasmids (Aoki *et al.*, 1971; Prescott *et al.*, 1990). R-plasmids are self-transmissible, hence conjugation is a regular practice which transfers the plasmid from one bacteria to the other causing rapid spread of the drug resistance. The incidence of R-plasmid transfer associated with aquaculture has been reviewed by Aoki (1992).

Plasmid-mediated resistance has been reported in a variety of bacteria, including human pathogenic forms known to be associated with seafoods, such as *E. coli*, *Salmonella* spp., *Vibrio anguillarum, V.parahaemolyticus, Aeromonas salmonicida, A. hydrophila, Edwardsiella tarda* and *Pasteurella* (Datta *et al.*, 1984; Aoki *et al.*, 1985, 1986; Inglis *et al.*, 1993a; Chakrabarti *et al.*, 1994; Twiddy and Reilly, 1995). Transferable R-plasmids encoding resistance to chloramphenecol, tetracycline, streptomycin and sulphamonomethoxine have been demonstrated in *A. salmonicida*

isolated from cultured salmonid in Japan by Aoki (1988). Inglis *et al.* (1993 b) reported the presence of transferable R-plasmids encoding oxytetracycline resistance in more than a quarter of strains of *A. salmonicida* isolated from outbreak of furunculosis in Atlantic Salmon in Scotland. They also observed that the resistance transferred was multiple: to OTC, streptomycin, sulphamethoxine and trimethoprim, or to OTC and one or two of these combinations. From the Southeast Asia, Twiddy and Reilly (1995) detected plasmids in Pseudomonas spp. and Enterobacter sp. resistant to chloramphenicol and/or tetracycline and *Salmonella* resistant to tetracycline in integrated fish farms.

Information on occurrence of plasmids in bacteria associated with farmed shrimp or shrimp culture environment is scanty. Fonseka *et al.* (1995), while investigating on *Salmonella* contamination in farm-raised shrimp (*P. monodon*) in Sri Lanka, came across plasmid DNA in only a single case of *Salmonella* strains isolated by them, and concluded a low plasmid carriage rate for that bacterium in farm shrimp.

2.9 Hazard Analysis and Critical Control Points (HACCP) in shrimp aquaculture

The rapid growth in aquaculture production during the past two decades has helped in augmenting supply of luxury seafood items like shrimp in the international markets , and indications are that demand for such food of gourmet will increase in the coming years (Anon.,1997).While finfish and crustaceans harvested from the open ocean are generally considered as more safe from human health point of view, such products from aquaculture have been associated with many food safety issues, as the risk of

contamination of products by chemical and biological agents is greater in culture ecosystem than in open sea (Reilly and Kaferstein,1997). With the increasing contribution of aquaculture to food supplies and to regional and international trade , proper assessment and regulation of food safety hazards associated with products from aquaculture have become important (Reilly *et al.*, 1998). The principle of Hazard Analysis and Critical Control Points (HACCP) permits a systematic approach to the identification and assessment of hazards and risks associated with production ,distribution and use of aquatic foods ,and puts the responsibility for farming safe aquatic foods into the hands of the aquaculture sector (Reilly and Kaferstein , 1997 ; Reilly *et al.*, 1998). HACCP is based on seven principles for production of hazard free food items, such as (1) Conduct hazard analysis and identify preventive measures ,(2) Identify the control points(CCPs), (3) Establish critical limits (4) Monitor each CCP, (5) Establish corrective action to be undertaken when a critical limit deviation occurs , (6) Establish record keeping systems , and (7) establish verification procedures (Avault, 1995; Cato and Santos, 1998; Gopakumar, 1998).

Reilly and Kaferstein (1997) reviewed the food safety hazards associated with finfish and crustaceans from aquaculture and suggested improved fish farm management practices based on HACCP system as the desirable option to ensure safe food products from aquaculture. Recently Gopakumar (1998) has discussed the risks associated with contaminated fish from aquaculture and their control measures,and urged the fish farmers in India to try to implement the HACCP system for the safety of the consumers.

While the implementation of HACCP–based safety assurance programmes are well advanced in the fish processing sector (Beer and Mclachlan , 1998 ; Debevere and Neyts , 1998) such programmes at the fish farm to enhance food sefety is in its infancy. Adoption of the HACCP system in the seafood processing sector has become mandatory almost in international level (Henry , 1996), and such requirements will impact on the aquaculture sector with respect to raw material standards and products moving in international trade .The introduction of HACCP – based food safety assurance programmes at fish-farm level would be a major challenge to the aquaculture sector (Reilly *et al.*,1998)

3. MATERIAL AND METHODS

A comparative assessment of the environmental parameters and bacteriological profile between traditional shrimp culture systems, using no artificial feed, and semi-intensive systems applying commercial shrimp feed, formed the basis of this study. The various physico-chemical parameters of pond water assessed included salinity, temperature, pH, dissolved oxygen, turbidity, ammonia, nitrite, nitrate, total phosphorus and hydrogen sulphide.

3.1 Study area and scheme of sampling

3.1.1 Study area, description of farms and culture operations

Samples were collected at different stages of farming from eight selected shrimp farms, four traditional farms and four semi-intensive farms (Fig.1), located around Cochin backwater in Kerala (Lat. 9° 50'N – 10° 11'N & Long.76° 10'E – 76° 23'E) during the period January 1997 to April 1998. The different farms under each category were situated at different salinity gradients of the backwater.

Two of the traditional farms (Farm-A & Farm-B) were located at Munambam, one (Farm-C) at Edavanakkad and one (Farm-D) at Kumbalangi. These farms were perennial farms, which carried out shrimp farming throughout the year in the traditional style as against the seasonal prawn filtration process in paddy ('Pokkali') fields (Muthu,

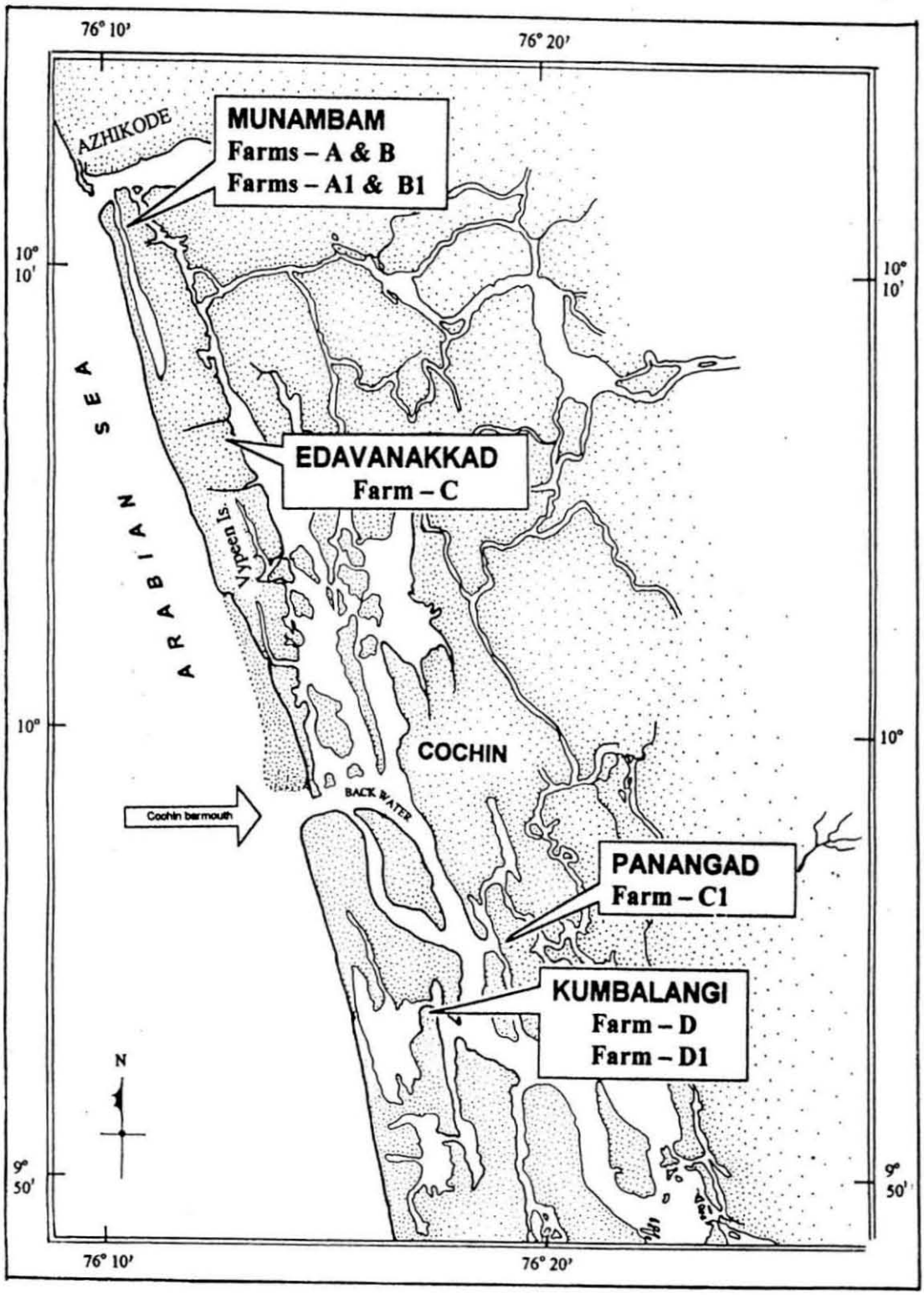

Fig. 1 : Map showing locations of shrimp culture systems

1980). Farm-A had a water-spread area of 5 ha, Farm-B 6 ha, Farm-C 2.5 ha and Farm-D 3.5 ha. The depth of water varied from 1m to 2m. While Farms–A and B were open to the southern extension of Munambam channel that connected sea and backwater, Farms-C and D were directly open to the backwater. The flow of tidal water was regulated through a sluice gate fixed in each pond. Farming involved autostocking of young prawns through incoming tides, and harvesting them periodically by filtration (sluice net operation) according to the lunar phase called 'Thakkom' (Menon, 1954; George *et al.*, 1968). Due to the non-selective nature of culture practice, the catch from these farms consisted of mixed varieties of fish and shrimp. The dominant shrimp species were *Metapenaeus dobsoni, M. monoceros* and *Penaeus indicus* of which *P. indicus* formed approximately 30-40%. During the period of observation, the monthly production of shrimp worked out to 35 to 100 kg/ha during the non-monsoon period and 20 to 70 kg/ha during the monsoon period.

Of the four semi-intensive farms selected for study, two (Farm-A1 & Farm-B1) were located at Munambam, one (Farm-C1) at Panangad and one (Farm-D1) at Kumbalangi. Farms A1 and B1 were situated near to Farms-A and B, and obtained water from the southern extension of the Munambam channel, while Farms-C1 and D1 were connected to the open backwater. Farm-A1 had a water-spread area of 1 ha, Farm-B1 0.9 ha, Farm-C1 0.4 ha and Farm-D1 2.21 ha, with mean depths of 1.25, 1.20, 1.35 and 1.40 m respectively. As in the traditional farms, water exchange was done through a single sluice gate provided in each farm. Shrimp culture in semi-intensive manner was carried

out from December/January to March/April in all the four farms every year. Farm-D1, however, undertook one more crop during the monsoon period of May/June to September/October. The present study was conducted during the non-monsoon crop of Farms-A1, B1 and C1 and during the monsoon crop of Farm-D1. Monoculture of *P. indicus* was carried out in these farms, although *P. monodon* was also cultured in other farms of the region either alone or in combination with *P. indicus*. Standard farming procedures such as draining of pond water, sun-drying and filling, pond fertilization etc. were followed before stocking of seed. After sufficient phytoplankton growth was achieved by adding fertilizers (Poultry manure 200-500 kg/ha or cow dung 500-1000 kg/ha + urea 40-80 kg/ha + Super phosphate 10-20 kg/ ha) in the water, seeds of *P. indicus* (PL 20) obtained from hatcheries were stocked at the rates of $15/m^2$ in Farm-A1 (1,50,000 nos.) and B1 (1,35,000 nos.), $12/m^2$ in Farm-C1 (48,000 nos.) and $11/m^2$ in Farm-D1 (2,43,000), and reared for 100 (Farm-C1) to 110 (Farms-A1 & B1) days during non-monsoon season and about 120 days (Farm-D1) during monsoon season. Supplementary feeding was given using "HIGASHI" commercial shrimp feeds at the rates of about 2 to 7 % of body weight depending on the stage of culture. The feeds were broadcast in the ponds 2-5 times a day. Water exchange was done through tide as well as by pumping, which normally commenced 10 to 15 days after seed stocking. The rate of daily water exchange varried from 5 to 10% during the early phase of culture and from 10 to 15 % in Farms-A1, B1 & C1 and 10 to 20 % in Farm-D1 during the advanced stages of culture operations. Shrimp productions from these farms were 2100 kg (2100 kg/ha) for

Farm-A1, 1945 kg(2160 kg/ha) for Farm-B1, 760 kg (1900 kg/ha) for Farm-C1, and 2920 kg (1320 kg/ha) for Farm-D1.

3.1.2 Scheme of sampling

Regular observations on environmental factors and microbiological characteristics from semi-intensive farms were carried out during the non-monsoon crop of Farms-A1 & B1 extending from December, '96 to April, '97 and that of Farm-C1 from December, '97 to March, '98, and during the monsoon crop of Farm-D1 extending from June to September, '97. Observations from traditional farms were carried out at monthly intervals from January to March, '97 in Farms-A, B and C and from June to September, '97 in Farm-D, which coincided with the period of observations in the semi-intensive farms. The schedule of sampling is shown on page 47.

Collection of samples and on-spot observations in the farms were carried out in the morning hours between 7 and 9 a.m. On each day of observation in a farm, readings were taken first for temperature, turbidity, and hydrogen sulphide concentration of water. Temperature was measured using a mercury thermometer graduated in 0-50^{o}C. The thermometer was dipped into water contained in narrow-mouth polypropylene bottle immediately after collection at the culture site and the temperature reading was noted. Turbidity of water was measured by using Secchi disc, the depth of which in the water column was inversely related to turbidity.

Schedule of sampling

Farms	Period of culture	Dates of observation			
		I Obser.	II Obser.	III Obser.	IV Obser.
Traditional					
Farm-A	Continuous	January '97 First week	February '97 First week	March '97 First week	-
Farm-B	Continuous	January '97 First week	February '97 First week	March '97 First week	-
Farm-C	Continuous	January '97 Second week	February '97 Second week	March '97 Second week	-
Farm-D	Continuous	June '97 Second week	July '97 Second week	August '97 Third week	September '97 Third week
Semi-intensive					
Farm-A1	110 days	January '97 25th DoC	February '97 60th DoC	April '97 105 DoC	-
Farm-B1	110 days	January '97 25th DoC	February '97 60th DoC	April '97 105th DoC	-
Farm-C1	100 days	January '98 25th DoC	February '98 60th DoC	April '98 95th DoC	-
Farm-D1	120 days	June '97 21st DoC	July '97 52nd DoC	August '97 85th DoC	September '97 110 DoC

DoC = Day of culture

Keeping the line vertical, the secchi disc was slowly lowered into the water and recorded the depth at which it just disappeared (d_1m), and then raised it until it just reappeared and then recorded the depth (d_2m). The secchi disc depth was the average of these two readings, i.e. $(d_1+d_2)/2$ m. Hydrogen sulphide concentration in water was determined by

using 'AQUAQUANT 14416' of E.merk, Darmstadt (Germany). After recording temperature, turbidity and hydrogen sulphide, water samples were collected for estimation of oxygen, salinity, pH and nutrients in the laboratory. In order to get an average picture of the environmental parameters of the entire culture system, observations were taken from four different areas of each of the farms and the mean values of the various parameters worked out. The water samples for oxygen determination were fixed at the farm-site itself immediately after collection. For microbiological analysis, column water samples were collected in sterile glass bottles of 250-ml capacity. Sediment samples collected from five different locations of the pond in sterile polythene bags were brought to the laboratory and were mixed thoroughly before analysis. Shrimp samples (*P.indicus*) were collected by cast netting and placed immediately in sterile polythene bags. Immediately after collection, all the samples were carefully packed in between ice layers in an insulated container and brought to the laboratory within 90 min. Care was taken to see that ice did not come into direct contact with the samples during transportation.

3.2 Media and chemicals used

Media:

The bacteriological media used in this study included dehydrated media and media compounded in the laboratory.

Dehydrated media

The following dehydrated media supplied by M/s Hi-media, Bombay were used.

a) Selenite systine(SS) broth

b) Tetrathionate (TT) broth

c) Xylose lysine desoxycholate (XLD)agar

d) Hektoen enteric agar (HEA)

e) Bismuth sulfite agar (BSA)

f) Thiosulphate-citrate-bile salt-sucrose (TCBS) agar

g) Tryptic soya agar (TSA)

h) Baird Parker (BP) medium

i) Muller Hington agar

Media compounded in the laboratory

All the media used were prepared according to methods outlined in FDA Bacteriological Analytical Manual (1992).

Tripe Sugar Iron (TSI) agar

Ingredients:

Polypetone	- 20 g
Sodium chloride	- 5 g
Lactose	- 10 g
Sucrose	- 10 g
Glucose	- 1 g
$Fe(NH_4)_2(SO_4)6H_2O$	- 0.2 g
$Na_2S_20_3$	- 0.2 g
Phenol red	- 0.025 g
Agar	- 13 g
Distilled water	- 1 litre

Suspended the ingredients in distilled water, mixed thoroughly and heated with occasional agitation. Boiled for about 1 min. to dissolve the ingredients. Filled 16-150

mm tubes 1/3 full and plugged to maintain aerobic condition. Autoclaved the medium at 118°C for 15 min. Before the media solidified the tubes were inclined to obtain 4-5 cm slant and 2.3 cm butt. Final pH was 7.3 ± 0.2.

Lysine iron agar (LIA)

Ingredients:

Peptone	- 5 g
Yeast extract	- 3 g
Glucose	- 1 g
L-lysine hydrochloride	- 10 g
Ferric ammonium citrate	- 0.04 g
Sodium thiosulphate (anhydrous)	- 0.04 g
Bromocresol purple	- 0.02 g
Agar	- 15 g
Distilled water	- 1 litre

Heated to dissolve the ingredients. Dispensed in 4 ml proportions into 13 x 100 mm tubes. Autoclaved for 12 min. at 121°C. Solidified in slanted position for 4 cm butts and 2.5 cm slants. Final pH was 6.7±0.2.

Lactose broth (LB)

Ingredients:

Beef extract	- 3 g
Peptone	- 5 g
Lactose	- 5 g
Distilled water	- 1 litre

Dispensed 225 ml portions into 500 ml Erlenmeyer flasks. After autoclaved for 15 min. at 121°C and just before use, aseptically adjusted volume to 225 ml. Final pH adjusted to 6.9 ± 0.2.

1% Trypone, 1% Sodium chloride (T_1 N_1) agar

Ingredients:

Tryptone	- 10 g
Sodium chloride	- 10 g (30 g, T_1N_3)
Agar	- 20 g
Distilled water	- 1 litre

Suspended ingredients and boiled to dissolve agar. Autoclaved for 15 min. at 121°C. Cooled the medium for plates to 45-50°C and poured into sterile petridishes.

Kligler iron agar (KIA)

Ingredients :

Peptone	- 20 g
Lactose	- 20 g
Dextrose	- 1 g
Sodium chloride	- 5 g
Ferric ammonium citrate	- 0.5 g
Sodium thiosulphate	- 0.5 g
Phenol red	- 0.025 g
Agar	- 15 g
Distilled water	- 1 litre

Heated with agitation to dissolve. Dispensed into 13 x 100 mm tubes and autoclaved for 15 min. at 121°C. Cooled and started to form deep butts. Final pH adjusted to 7.4 ± 0.2.

Gelatin agar (GA)

Ingredients :

Peptone	- 4 g
Yeast extract	- 1 g
Gelatin	- 15 g
Agar	- 15 g
Distilled water	- 1 litre

Suspended ingredients with constant stirring to prevent scorching gelatin, and boiled to dissolve gelatin and agar. Adjusted pH to 7.2 ± 0.2. Autoclaved for 15 min. at 121°C. Cooled to 45° - 50°C.

Gelatin salt agar (GSA)

Prepared the gelatin agar, with an addition of 30 g of Sodium chloride/l. Suspended ingredients and boiled to dissolve gelatin and agar. Adjusted to pH 7.2 ± 0.2. Autoclaved 15 min. at 121°C. Cooled to 40°-50°C. Pour plated.

Hugh-Leifson glucose medium (H&L)

Ingredients :

Peptone	- 10 g
Sodium chloride	- 5 g
K^2HPO^4	- 3 g

Glucose - 10 g
Phenol red - 1 g
Agar - 3 g
Distilled water - 1 litre

Heated with agitation to dissolve the agar. Adjusted the pH to 7.2 ±0.2. Autoclaved for 10 min. at 10 lbs

Alkaline peptone water (1%) & (3%) (APW)

Ingredients :

Peptone - 10 g
Sodium chloride - 10 g (1%)/3 g (3%)
Distilled water - 1 litre

Adjusted the pH so that the value after sterilization was 8.5 ± 0.2. Autoclaved for10 min. at 121°c.

Modified Mackonkey Broth

Ingredients :

Bile salt No.3 - 5 g
Peptone - 20 g
Lactose - 10 g
Sodium chloride - 5 g
Bromocresol purple - 0.01 g
Cystal violet - 0.5 g
Distilled water - 1 litre

Double strength and single strength of the broth were prepared and distributed into tubes and autoclaved for 10 min. at 120°C. pH adjusted to 7.3 ± 2.

Brilliant green lactose bile broth, 2% (BGLB)

Ingredients :

Peptone	- 10 g
Lactose	- 10 g
Bile salt No.3	- 20 g
Brilliant green	- 13.3 ml
Distilled water	- 1 litre

Dissolved the ingredients by boiling. Distributed to small tubes with inverted durams tubes inside. Sterilized at 10 lbs. for 20 min.

E.C. Broth

Ingredients :

Tryptone	- 20 g
Bile salt No.3	- 1.5 g
Lactose	- 5 g
K_2HP0_4	- 4 g
KH_2PO_4	- 1.5 g
Sodium chloride	- 5 g
Distilled water	- 1 litre

Distributed 8 ml portions to 16 x 150 mm test tubes containing inverted 10 x 75 mm fermentation tubes. Autoclaved for 15 min. at 121°C. Final pH was adjusted to 6.9 ±0.2.

Indole medium

Ingredients :

Tryptone	- 10 g
Distilled water	- 1 litre

Dissolved and dispensed 5 ml portion into 16 x 125 or 16 x 150 mm test tubes. Autoclaved for 15 min. at 121°C. Final pH was adjusted to 6.9 ± 0.2.

Tryptone glucose agar (TGA)

Ingredients :

Tryptone	- 10 g
Beef extract	- 3 g
Glucose	- 1 g
Sodium chloride	- 5 g
Agar	- 15 g
Distilled water	- 1 litre

Heated the ingredient to dissolve. Adjusted pH to 7.1 ± 0.2 and autoclaved for 15 min. at 15 lbs.

Chemicals:

Ingredients such as peptone, proteose peptone, tryptone, beef extract, yeast extract, agar powder & gelatin for bacteriological media and antibiotics and antimicrobials (with disc contents in parenthesis), such as Amoxycillin (30 mcg), Ampicillin (10 mcg), Bacitracin (10 mcg), Chloramphenicol (30 mcg),

Chlortetracycline(30 mcg), Erythromycin (15 mcg) , Gentamycin (10 mcg), Kanamycin (30 mcg), Novobiocin (30 mcg), Neomycin (30 mcg), Oxacillin (1 mcg), Oxytetracycline (30 mcg), Penicillin-G (10 units) Streptomycin (10 mcg), Tetracycline (30 mcg), Trimethoprim (10 mcg), Furazolidone (50 mcg) , Nalidixic acid (30 mcg), Nitrofurantoin (300 mcg), Polymyxin-B and Sulphadiazine (300 mcg) were supplied by Hi-media, Bombay. Lysozyme used was from Sigma (USA) brand and other chemicals were of BDH (England), Sisco (India), E-merk (India), and Hi-media (India) brands.

3.3 Analytical procedures

3.3.1 Estimation of water quality parameters

Salinity of water samples was determined by Mohr's titration method (Strickland and Parsons, 1968). The pH of water samples was measured using a digital pH meter (Elico Li-10). Dissolved oxygen was estimated by Winkler method (Strickland and Parsons, 1968). Ammonia-Nitrogen (NH_3-N) was determined following the phenol-hypochlorite method (Solorzano, 1969). Nitrite-Nitrogen (NO_2-N) was determined by the Azo-dye method. Nitrate-nitrogen (NO_3-N) was estimated using cadmium reduction column as per Solyom and Carlberg (1975). The total phosphorus content of water was estimated using the method described by Strickland and Parsons, 1968.

3.3.2 Analysis of shrimp feed

The proximate composition of shrimp feed samples was determined using standard methods (AOAC, 1990) as described below.

Moisture:

Moisture in the feed was determined gravimetrically by oven drying the samples at 100^{o}C till concurrent dry weights were obtained. Percentage moisture in the samples was calculated.

Crude protein:

This was determined by the micro-kjeldahl method (AOAC, 1990) by measuring the total nitrogen content in the sample and converting it to a total crude protein value using the empirical conversion factor of 6.25

To determine the crude protein, weighed samples were digested in digestion tubes containing a catalyst mixture of 15 g of K_2SO_4, 0.05 g anhydrous $CuSO_4$ and 10 ml Con. H_2SO_4 for 6 hrs at 120^{o}C till the solution became clear. After cooling to room temperature it was diluted with ammonia-free distilled water and made upto 100 ml. 10 ml of aliquot sample mixed with 10 ml of 40% NaOH was distilled in Kjeldahl apparatus for 15 minutes and distillate collected in the boric acid indicator solution, prepared by mixing 10 ml of 2% boric acid with Tashiro's indicator (0.2% methylred and 0.2%

methylene blue in ethanol) which changes its colour from light purple to green. Distillation was continued until no trace of ammonia, as confirmed by litmus paper, was noticed. The ammonia absorbed was titrated against 0.2 N H_2SO_4, until the colour turned purple from green. Similarly, nitrogen content in standard and blank solutions was estimated. For determining blank values, double distilled water was used by the above procedure and corrections for blank were made. Percentage nitrogen was determined using the formula: A-B x N x 0.14, where A and B are the volume of standard acid used in sample and blank titration respectively. N the Normality of the acid and 0.14 a constant.

Crude fibre:

To dried, ground fat-free samples of known weight, 200 ml of boiling 1.25% H_2SO_4 and a drop of antiform actanol were added in beakers of 1 litre capacity. A set of six such beakers were placed on digestion apparatus with pre-adjusted hot plate and boiled exactly for 30 minutes, rotating the beakers periodically to keep solids from adhering to sides. The contents were subsequently filtered and rinsed repeatedly thrice, using 50 ml of boiling water each time. To the residue, 200 ml of boiling (1.25%) NaOH was added and boiled exactly for 30 minutes. This digest was again filtered and rinsed as above. The residue was then washed with 25 ml of boiling H_2SO_4 (1.25%), rinsed 3 times with 50 ml each of boiling water, drained, washed with 25 ml of alcohol and dried for 2 hrs at 130^{o}C. Subsequently it was transferred to crucibles of known weight, cooled in a

desiccator and reweighed. The loss of weight in sample due to ignition (at 600^{o}C for 6 hrs) made into 100 g gives the percentage of crude fibre.

Crude fat:

Weighted, dried sample of feed in a thimble was placed in soxhelet apparatus, having a round bottom flask of known weight. The lipid was extracted 16 hrs using petroleum-ether (b.p. 40-60^{o}C) as the solvent, adjusting the heat to give a condensation rate of 2-3 drops per second. On completion of extraction, the flask with the residue was oven dried as 105^{o}C for 30 min and then cooled in a desiccator and weighed. The difference in weight expressed as percentage in the weight of sample studied accounts for the lipid content in the sample.

Ash:

Dried samples of known weight were ashed in silica crucibles at 600^{o}C for 6 hrs in a muffle furnace and percentage of ash was determined.

Nitrogen free extract:

This comprises of soluble carbohydrate such as sugars and starch, and it was estimated by subtracting the values of moisture, crude protein, crude fibre, crude fat and ash from the total value of the sample.

All the analyses were carried out in six replicates and the average values are presented on dry matter basis.

3.3.3 Estimation of microbiological parameters

3.3.3.1 Quantitative estimation

Water samples collected aseptically from the farms were serially diluted in sterile physiological saline and pour plated on TGA for total plate count (TPC) as per procedure described in the Bacteriological Analytical Manual of the FDA (Peeler and Maturin, 1992).

Aseptically weighed 25-g samples of mud into a sterile polythene bag and added 225-ml physiological saline, and homogenized for 1 min. All serial dilutions were prepared in sterile physiological saline and pour plated on TGA for TPC.

In the case of farmed shrimp, bacteriological analysis was carried out separately for whole shrimp, shrimp surface and shrimp gut. Whole shrimp samples weighing 25 g were homogenized with 225 ml of physiological saline and the homogenate was used for estimating TPC. For shrimp surface, ten shrimps were rinsed in 100 ml physiological saline and this saline was used for estimating TPC. Shrimps were deveined to get 6 g gut and the same was homogenized in 54 ml physiological saline for estimating TPC.

Commercial pelletised feed samples collected from shrimp farm A1-D1 was powdered using a sterile motor and pestle. Weighed 25 g of the powdered feed into a

sterile stomacher bag and added 225 ml sterile physiological saline, and homogenized for 1 min. Serial dilutions were prepared and pour plated on TGA for TPC.

All the TGA plates were incubated aerobically at 30°C for 48 hrs. After incubation, colony-forming units were counted. Readings obtained between 30 and 300 colonies on plates at the temperature of incubation were used for calculation of the bacterial populations as shown below and the results recorded as CFU per unit of sample.

$$\text{No. of bacteria/gm or No. of bacteria/ml or No. of bacteria/cm}^2 = \frac{\text{No. of colonies x reciprocal of dilution x 100}}{\text{Weight of sample}}$$

3.3.3.2 Qualitative estimation

A total of 20-30 colonies were isolated and purified from each sample and carried out the morphological and Biochemical tests to identify and enumerate the isolates to generic level using the scheme adopted by Buchanan and Gibbon (1974) and Surendran and Gopakumar (1981).

3.3.4 Detection of *Salmonella*

Salmonella was screened as per procedure described in the Bacteriological Analytical Manual of the FDA (Andrews *et al.*, 1992). Aseptically weighed 25 g whole shrimp/sediment/feed sample (25 ml pond water sample) into sterile blending container. Added 225 ml sterile lactose broth and blended for 2 min. Aseptically transferred

homogenized mixture to sterile wide-mouth conical flask (500 ml) and let stand 60 min. at room temperature with the flask tightly plugged. Mixed well and loosened the plug. Incubated for 24 hrs at 35°C.

Isolation

1. Transferred 1 ml of above mixture to 10 ml selenite cystine (SC) broth and another 1 ml mixture to 10 ml tetrathionate (TT) broth.
2. Incubated SC and TT broths for 24 hrs at 35°C.
3. Mixed and streaked 3 mm loopful incubated TT broth on Bismuth sulphite (BS) agar, Xylose lysine desoxycholate (XLD) agar, and Jektoen enteric (HE) agar.
4. Prepared BS, XCD and HE plates the day before streaking and stored in dark at room temperature until streaked.
5. Incubated the plates 24 hrs ± 2 hrs at 35°C.
6. Examined plates for presence of colonies suspected to be *Salmonella*
7. Selected 2 or more colonies typical of *Salmonella* from each selective agar. Inoculated into triple sugar iron (TSI) agar and lysine iron agar (LIA).
8. Lightly touched the very centre of the colony to be picked with sterile inoculating needle and inoculated to TSI agar slant by streaking slant and stabbing butt. Without flaming inoculated LIA by stabbing butt twice and then streaking slant.
9. Incubated TSI agar and LIA slants at 35°C for 24+2 hrs and 48 + 2 hrs, respectively.

Salmonella in culture typically produces alkaline slant and acid butt, with or without production of H^2S in TSI agar. In LIA, *Salmonella* typically produces alkaline reaction in butt of tube.

3.3.5 Detection of *Vibrio cholerae*

Vibrio cholerae was screened as per procedure described in the Bacteriological Analytical Manual of the FDA (Elliot *et al.*, 1992)

Enrichment:

Aseptically weighed 25 g of whole shrimp/sediment/feed sample (25 ml pond water sample) sample into sterile blender jar. Mixed with 225 ml of alkaline peptone water. Left blended solution into loosely plugged sterile 500 ml flasks and incubated for 6-8 hrs at 35-37^{o}C.

After incubation, and without shaking flask, transferred 3-5 mm loopful of inoculum from pellicle onto one plate of selective plating medium: TCBS agar. Incubated TCBS agar for 18-24 hrs at 35-37^{o}C. Carefully picked 3 or more suspected colonies from each plate (large, smooth, yellow, sucrose-positive, and slightly flattened colonies with opaque centres and translucent peripheries), streaked for isolation on T_1N_1 agar and incubated for 12-18 hrs at 35-37^{o}C.

Characterization :

The preliminary identification tests included in TSI, KIA, T_1N_0, T_1N_3, gelatin agar, gelatin salt agar, Hugh-Leifson glucose medium, oxidase test and Grams

stain. The biochemical reactions included growth in 0%, 3%, 6%, 8% and 10% Sodium chloride, growth at 42°C, acid from sucrose, lactose, mannose and mannitol, ONPG, Voges-Proskauer, arginine hydrolase, lysine decarboxylase and ornithine decarboxylase tests, sensitivity to $10_{\mu}g$ 0/129 and $150_{\mu}g$ 0/129, urease test and serological test.

3.3.6 Detection of *Vibrio parahaemolyticus* and *V. vulnificus*

Vibrio parahaemolyticus and *V. vulnificus* was screened as per procedure described in the Bacteriological Analytical Manual of the FDA (Elliot *et al.*, 1992)

Enrichment:

Aseptically weighed 25 g of whole shrimp/sediment/feed sample (25 ml pond water sample) sample into sterile blender jar. Added 225 ml 3% Sodium chloride alkaline peptone water and blended for 2 min. Incubated for 16-18 hrs at 35-37°C.

Isolation:

After incubation, streaked to TCBS agar with 1 loopful from top 1 cm of enrichment broth. Incubated at 35-37°C for 18-24 hrs. Examined TCBS for typical *V. parahaemolyticus* and *V. vulnificus* colonies (Round, 2-3 mm diameter, green or blue-green). Picked 3 or more typical colonies and streaked to T_1N_2 agar for isolation. Incubated at 35-37°C for 18-24 hrs.

Characterization:

The preliminary identification tests included, oxidase test, motility, reaction is TSI, 0/129 (10$_{\mu}$g), 0/129 (150 $_{\mu}$g), ONPG and Grams stain.

The biochemical reaction included growth in 0%, 3%, 6%, 8% and 10% Sodium chloride, growth at 42^{o}C, acid from sucrose, D-cellulose, lactose, arabinose, mannose and mannitol, Voges-Proskauer, arginine dehydrolase, lysine decarboxylase, ornithine decarboxylase, gelatinese and urease tests, and Kanagawa phenomenon for *V. parahaemolyticus* were studied on Wagatsuma agar.

3.3.7 Detection and enumeration of *Staphylococcus aureus*

Staphylococcus aureus was screened as per procedure described in the Bacteriological Analytical Manual of the FDA (Bennett and Lancette, 1992)

Isolation and enumeration:

For each dilution to be plated aseptically transferred 1 ml of the sample suspension to 3 plates of Baird-Parker agar, distributing 1 ml of inoculum equitably to 3 plates (e.g. 0.4 ml, 0.3 ml and 0.3 ml). Spread inoculum over surface of agar plate, using sterile, bent glass-streaking rod. Retained the plates in upright position until inoculum was absorbed by the agar. Inverted the plates and incubated for 45-48 hrs at 35^{o}C. Selected plates containing 20-200 colonies, unless only plates at lower dilutious (>200 colonies) had colonies with typical appearance of *S. aureus*. Colonies of *S. aureus* were circular, smooth, convex, moist, 2-3 mm in diameter on uncrowded plates, grey to jet-

black, frequently with light-coloured (off-while) margin, surrounded by opaque zone and frequently with an outer clear zone; colories had butter to gummy consistency when touched with inoculating needle.

Several types of colonies appeared to be *S. aureus*, counted numbers of colonies of each type. Used plates of lower dilution plated, which contained <20 colonies. Selected more than one colony of each type counted and tested for coagulase production. Added number of colonies on set of triplicate plates represented by colonies giving positive coagulase test and multiplied by sample dilution factor. Taken this number as number of *S. aureus*/g of the sample tested.

3.3.8 Detection and enumeration of total coliforms, faecal coliforms and *E. coli*

Total coliforms, faecal coliforms and *E. coli* was screened as per procedure described in the Bacteriological Analytical Manual of the FDA (Hitchins *et al.*, 1992)

Presumptive test for coliform bacteria:

Weighed 25 g sample into sterile blender jar. Added 225 ml Butterfield's phosphate-buffered dilution water and blended 2 min. Prepared decimal dilutions with 90 ml sterile dilution water plus 10 ml from previous dilution. Number of dilutions prepared depended on anticipated coliform density. Shaked well all suspensions 25 times in 30 cm are for 7 seconds. Transferred 1 ml portion to modifid MacConkey broth tubes for each dilution for 3 consecutive dilutions. Incubated tubes for 48 ± 2 hrs at 35°C. Examined tubes at 24 h ± 2 hrs for gas. Reincubated negative tubes for additional 24 hrs.

Examined a second time for gas. Performed a confirmed test on all presumptive positive (gassing) tubes.

Confirmed test for coliforms:

Gently agitated each gassing modified MacConkey broth tubes and transferred loopful of suspension to tube of BGLB Broth. Incubated tubes for 48 ± 2 h at 35°C. Examined for gas production and recorded, calculated most probable number (MPN) of coliforms based on proportion of confirmed gassing modified MacConkey broth tubes for three consecutive dilutions.

Confirmed tests for faecal coliforms and *E. coli*:

Gently agitated each gassing modified MacConkey broth tube and transferred loopful of each suspension to tube of EC broth. Incubated EC tubes for 48 ± 2 hrs at 45.5 ± 0.2°C. Examined for gas production at 24 ± 2 hrs; when negative, examined again at 48 ± 2 hrs. Results of this test were used to calculate faecal coliform MPN.

From each gassing modified MacConkey broth tube transferred loopful of each suspension to tube of Indole medium and incubated for 24 ± 2 hrs at 35°C. Tested for indole by adding 0.2-0.3 ml of Kovac's reagent. The biochemical tests included nitrate reduction, oxidase test, fermentative (TS1), mannitol, lactose, malonate, H_2S production, urease test, citrate test, VP test, ONPG test and Methyl Red test.

3.3.9 Detection and enumeration of Faecal Streptococci

Faecal Streptococci was screened as per procedure described in the Bacteriological Analytical Manual of the FDA (1978)

Isolation and enumeration:

For each dilution to be plated, aseptically transferred 1 ml of the sample suspension to 3 plates of KF agar, distributing 1 ml of inoculum equitably to 3 plates (eg. 0.4 ml, 0.3 ml and 0.3 ml). Spread inoculum over surface of agar plate, using sterile, bent glass-streaking rod. Retained the plates in upright position until inoculum was absorbed by the agar. Inverted the plates and incubated for 45-48 hrs at 35^{o}C. Colonies of Faecal streptococci were circular, small and pink. Added number of colonies on set of triplicate plates and multiplied by sample dilution factor. This number was taken as number of faecal streptococci/g of the sample tested.

3.3.10 Antibiotic sensitivity studies on bacterial isolates

Antibiotic sensitivity studies were done using Bauer-Kirby method for rapidly growing aerobic organisms (Bauer *et al..*, 1966). The results were interpreted by using the zone size interpretative chart Performance standards for antimicrobial disk susceptibility test.

Plates were prepared with Muller Hington Agar. Pure cultures were used as inoculum. Three to four similar colonies were selected and transferred into about 5 ml of nutrient broth and incubated at 35^{o}C for 2-8 hrs till light to moderate turbidity developed. A sterile cotton swab was dipped into the properly prepared inoculum and rotated firmly

against the upper inside wall of the tube to express excess fluid. The entire agar surface of the plate was streaked with the swab three times turning the plates 60^{o} between each streaking. The antibiotic discs were applied using aseptic technique. The discs were deposited with centre at least 24 mm apart. The plates were immediately incubated at 37^{o} and examined after 24 hrs. Only zones showing complete inhibition were measured and the diameters of the zones were recorded in millimeter. Multiple antibiotic resistance (MAR) indexing was calculated as described by Krumperman (1983)

3.3.11 Detection of plasmids

Strains of *V. parahaemolyticus* isolated and stored have been used for plasmid screening by employing the modified method of Maniatis *et al.* (1989).

The bacterial strains were harvested from the nutrient broth culture during the post logarithmic phase by spinning at 10K; 4^{o}C, 10' in 1.5 ml eppendorf tube in a refrigerated high speed centrifuge. The supernatent was carefully drained out and the pellet was suspended in 100 μl of TEG buffer (pH: 8) containing lysozyme (5 mg/ml). The cell suspension was vortexed in a vortex mixture and incugated at 4^{o}C for 10 min. After the bacterial cell wall lysis in TEG buffer containing lysozyme, 200 μl of alkaline lysis buffer containing 0.2 M NaOH and 1% SDS was added. The solution was mixed gently and incubated at 4^{o}C for 15 min. The solution was gently shaken to mix the contents at every five minutes. The nuclear DNA and proteins got denatured during

alkaline lysis and the solution became viscous. To that viscous solution, 150 μl of 3 M Potassium acetate (pH 5.2) was added and kept at 4^{o}C for 10 min. The contents were mixed well. A network of precipitated proteins and nuclear DNA was formed. After 10 minutes the preparation was centrifuged for 15 minutes at 10K, 4^{o}C. After centrifugation, the clear supernatant containing plasmid DNA was collected in another micro-centrifuge tube. To that, equal volume of neutral phenol was added to precipitate any protein present in the solution. The solution was mixed by gentle shaking and kept undisturbed for 10 minutes to precipitate the proteins. The preparation was then centrifuged at 10K, 4^{o}C, 10'. Protein layer got precipitated in the aqueous-organic interphase. The aqueous phase was carefully pipetted out and transferred to another eppendorf, tube and the neutral phenol extraction was repeated again. To the aqueous phase collected, equal volume of chloroform-isoamyl alcohol was added to remove traces of phenol and other impurities, if any. The mixture was shaken well and centrifuged and 10K, 4^{o}C, 5". The aqueous phase was transferred to another eppendorf tube. The quantity was measured and 1/10th volume of 3 M sodium acetate was added and mixed well. To this mixture, 2-2.5 volume of absolute ethanol was added. The mixture was shaken well and kept at -20^{o}C overnight for precipitating plasmid DNA. The ethanol-precipitated preparation was centrifuged at 10K, 4^{o}C 15'. The supernatant was discarded carefully and the precipitate was washed with 70% ethanol to dissolve the salt (sodium acetate). Then the solution was centrifuged at 10K, 4^{o}C, 10'. The supernatant was

discarded completely free of moisture, it was dissolved in minimum quantity of TE buffer (pH: 8). The plasmid DNA thus obtained was stored at -20^{o}C.

Plasmids isolated from the bacteria were subjected to agarose gel electrophoresis to resolve the plasmids according to their size. The duration of electrophoresis was four hours. The gel was stained in ethidium bromide in darkness for 20 minutes. The stained gel was dipped in distilled water to remove excess stain. Then the gel was viewed by wing a UV transilluminator. Plasmid DNA appeared as reddish orange bands and the molecular weight of the plasmid was determined by comparing it with standard DNA marker.

3.3.12 Statistical analysis

In order to find out the relationship between environmental parameters and microbiological parameters like Total Plate Count in water, sediment, whole shrimp and shrimp surface of the traditional and semi-intensive farms, their values were subjected to statistical analysis in a computer programme 'Stasistica' for the estimation of correlation coefficient 'r' and the significance 'p' of correlation coefficient 'r' of the different parameters were tested at 5 % levels.

4. RESULTS AND DISCUSSION

4.1 Characteristics of environmental parameters in shrimp culture systems

4.1.1 Traditional farms

The temporal values of environmental parameters recorded for the different culture systems are shown in Table 1 and their mean values in Table 2.

Table 1: Temporal dustribution of environmental parameters in traditional shrimp farms

Months of observation	Sal (ppt)	Temp (°C)	pH	DO (mg/l)	Ammonia ($\mu g.at NH_3$-N/l)	Nitrite ($\mu g.at NO_2$-N/l)	Nitrate ($\mu g.at NO_3$-N/l)	Phos ($\mu g.at$ P/l)	H_2S (ppm)
Farm A									
January '97	33.20	30.0	8.1	4.5	14.28	2.14	7.86	8.06	n.d
Feb '97	24.50	31.0	8.2	6.7	43.57	3.36	4.03	6.45	0
March '97	28.20	32.0	8.8	6.2	35.71	3.57	8.07	6.67	0
Farm B									
January '97	34.20	29.0	8	4.8	15	2.13	7.06	8.76	n.d
Feb '97	34.30	31.0	7.9	6	45.14	3. 56	4.57	7.01	0
March '97	28.00	32.0	8.7	8.5	12.85	2.14	4.05	3.87	n.d
Farm C									
January '97	15.93	28.0	8.5	7.6	14.35	2.12	7.76	7.14	0
Feb '97	16.83	30.0	8.8	8	14.28	3.21	8.79	7.86	0
March '97	17.23	32.5	9.2	8.2	15	3.57	n.d	6.13	n.d
Farm D									
June '97	1.01	28.0	7	5.7	16.42	6.12	15.01	17.56	n.d
July '97	0.01	28.5	7.6	7.8	25	7.01	15.96	17.97	n.d
Aug '97	1.13	28.0	7.5	5	47.85	7.23	16.05	18.12	n.d
Sept '97	2.03	30.0	7.7	4.8	65	7.14	16.14	18.7	n.d

n.d = Not detected

Table 2: Mean values of environmental parameters in traditional shrimp farms

Parameters	Non-monsoon season (January-April 1997)			Monsoon season (June -Sept.'97)
	Farm A	Farm B	Farm C	Farm D
Salinity (ppt)	31.96 ± 3.33	32.17 ± 3.61	16.66 ±0.67	1.05 ±0.83
Temperature (°C)	31.00 ±1.00	30.66 ±1.52	30.17 ±2.25	28.63 ±0.95
pH	8.36 ±0.38	8.20 ±0.44	8.83 ±0.35	7.45 ±0.10
Dissolved oxygen (mg/l)	5.79 ±1.15	6.42 ±1.87	7.93 ±0.30	5.82 ±1.38
Ammonia (µg.at NH_3- N / l)	31.18 ±15.16	24.33 ±18.05	14.54 ±0.40	38.57 ±22.05
Nitrite (µg.at NO_2- N / l)	3.02 ±0.77	2.61 ±0.82	2.97 ±0.75	6.88 ±0.51
Nitrate (µg.at NO_3- N / l)	6.66 ±2.27	5.22 ±1.60	8.27 ±0.73	15.79 ±0.52
T.Phosphorus (µg.at P / l)	7.06 ±0.87	6.65 ±2.48	7.05 ±0.87	18.09 ±0.47
H_2S (ppm)	0.02	0.007	0.013	n.d

n.d = Not detected

Salinity was maximum in Farms - A & B, where it varied from 24.5 ppt (Farm-A) to 34.3 ppt (Farm-B) during the observation period from January to March. The mean values were 31.96 ppt in Farm-A and 32.17 ppt in Farm-B. In Farm-C, it remained at a medium level of 16.66 ± 0.67 ppt during the same period, while in Farm-D the salinity during the monsoon season (June-September) was as low as 0.01-2.03 ppt, with a mean value of 1.05 ppt. Water temperatures varied from 28°C to 32.5°C, the mean values

being 31°C in Farm-A, 30.66°C in Farm-B, 30.17°C in Farm-C and 28.63°C in Farm-D. The pH fluctuated between 7 and 9.2 and showed very little variation between farms. Dissolved oxygen ranged from 4.50 to 8.46 mg/l, with mean values of 5.79 mg/l in Farm -A, 6.42 mg/l in Farm-B and 7.93 mg/l in Farm-C during non-monsoon season and 5.82 mg/l in Farm-D during monsoon season. The secchi disc visibility depths of pond water varied between 40 and 52 cm, showing no trend in any of the farms studied. Ammonia (NH_3-N) always dominated among the nitrogenous nutrients monitored. It varied from 12.85 to 45.14 µg.at N/l in the farms during non-monsoon season, with mean values of 31.18 µg.at N/l in Farm-A, 24.44 µg.at N/l in Farm-B and 14.54 µg.atN/l in Farm-C. In Farm-D, the ammonia concentration during monsoon season showed a regular increase from 16.42 µg.at N/l in June to 65 µg.at N/l in September, and recorded the mean value of 38.57 µg.at N/l. Nitrite (NO_2-N) content varied from 2.12 µg.at N/l (Farm-C) to 7.23 µg.at N/l (Farm-D) showing considerably higher level during monsoon season (6.88±0.51 µg.at N/l) in Farm-D than during non-monsoon season (2.61 ± 0.82 to 3.02 ± 0.77 µg.at N/l)in the other three farms. Nitrate (NO_3-N) level also showed more or less similar pattern of distribution farm-wise as well as season-wise, the monthly values varying from 4.03 to 8.79 µg.at N/l in Farms-A, B & C and 15.01 to 16.14 µg.at N/l in Farm-D. The mean values ranged between 5.22 µg.at N/l in Farm-B and 15.79 µg.at N/l in Farm-D. The concentration of total phosphorus in water (3.87 - 18.70 µg.at P/l) was maximum during monsoon season with a mean value of 18.09 µg.at P/l recorded in Farm-D and

minimum during non-monsoon season (6.65 µg.at P/l) recorded in Farm-B. Hydrogen sulphide was noted in detectable level (0.02 – 0.04 ppm) in Farms-A, B, & C.

The environmental characteristics and productivity of traditional shrimp culture systems around Cochin backwaters have been studied by several workers (Gopinathan *et al.*, 1982; Sankaranarayanan *et al.*, 1982; Joshi, 1990; Vasudevappa, 1992; Sheeba and Menon, 1993; Panigrahi and Misra, 1995) and it has been generally observed that the physico-chemical conditions of the farm waters exhibit considerable variations over different periods of the year as well as in different regions of the backwater system. As the shrimp farms are mostly extensions of backwater and connected estuarine areas (Sankaranarayanan *et al.*, 1982) the variations in the farms may be a reflection of what is happening in the backwater environment. Cyclic natural events such as semidiurnal incursions of tidal waters through two permanent bar mouths, monsoon rains and consequent land run-off and the large-scale freshwater discharges and organic and inorganic inputs from three major rivers (Periyar, Pampa and Muvattupuzha rivers) make the whole backwater system highly dynamic (Qasim and Gopinathan, 1969; Qasim *et al.*, 1969; Sankaranarayanan and Q asim, 1969; Gopinathan *et al.*, 1982; Sankaranarayanan *et al.*, 1982).

The present data on physico-chemical charecteristics of the four traditional shrimp farms (Tables 1 & 2) are within the ranges observed by other workers for same type of

farms in Cochin and neighbouring areas. Farms-A and B depicted more or less identical features and characterized a marine or nearly marine pond condition throughout the culture period. The salinity of these farms remained at the maximum level (24.5 – 34.0 ppt) due to their proximity with the sea and the bar opening at Azhikode which would have facilitated constant entry of sea water into the farms through tidal flow. In Farms-C and D the salinity was of medium (15.93 - 17.23 ppt) and nearly freshwater (0.01 – 2.03 ppt) levels respectively showing relatively narrow fluctuation during the culture period. During non-monsoon period (December – May) Gopinathan *et al.* (1982) observed salinity above 15 ppt at station 29 near Farm-C in the northern region of Vembanad lake. Normal shrimp farming and production levels under very low salinity conditions of traditional culture systems have been reported by George (1974) and Vasudevappa (1992) although the ideal salinity range recommended for the culture of *P.indicus* is 10 – 35 ppt (Muthu, 1980).

The values of water temperature indicated relatively warmer condition (28 –32.5 °C) in Farms-A, B and C during the non-monsoon period. In Farm-D, however, the temperature was slightly lower (28 – 30 °C) and remained almost stable during major part of the monsoon period (June to August). Though the temperature of the farm waters in the area has been reported to vary widely between 26 °C and 36 °C (Sheeba and Menon, 1993), a range of 26 – 33 °C was the condition generally noticed by most of the earlier workers (Gopinathan *et al.*, 1982; Joshi, 1990; Vasudevappa, 1992). Relatively low values of temperature during monsoon period were also observed by Sankaranarayanan *et*

al., (1982), Joshi, (1990) and Vasudevappa, (1992). Wide seasonal fluctuations in salinity and temperature were observed in Cochin Backwaters by Sankaranarayanan and Qasim, (1969) who reported maximum values for these parameters during January to April / May and the lowest during June to August.

Data on hydrogen ion concentration revealed that in all the farms water had an alkaline character throughout the period of observation. During non-monsoon period pH values remained uniformly high (7.9-9.2) in all the three farms monitored, whereas during the monsoon period Farm-D showed conspicuously lower values (7.0-7.7). Sankaranarayanan *et al.* (1982) noticed that pH values in the traditional shrimp farms studied by them were higher during the premonsoon season when the salinity values were also high. Low pH values, according to them, were confined to the SW monsoon period when the system was dominated with freshwater. All the shrimp farms appeared to be well oxygenated. The dissolved oxygen content of water (4.5-8.46 mg/l) showed minimum variation in Farm-C (0.57 mg/l) and maximum in Farm-B (3.67 mg/l). In Farm-D relatively low level of oxygen content prevailed for most part of the monsoon period. A wider range of dissolved oxygen content varying between 2 and 10.29 mg/l was reported by other workers (Gopinathan *et al.*, 1982; Sankaranarayanan *et al.*, 1982; Sheeba and Menon, 1993). In Ramanthuruth farms, Sankaranarayanan *et al.*, (1982) noticed high oxygen values during the premonsoon months and low values (< 4 ml/l) during the SW monsoon period. Gopinathan *et al.*, (1982) observed that the traditional prawn culture fields of the northern region of Cochin backwaters had high or optimal

levels of dissolved oxygen (4-6 mg/l) as compared to those of the southern region (2-3 mg/l).

A perusal of the nutrient profile of shrimp farms studied during the present investigation would reveal that concentrations of all the four chemical parameters (ammonia, nitrite, nitrate and total phosphorus) are distinctly different in farms operating in different seasons of the year. During the non-monsoon season (January-March) the mean values of ammonia, nitrite, nitrate and total phosphorus content of water ranged from 14.54 to 31.18, 2.61 to 3.02, 5.22 to 8.25 and 6.65 to 7.06 µg-at /l respectively in Farms-A, B and C. During monsoon season (June – September) in Farm-D, on the other hand, the corresponding mean values of the nitrogenous compounds and phosphorus were 38.57, 6.88, 15.79 and 18.09 µg-at/l whereby showing a much higher nutrient concentration in the culture environment. It is all the more significant to note that concentration of all the individual parameters showed successive increase from beginning to end of the season except for a marginal decrease of nitrite in September. This however, was not the case during the non-monsoon season when the values of none of the nutrients showed any definite trend in their monthly distribution. Nutrient concentrations as high as 164.93 µg-at /l of ammonia, 7 µg-at /l of nitrite, 74 µg-at /l of nitrate and 66 µg-at /l of total phosphorus have been reported from traditional shrimp farms of Cochin by earlier workers (Sheeba and Menon, 1993; Panigrahi and Misra, 1995). Monsoon months June to August was also found to be the peak period for nutrient concentration in shrimp farms by Sankaranarayanan *et al.*, (1982) and Joshi (1990).

Accumulation of inorganic nutrients in shrimp aquaculture systems can take place in several ways such as entry through source water, land run-off and anthropogenic activities like excess feeding and feed waste, fertilizers, application of chemicals etc., undigested feed residues, decomposition of organic matter by shrimps and other living communities in the system (Pillay, 1992; Bergheim and Asgard, 1996; Boyd, 1999). As the traditional shrimp culture practices in Kerala do not involve application of fertilizers to stimulate production of natural food organisms or addition of any manufactured supplementary feed in the ponds the possible way of nutrient accumulation in Farms - A to D could be mainly, if not wholly, from the respective source waters of marine, estuarine or freshwater origin. Sankaranarayanan and Qasim (1969) have observed that the nutrient distribution in Cochin backwater is dependent upon two main components – the marine influence and the freshwater discharge. During the period when the system remains predominantly marine, the nutrient concentrations are low and remain homogenous throughout the water column, but during the period of freshwater discharge, high concentrations of nutrients occur, with gradient zones within the system. They also noted that large quantities of organic matter are brought into the estuary by the land runoff. These are probably decomposed at the bottom and, as a consequence, greatly influence on the nutrient distribution.

4.1.2 Semi-intensive farms

4.1.2.1. Evaluation of shrimp feed used

A total of 8 samples of Indian shrimp feed ('Higashi' 7 samples and 'Lux' 1 sample) and 7 samples of imported feed ('CP' 6 samples and 'Grobest' 1 sample) which were in use in the semi-intensive shrimp farms of the study area have been analyzed for proximate composition and the results are presented in Table - 3. Higashi-3000, which was the only feed used in the semi-intensive farms selected for the present study, showed a proximate composition of 36.5 – 40.6% protein, 7.3 - 7.7% fat, 8.5 – 9.9% moisture, 1.5 – 2.0% fibre, 12.3 – 14.1% ash and 29.6 – 30.0% nitrogen free extract. The protein content was maximum (40.6%) in starter feed and it showed a successive reduction in grower and finisher feeds. A comparison of the biochemical composition of the various feed samples analyzed would show that in Higashi-3000 the protein content was lower by 3.6 to 7.5% and nitrogen free extract (NFE) was higher by 4.4 to 4.8% than in Higashi–6000 and Lux which were also occasionally used in the large semi-intensive farms of the area. Similar differences could also be noticed in protein and nitrogen free extract levels between Higashi–3000 and the imported feeds used in shrimp farms of Cochin and neighbouring areas.

As was the usual practice, supplementary feeding in the shrimp farms began about 10–15 days after seed stocking. Based on feeding rates and surviving shrimp mass at different stages of farming, the total feed input during the entire culture period has been estimated to be 3570 kg in Farm-A1 (3570 kg/ha), 3210 kg in Farm-B1 (3560 kg/ha), 1140 kg in Farm-C1 (2850 kg/ha) and 5840 kg in Farm-D1 (2640 kg/ha). The food conversion ratios (FCR) for the nutritional supplement estimated by dividing the total

weight of feed added to the pond by the total shrimp biomass harvested (Parker, 1987) ranged from 1.5:1 in Farm-C1 to 2: 1 in Farm-D1.

Table -3: Proximate composition of shrimp feed used in semi-intensive farms and other commercial feeds

Name, grade & type of feed	Percentage of biochemical constituents					
	Protein	Fat	NFE	Fibre	Moisture	Ash
"HIGASHI" (Indian)						
Gr. 1000	30.5	6.2	36.6	2.8	9.4	14.5
Gr.3000 Starter	40.6	7.5	29.6	1.5	8.5	12.3
Gr.3000 Grower	37.9	7.3	30.0	1.8	9.4	13.6
Gr.3000 Finisher	36.5	7.7	29.8	2.0	9.9	14.1
Gr.6000 Starter	44.2	7.8	25.2	1.6	8.7	12.5
Gr.6000 Grower	43.6	7.6	25.2	1.8	9.1	12.6
Gr.6000 Finisher	42.4	7.3	25.0	1.7	10.1	13.5
"LUX"(Indian)	45.8	7.9	22.2	1.2	9.3	13.6
"CP" (Imported)						
Gr. S 9001	40.5	9.2	18.9	1.2	6.8	12.3
Gr. S 9002	45.2	9.8	23.1	1.3	7.1	13.5
Gr. S 9003	50.2	9.6	17.4	2.2	7.5	13.1
Gr. S 9004	48.8	10.	18.2	1.5	7.6	13.8
Gr. S 9005	47.2	10.	18.4	1.7	8.3	13.9
Gr. S 9006	49.5	9.0	18.3	1.9	8.2	13.1
"GROBEST" (Imported)	42.2	7.8	26.1	1.9	9.5	12.5

NFE = Nitrogen free extract

4.1.2.2 Evaluation of water quality parameters

The temporal values of environmental parameters recorded for the different culture systems are shown in Table-4 and their mean values in Table-5. Salinity registered mean values of 31.90 ppt in Farm-A1, 31.57 ppt in Farm-B1, 13.68 ppt in Farm-C1 and 0.53 ppt in Farm-D1. During the course of culture operation, the salinity

varied by 5.7 - 6.0 ppt in Farms-A1 & B1, 5.6 ppt in Farm-C1 and 1.2 ppt in Farm-D1, showing relatively narrower fluctuation in Farms-A1, B1 & D1 than in the corresponding traditional farms. Water temperature varied from 27.5 to 32.5°C, showing mean values of 31.57°C in Farm-A1, 31.87°C in Farm- B1 and 31.73°C in Farm-C1 during non-monsoon season and 29°C in Farm-D1 during monsoon season. The pH varied from 7.4 to 8.8 showing slightly higher levels in Farms-A1 and B1. Dissolved oxygen content fluctuated between 2.57 and 7.01 mg/l, with mean values of 4.62 mg/l in Farm-A1, 5.09 mg/l in Farm-B1, 4.29 mg/l in Farm-C1 and 4.14 mg/l in Farm-D1. The turbidity values of pond

Table 4 : Temporal distri bution of environmental parameters in semi-intensive shrimp farms

Sequence of obsevations	Sal (ppt)	Temp (°C)	pH	DO (mg/l)	Ammonia (μg.atNH_3-N/l)	Nitrite (μg.atNO_2-N/l)	Nitrate (μg.atNO_3-N/l)	Phos (μg.at P/l)	H_2S ppm
Farm A1									
Obser. I	30.12	30.2	8.3	5.13	46.42	1.64	2.03	6.45	0.02
Obser. II	34.2	32.5	7.7	2.57	35.71	3.71	32.28	11.3	n.d
Obser. III	28.2	32.0	8.7	6.17	12.87	3.41	16.14	6.77	n.d
Farm B1									
Obser. I	30.0	32.1	8.2	5.42	51.42	1.73	2.01	6.87	0.02
Obser. II	35.2	31.0	7.5	2.83	37.85	3.87	35.23	11.67	n.d
Obser. III	29.5	32.5	8.8	7.01	15.00	3.71	16.64	7.01	n.d
Farm C1									
Obser. I	15.0	31.3	7.8	3.8	12.85	2.62	5.63	6.82	n.d
Obser. II	15.8	31.8	7.9	4.81	20.00	1.85	8.25	7.05	0.01
Obser. III	10.3	32.1	7.5	4.25	15.71	3.21	6.03	5.62	n.d
Farm D1									
Obser. I	0.1	29.0	7.4	3.82	20.35	10.71	16.14	8.06	n.d
Obser. II	0.1	28.8	8.3	5.93	14.28	3.57	2.06	6.13	n.d
Obser. III	1.2	27.5	7.6	3.01	15.26	3.71	4.03	18.7	n.d
Obser. IV	0.8	30.7	7.9	2.83	10.85	3.28	8.07	8.71	n.d

n.d = Not detected

water varied between 30 and 48 cm, showing no definite trend over the culture period. Concentration of ammonia, which dominated among the three nitrogenous nutrients, showed maximum values in Farms-A1 & B1 (Mean 31.67 & 34.76 μg.at N/l) followed

by Farm-C1 (16.19 µg.at N/l) and Farm-D1 (15.19 µg.at N/l). Nitrite level was the least in all the farms and its maximum value (10.71 µg.at N/l) was recorded in the initial phase of culture in Farm-D1 during monsoon season. Nitrate content in water varied widely (2.01 to 35.23 µg.at N/l), showing mean values of 16.82 µg.at N/l in Farm-A1, 17.96 µg.at N/l in Farm-B1, 6.64 µg.at N/l in Farm-C1 and 7.58 µg.at N/l in Farm-D1. Maximum concentration of the variable was observed after about the middle of the culture period in Farms-A1, B1 & C1. In Farm-D1, however, the nitrate level was highest (16.14 µg.at N/l) in the initial phase of culture. The level of total phosphorus was maximum in Farm-D1 (10.40 ± 5.64 µg.at P/l) during monsoon season. Hydrogen sulphide was recorded at detectable level (0.01 to 0.02 ppm) on one occasion each in Farm-A1, B1 & C1 during non-monsoon season.

In semi-intensive farms salinity was almost identical and more stable in Farms-A1 and B1 than in the neighbouring traditional farms. This may probably be due to the controlled water exchange with relatively stable and less-mixed source water. In Farms-C1 and D1, the mean values of salinity were lower than in Farms-C and D by about 3 ppt and 0.5ppt respectively. All the four farms showed slightly warmer pond conditions than traditional farms as evident from relatively higher mean values of water temperature (29.0 °C to 31.87 °C) during the growout period. Temperatures between 25 °C and 32 °C are considered as ideal for shrimp growth in tropical aquaculture systems (Boyd and Pillai, 1985). In general, chemical and biological processes in aquatic environment are faster when water temperature is relatively high (Pillay, 1992). The pH values (7.4 to

8.8) were invariably within the range considered to be the most favourable for shrimp production (Boyd, 1981). According to Boyd (1995) brackishwaters are well buffered against pH change and pH seldom falls below 6.5 or rise above 9.0. The pH also fluctuated closely with dissolved oxygen content of the water (Table-4). Dissolved oxygen concentration in all the semi-intensive farms remained lower than in the traditional farms (Tables-1and 2).

Table 5 : Mean values of environmental parameters in semi-intensive shrimp farms

Parameters	Non-monsoon season (January-March 1997)			Monsoon season (June-Sept'97)
	Farm A	Farm B	Farm C	Farm D
Salinity (ppt)	31.9 ±2.67	31.57 ±3.15	13.68 ±3.00	0.53 ±0.59
Temprature (°C)	31.57 ±1.20	31.87 ±0.78	31.73 ±0.40	29.00 ±1.31
pH	8.23 ±0.50	8.17 ±0.65	7.73 ±0.21	7.80 ±0.39
Dissolved oxygen (mg/l)	4.62 ±1.85	5.09 ±2.11	4.29 ±0.51	4.14 ±1.08
Ammonia (µg.at NH_3- N / l)	31.67 ±17.14	34.76 ±18.41	16.19 ±3.60	15.19 ±3.93
Nitrite (µg.at NO_2- N / l)	2.92 ±1.12	3.11 ±1.19	2.56 ±0.68	5.32 ±3.60
Nitrate (µg.at NO_3- N / l)	16.82 ±15.14	17.96 ±16.65	6.64 ±1.41	7.58 ±6.23
T.Phosphorus (µg.at P / l)	8.17 ±2.71	8.52 ±2.73	6.50 ±0.77	10.40 ±5.64
H_2S (ppm)	0.007	0.007	0.003	n.d

n.d -Not detected

As the concentration of dissolved oxygen varies with barometric pressure, temperature and salinity (Boyd, 1989), an attempt is made to estimate the percent

saturation of oxygen in water (Colt, 1984) at prevailing temperature and salinity conditions for all the individual observations in the two types of farms (n = 13 in each type) for sake of comparison. It has been found that in the traditional farms the water was fully saturated or supersaturated with dissolved oxygen (100.1 to 136.7 %) in six of the observations, while in the rest, the dissolved oxygen levels in water were as high as 64 % to nearly 100 % saturation. In semi-intensive farms, on the contrary, the water was supersaturated with dissolved oxygen (116.3 %) in only one instance in Farm-B1, while in all other observations the oxygen saturation levels were below 100 %. In the latter cases, it was further noticed that the dissolved oxygen levels in water remained below 50 % saturation in as many as five observations at different stages of culture. All these suggest that a greater amount of dissolved oxygen available in the farm water was consumed disproportionate to the amount of oxygen that might have been produced within the system. Lowering of dissolved oxygen content in water during growout period has been observed by Joseph *et al.* (1999) in semi-intensive shrimp farms and Kumaresan *et al.* (1997) and Oswin and Rahman (1997) in shrimp farm effluents on the east coast of India. It is well known that dissolved oxygen plays a very important role on the healthy survival of organisms in aquaculture systems, and that its production and reduction are influenced by the physical, chemical and biological processes of the environment. While photosynthesis by phytoplankton and diffusion from atmosphere are the two important sources of dissolved oxygen in culture systems, its major losses are caused by respiration of organisms living in the water as well as at the bottom of the pond, microbial oxidation of organic and inorganic substances and diffusion into the air (Boyd, 1989; Pillay, 1992;

Boyd and Clay, 1998; Boyd and Tucker, 1998). A perceptive decrease in dissolved oxygen content in shrimp culture environment due to increase in organic matter caused by excess feeding and its oxidation by bacteria and an increase in metabolic wastes was reported by Boyd (1990). Moriarty (1987) is of the view that bacteria may account for the bulk of oxygen uptake in shrimp culture ponds were detrital food chains operate. Their respiration, simply by lowering oxygen concentration in the water, may be the most important factor that limits the production of prawns on detrital food chains.

The distribution of temporal and mean values of inorganic nutrients in semi-intensive farms depicts trends apparently characterizing allochthonous systems in which feed-derived detrital food chain operates involving microbial degradation of organic wastes and nutrient regeneration in farm environment (Fry, 1987; Moriarty, 1987). Nitrogen and phosphorus are the two important chemical elements contributing to the nutrient load in aquaculture systems. Among the nitrogenous nutrients monitored, ammonia (NH_3 - N) formed the principal constituent in water followed by nitrate and nitrite in the order of concentration (Tables 4 & 5). Production of ammonia nitrogen and phosphorus in semi-intensive farms can take place from feed as well as from fertilizers, which are routinely incorporated in ponds during farming. From feeds added to this environment considerable amounts of organic wastes in the form of uneaten feed (feed waste) and undigested feed residues (fecal waste) accumulate on the pond bottom. These feed-derived wastes and other organic substances like dead phytoplankton formed in the

culture system are decomposed by microorganisms and converted to ammonia, phosphate and carbondioxide (Pillay, 1992; Bergheim and Asgard, 1996; Boyd, 1999). Large amounts of ammonia also reaches the culture system by deamination and transamination of catabolic products of organic nitrogen ingested and assimilated by the culture animals (Armstrong, 1979). In crustaceans including shrimp, ammonia constitutes more than half the nitrogenous wastes excreted from metabolic breakdown of absorbed food (Kinne, 1976; Regnault, 1987). Diffusion of un-ionized ammonia is the principal route of excretion as blood levels are normally much higher than ambient water concentrations (Kinne, 1976; Chen and Kou, 1993). Ammonia is also produced from nitrate by nitrate dissimilation, which is probably important only in strongly anaerobic habitats (Fry, 1987). In an organically rich coastal sediment 52 % of the nitrate reduced was converted to ammonia by nitrate dissimilation (Herbert, 1982). In aerobic habitats nitrification converts ammonia to nitrate through nitrite using oxygen by bacteria (Boyd, 1982; Fry, 1987; Pillay, 1990; Russo and Thurston, 1991), and this process can account for 15 – 20 % of oxygen removal from the hypolimnion (Hall and Jeffries, 1984). In the present study, ammonia concentration in semi-intensive farms was found to be higher (16.19 ± 3.60 to 34.76 ± 18.41 µg.at/l) than in traditional farms (14.54 ± 0.40 to 31.18 ± 15.16 µg.at/l) especially during the non-monsoon period (Tables 2 & 5). This may be due to the application of nitrogen fertilizers, ammonification of unutilized feed, fecal wastes etc., and through the deamination of shrimps' excretion in the restricted water exchange systems. While the nitrite values did not vary drastically between the two types of farms, levels of nitrate content in water were much higher in Farms-A1 and B1 (16.82 ± 15.14 to

17.96 ± 16.65 µg. at/l) than in Farms-A and B (5.22 ± 1.60 to 6.66 ± 2.27 µg. at/l). A perusal of the temporal values of the three types of nutrients (ammonia, nitrite and nitrate) in Table 2 would show that their increase and decrease in water column occurred in sequential manner which is not the case in the traditional systems. In most cases ammonia occurred in peak concentrations in the initial phase of farming (Obser. I) and subsequently (Obser. II – IV) its concentration declined along with an increase in nitrite and nitrate levels in the pond water. From this it can be inferred that nitrification was actively operative in the semi-intensive farms. It is also noteworthy that a lowering of ammonia content and increase in nitrite and nitrate contents in pond water was generally accompanied by a reduction of dissolved oxygen in water. The possible increase in organic matter from uneaten feed, fecal wastes and phytoplankton (as evident from lower values of water transparency) in the semi-intensive shrimp farms, and its decomposition and successive oxidation processes mediated by bacteria may account for this oxygen depletion. The unusually high value of nitrite content (10.71 µg.at/l) recorded in the initial phase of shrimp culture during the monsoon season (Farm-D1) might have been due to the combined effect of oxidation of ammonia and the use of nutrient-enriched source water. The present observations are in conformity with the finding of Gopalakrishnan *et al.* (1997) and Padmavathi *et al.* (1997) that ammonia levels in more or lass similar shrimp farming conditions at Tuticorin attained peak at the end of the first month and nitrite at the end of second month of the culture period extending about 120-140 days. Rajendran *et al.* (1972) reported that oxidation of organic matter to ammonia took 2-3 weeks and nitrification proceeded upto 120 days. These authors further noted

that nitrate content increased progressively from beginning to the end of culture with the result peak concentration of nitrate was recorded at the final stage of farming. Of the three different types of inorganic nitrogen analyzed, Gopalakrishnan *et al.* (1997) and Padmavathi *et al.* (1997) observed nitrate to be the dominant one followed by ammonia and nitrite in the order of their concentration in the semi-intensive *P.indicus* farms. During the present investigation, however, the amount of nitrate produced exhibited a declining trend after about the middle of the culture period, although in some cases (Farms-A1 and B1) its concentration was marginally higher than that of ammonia. Chattopadhyay and Mandal, as quoted by Padmavathi *et al.*, (1997), observed that the amount of nitrate was lower than the amount of ammonia in brackishwater environments and according to them (C & M) it was due to the prevailing anaerobic condition.

Like nitrogen, phosphorus is considered as an essential plant nutrient which decides the biological productivity and shrimp yield in aquaculture systems (Boyd, 1989). Generally in brackishwater farms, however, phosphorus is found in much lesser concentrations than nitrogen (Beveridge *et al.*, 1991; Phillips *et al.*, 1993). Although addition of phosphorus in shrimp farms takes place from various sources such as fertilizers, inlet water, runoff, rain, shrimps' excretory products, feed wastes and fecal matter, the major input is from fecal deposits (Boyd, 1989: Bergheim and Asgard, 1996). Phosphorus, in oxidized (particulate) form, is strongly adsorbed and retained by the pond sediments (Pillay, 1992; Boyd, 1999). The mean concentration of total phosphorus recorded during the present investigation was higher in Farms-A1 and B1 than in the

corresponding traditional farms (Farms-A and B). In all the semi-intensive farms this nutrient showed a single peak in the second or third month of farming. Its temporal values (5.62-18.70µg.at/l) were comparable to those reported by Padmavathi *et al.* (1997) for similar farming operations. The level and frequency of hydrogen sulphide detected in semi-intensive farms were interestingly lower than in the traditional farms.

4.2 Bacteriological profile of shrimp feed used

Microbial contamination of aquafeeds in any measurable levels is considered undesirable as it can affect the quality and storage life of the feed or play a role in the contamination of the farmed animal and its environment (Trust, 1971; Reilly and Twiddy, 1992). In order to assess the bacteriological condition of the commercial feed applied in the shrimp farms, a total of eight samples of pelleted feed (Higashi-3000) collected randomly from Farms-A1 to D1 (2 each) were analyzed quantitatively and qualitatively.

The mean TPC of feed ranged from 1.1×10^3 to 3.1×10^5/g the maximum bacterial load being noticed in the feeds applied in Farms-A1 and B1. Qualitative study of the flora from 164 isolates indicated dominance of gram positive types (46-71 %). The microbial population consisted of *Bacillus* (25-43%), Enterobactereaceae (24-38%), Micrococcaceae (6-38%), *Alcaligenes* (5-17%) and *Aeromonas* (0.4-15%) among the major representatives followed by *Moraxella, Acinetobacter, Pseudomonas, Lactobacillus* and *Arthrobacter* in lesser percentage. The flora isolated were

comparatively richer in diversity in feed used in Farms-A1 and B1 than in the other two farms. *Bacillus*, Enterobactereaceae and *Arthrobacter* were present in all the feed samples tested. No pathogenic or indicator organisms were encountered in any of the samples tested.

Fish diets often contain the nutrients required for the growth of a wide range of bacterial species (Trust, 1971). Bacterial decomposition of material generally becomes evident in foods containing 10^6–10^8 organisms/g (Thatcher and Clark, 1968). Significant numbers of bacteria have been reported in dry fish feeds (10^3 – 10^7 / g) by Trust (1971) and in shrimp feeds (10^4 – 10^6 CFU/g) by Lalitha (1998). The bacterial load observed in shrimp feed during the present study was comparatively less than those recorded by the above workers. However, the predominance of Gram positive organisms, particularly *Bacillus*, in the shrimp diet is in agreement with the obsevations of Lalitha (1998).

4.3 Quantitative distribution of bacterial flora in shrimp culture systems

4.3.1. Traditional farms

A total of 52 samples, 13 each from water, sediment, whole shrimp and shrimp surface, collected periodically from the four traditional culture systems and four samples of shrimp gut collected form Farm-D were analysed for bacteriological parameters. The quantitative abundance of bacterial flora in the farm environment and shrimp body is

shown in Table-6. Fig. 2 depicts the trends in temporal distribution of the microflora in different habitats. The total plate counts (TPC) varied between 5.1×10^{2} and 6.2×10^{3} CFU/ml for water, 1.2×10^{3} and 5.4×10^{4} CFU/g for sediment, 2.5×10^{5} and 7.1×10^{6} CFU/g for whole shrimp and 2.2×10^{5} and 2.6×10^{6} CFU /cm^2 for shrimp surface. The mean bacterial counts (TPC) ranged from 1.4×10^{3}/ml (Farm-D) to 4.1×10^{3}/ml (Farm-A) in water, 6.8×10^{3}/g (Farm-C) to 3.2×10^{4}/ml (Farm-A) in sediment, 4.9×10^{5}/g (Farm-C) to 8.6×10^{6} (Farm-D) in whole shrimp and 3.4×10^{5}/cm^2 (Farm-C) to 3.4×10^{6}/cm^2 (Farm-D) in shrimp surface. The mean TPC of the gut samples collected from Farm-D ranged from 4.5×10^{6} to 4.9×10^{7} CFU/g with a mean value of 2.7×10^{7}/g. The bacterial counts of all types of samples showed less pronounced temporal variations in Farm-C and Farm-D than in the other two farms (Fig. 2).

Statistical analysis of data indicated no significant correlation between environmental parameters and microbial counts of water, whole shrimp and shrimp surface. However, there was significant negative correlation ($P< 0.05$) between the TPC of sediment and pH of water and TPC of sediment and dissolved oxygen content of water. It can be seen from the present study that the body of shrimp invariably harboured a larger population of microorganisms than in water and sediment of the traditional culture systems.

Table 6 : Total plate counts (TPC) of pond water, sediment and shrimp from traditional shrimp culture systems

Sample type	Plate counts		
	Minimum	**Maximum**	**Mean**
Farm A			
• **Water (/ml)**	2.4×10^3	6.2×10^3	4.1×10^3
• **Sediment (/g)**	2.3×10^3	5.2×10^4	3.2×10^4
• **Whole Shrimp (/g)**	6.3×10^5	7.1×10^6	2.8×10^6
• **Shrimp surface(/cm^2)**	2.3×10^5	6.0×10^5	4.5×10^5
Farm B			
• **Water(/ml)**	5.1×10^2	6.0×10^3	2.8×10^3
• **Sediment(/g)**	1.2×10^3	5.4×10^4	3.1×10^4
• **Whole Shrimp (/g)**	4.6×10^5	7.1×10^5	5.6×10^5
• **Shrimp surface(/cm^2)**	2.2×10^5	6.2×10^5	4.7×10^5
Farm C			
• **Water(/ml)**	3.5×10^3	4.0×10^3	3.8×10^3
• **Sediment(/g)**	6.2×10^3	7.5×10^3	6.8×10^3
• **Whole Shrimp (/g)**	2.5×10^5	6.3×10^5	4.9×10^5
• **Shrimp surface(/cm^2)**	2.2×10^5	6.0×10^5	3.7×10^5
Farm D			
• **Water(/ml)**	1.0×10^3	2.0×10^3	1.4×10^3
• **Sediment(/g)**	2.9×10^4	3.2×10^4	3.1×10^4
• **Whole Shrimp (/g)**	2.5×10^5	3.0×10^6	2.1×10^6
• **Shrimp surface(/cm^2)**	2.4×10^5	2.6×10^6	8.3×10^5

The bacterial counts of water samples remained more or less uniform (10^3 to 10^4/ml) in all the farms even though marked variations existed in some of the environmental parameters like salinity, temperature, pH and nutrients between monsoon (Farm-D) and nonmonsoon (Farms-A, B and C) seasons. This points to the possibility that the

environmental factors do not influence significantly to the bacterial load in water of traditional culture systems as is the case in the adjoining backwater reported by Singh (1986). Statistical analyses of relevant data also lend support to this view. While sediment samples also showed a similar pattern of microbial abundance in the different farms under study, they invariably harboured a characteristically richer population than in water, which is the normal case reported for similar farms (Ninawe and Raj, 1993; Ravi and Chandrika, 1993) and the adjoining backwater environment (Pradeep and Lakshmanaperumalsamy, 1986; Singh, 1986; Singh *et al.*, 1998). This is attributed to various factors such as gradual deposition of bacteria from the overlying water, increased propagation of the bacteria indigenous to the sediment; settlement of the particulate substrates during the process of sedimentation (Ninawe and Raj, 1993). On the body surface of shrimp (*P. monodon*), Surendran *et al.* (1995) recorded varying densities of bacterial flora ranging from $3.6x10^6$ to $4.6x10^6/cm^2$ in brackishwater culture pond while Singh *et al.* (1998) noticed slightly higher counts of $7.3x10^6/g$ in the body of juvenile prawns (*P. indicus*) obtained from Cochin backwater. The counts of shrimp surface recorded during the present study ($10^5/cm^2$) are comparable to those reported by Singh *et al.* (1998) but slightly less than those reported by Surendran *et al.* (1995). The TPC of whole shrimp samples (10^5 to $10^6/g$) and that of shrimp gut (10^6 to $10^7/g$) are also comparable to those reported by Singh *et al.* (1998). Since the whole-shrimp samples included the entire body of the animal (shell-on) the higher bacterial counts recorded could be due to contribution from the intestine and gills which harbour maximum number

of microorganisms in shrimp's body (Ivy Thomas, 1982; Singh, 1986; Rossama Philip and Perumalsamy, 1995; Singh *et al.*, 1998).

Table 7: Matrix of correlation of TPC of water, sediment and shrimp with environmental parameters of traditional shrimp culture systems

	Salinity	Temp	pH	DO_2	NH_3	NO_2	NO_3	P	H_2S	TPC-W	TPC -SD	TPC WS	TPC SS
Salinity	1.00												
Temp	0.55	1.00											
PH	0.50	0.71*	1.00										
DO_2	-0.05	0.33	0.59*	1.00									
NH_3	-0.26	0.04	-0.35	-0.44	1.00								
NO_2	-0.87*	-0.41	-0.66*	-0.24	0.58*	1.00							
NO_3	-0.77*	-0.73*	-0.76*	-0.41	0.36	0.82*	1.00						
P	-0.85*	-0.67*	-0.81*	-0.41	0.43	0.93*	0.93*	1.00					
H_2S	0.36	0.31	0.42	0.18	0.15	-0.32	-0.28	-0.46	1.00				
TPC-W	0.49	0.19	0.30	-0.17	0.02	-0.43	-0.46	-0.45	0.51	1.00			
TPC -SD	-0.17	-0.48	-0.70*	-0.57*	0.35	0.35	0.31	0.48	-0.38	0.25	1.00		
TPC WS	-0.23	-0.12	-0.25	-0.13	-0.01	0.39	0.38	0.32	0.17	-0.12	-0.14	1.00	
TPC SS	0.14	0.15	-0.05	-0.12	0.42	-0.08	-0.21	-0.13	0.18	0.34	0.41	-0.31	1.00

*correlations significant at $p < .05$

Table 8: Matrix of correlation of TPC of water, sediment and shrimp with environmental parameters of semi-intensive shrimp culture systems

	Salinity	Temp	pH	DO_2	NH_3	NO_2	NO_3	P	H_2S	TPC-W	TPC -SD	TPC WS	TPC SS
Salinity	1.00												
Temp	0.68*	1.00											
pH	0.35	0.31	1.00										
DO_2	0.25	0.23	0.80*	1.00									
NH_3	0.65*	0.20	-0.03	-0.01	1.00								
NO_2	-0.41	-0.40	-0.41	-0.19	-0.23	1.00							
NO_3	0.51	0.33	-0.22	-0.31	0.17	0.30	1.00						
P	-0.12	-0.50	-0.43	-0.56*	0.00	0.12	0.27	1.00					
H2S	0.37	0.14	0.25	0.29	0.75*	-0.45	-0.42	-0.28	1.00				
TPC-W	-0.04	0.45	-0.44	-0.20	-0.25	-0.08	0.18	-0.28	-0.24	1.00			
TPC -SD	-0.55*	-0.39	-0.89*	-0.73*	-0.19	0.41	0.18	0.33	-0.42	0.47	1.00		
TPC WS	-0.10	0.23	0.32	0.20	-0.48	-0.69*	-0.28	-0.13	-0.20	0.35	-0.09	1.00	
TPC SS	-0.05	-0.06	0.55	0.21	-0.38	-0.59*	-0.28	0.16	-0.20	-0.32	-0.38	0.73*	1.00

*correlations significant at $p < .05$

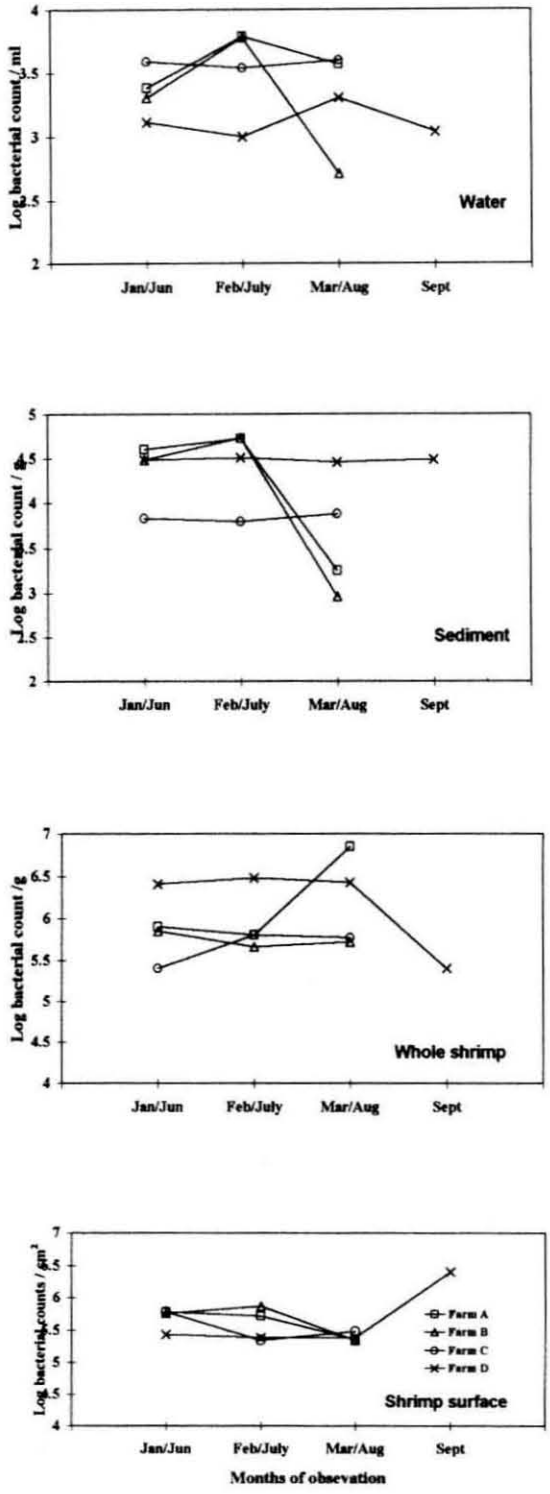

Fig. 2 Trends of temporal distribution of bacterial load in the environment and shrimp body of traditional culture systems

4.3.2 Semi-intensive farms

A total of 50 samples, 13 each from water and sediment, and 12 each from whole-shrimp and shrimp surface, collected at different stages of shrimp culture from the four selected semi-intensive farms and four samples of shrimp gut collected from Farm-D1 were analyzed for bacteriological parameters. The quantitative abundance of bacterial flora in the farm environment and shrimp body is shown in Table- 9. Fig. 3 shows the trends in the temporal distribution of bacterial counts in different habitats. The total plate counts varied between $1.1x10^3$ and $3.9x10^5$ CFU/ml for water, $1.3x10^3$ and $2x10^5$ CFU/g for sediment, $2.3x10^3$ and $2.5x10^5$ CFU/g for whole shrimp and $1x10^3$ and $5.2x10^4$ CFU/cm^2 for shrimp surface. The mean TPC ranged from $3.9x10^3$/ml (Farm-D1) to $2.3x10^5$/ml (Farm-C1) in water, $6.9x10^4$/g (Farm-A1) to $2.2x10^5$ (Farm-D1) in sediment, $8.7x10^4$/g (Farm-B1) to $1.6x10^6$/g (Farm-C1) in whole shrimp and $1.8x10^4$/cm^2 (Farm-C1) to $4.5x10^5$/cm^2 (Farm-D1) in shrimp surface. As in traditional farms, the bacterial load was invariably higher relatively more in pond sediment than in water. In the shrimp body, whole-shrimp samples (shell-on) showed greater abundance of bacterial flora than shrimp surface samples. Analysis of the gut samples of prawns collected from Farm-D1 indicated bacterial load ranging from $3.6x10^6$ to $2x10^7$ CFU/g with a mean TPC of $1.2x10^6$CFU/g. Temporal data of bacterial counts did not show any definite trend over the culture period either in the environment or in the body of the shrimp.

Statistical analysis of data (Table-8) showed that there existed significant negative correlation ($P<0.05$) between sediment TPC and water quality parameters such as salinity, pH and dissolved oxygen content. A similar relationship was also discernible between TPC of whole shrimp and nitrite and TPC of shrimp surface and nitrite. There was also statistically significant positive correlation ($P<0.05$) between TPC of whole shrimp and TPC of shrimp surface. However, no significant correlation could be seen between TPC of pond water and any of the environmental factors monitored from the semi-intensive farms.

Table 9: Total plate counts (TPC) of pond water, sediment and shrimp from semi-intensive shrimp culture systems

Sample type	Plate count		
	Minimum	**Maximum**	**Mean**
Farm A1			
• **Water(/ml)**	1.2×10^3	1.7×10^4	7.3×10^3
• **Sediment(/g)**	1.3×10^3	2.0×10^5	6.9×10^4
• **Whole Shrimp (/g)**	2.3×10^3	2.7×10^5	9.3×10^4
• **Shrimp surface(/cm^2)**	1.5×10^3	2.5×10^5	8.5×10^4
Farm B1			
• **Water(/ml)**	1.5×10^3	1.9×10^4	7.9×10^3
• **Sediment(/g)**	1.7×10^3	2.2×10^5	7.6×10^4
• **Whole Shrimp (/g)**	2.5×10^3	2.5×10^5	8.7×10^4
• **Shrimp surface(/cm^2)**	1.8×10^3	2.2×10^5	7.5×10^4
Farm C1			
• **Water(/ml)**	3.1×10^4	3.9×10^5	2.3×10^5
• **Sediment(/g)**	1.1×10^5	3.2×10^5	1.6×10^5
• **Whole Shrimp (/g)**	1.7×10^5	2.4×10^6	1.6×10^6
• **Shrimp surface(/cm^2)**	1.0×10^3	5.2×10^4	1.8×10^4
Farm D1			
• **Water(/ml)**	1.1×10^3	5.8×10^3	3.9×10^3
• **Sediment(/g)**	1.4×10^5	4.3×10^5	2.2×10^5
• **Whole Shrimp (/g)**	9.7×10^4	3.0×10^6	1.2×10^6
• **Shrimp surface(/cm^2)**	1.8×10^5	6.0×10^5	4.5×10^5

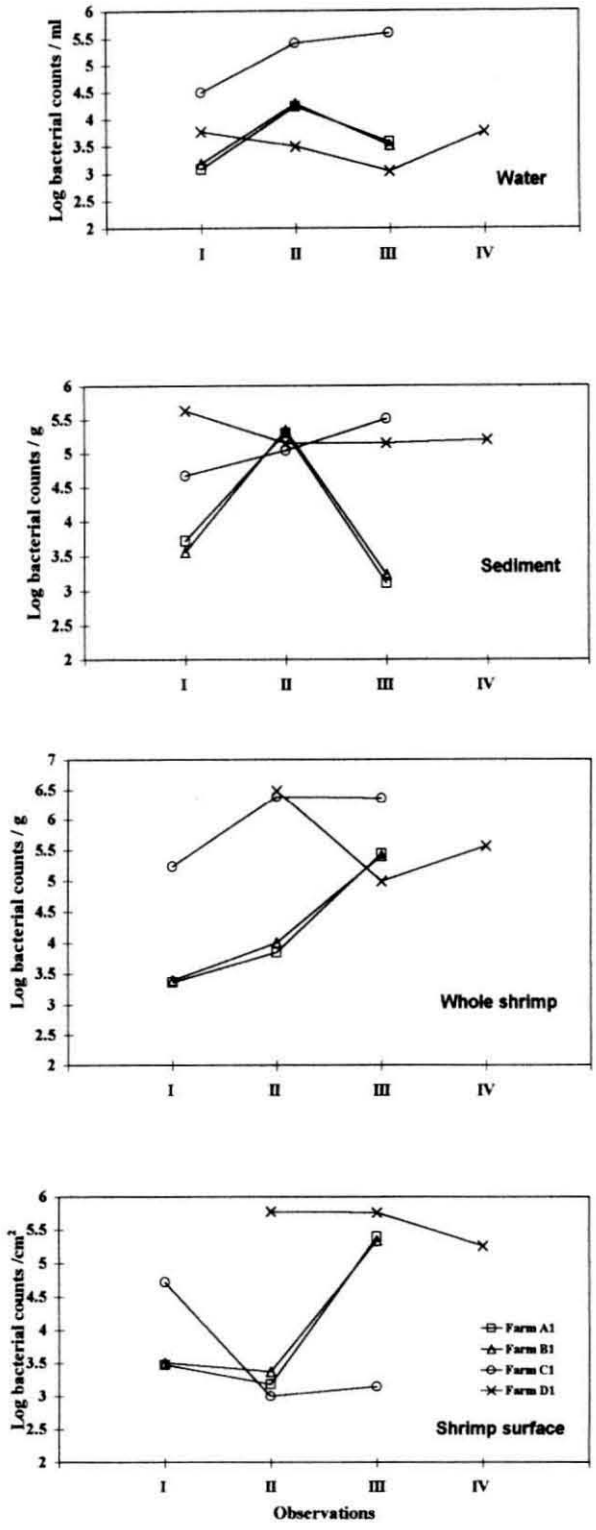

Fig. 3 Trends of temporal distribution of bacterial load in the environment and shrimp body of semi-intensive culture systems

It may be seen from the above results that the relative abundance of microorganisms associated with water, sediment and shrimp body (whole shrimp and shrimp surface) in semi-intensive culture systems was almost in the same manner as observed in the traditional farms. However, a comparison of the bacterial counts of abiotic samples (Tables-6 & 9) between the two types of culture systems would show a measurable increase in microbial load of both water and sediment in all the four semi-intensive farms studied. The bacterial counts of water recorded (10^3 to 10^5/ml) were comparable to the TPC levels reported by Surendran *et al.* (1995), Sharmila *et al.* (1996) and Lalitha (1998) from Indian coasts and Vanderzant *et al.* (1971) from Texas Gulf Coast. TPC of pond water lower than the above range has been reported by Nayyarahamed *et al.* (1995) from Karnataka and Tamilnadu coasts of India and Fonseka(1990) from the west coast of Sri Lanka. Higher levels of counts ranging 10^5 to 10^6 CFU/ml have also been recorded for semi-intensive shrimp farms by other workers like Putro *et al.* (1990) and Rattagool *et al.* (1990) from the South East Asian countries. The bacterial counts of sediment samples (10^4 to 10^5/g) were about 1-2 log units higher than those of the overlying water in all the farms except Farm-C1 in which the microbial density was nearly equal for both sediment and water. Highly varying TPC levels have been reported for pond sediments in semi-intensive shrimp culture operations from different tropical regions of the world. They included counts ranging from 10^3 to 10^6/g from Indian coasts (Nayyarahamed *et al.*, 1995; Surendran *et al.*, 1995; Sharmila *et al.* 1996; Lalitha, 1998) and 10^4 to 10^8/g from South East Asian countries (Llobrerra *et al.*,1990; Putro *et al.*, 1990). These variations in microbial load observed in shrimp

culture environment (water and sediment) from different Asian countries have been attributed to differences in the culture practices (Nayyarahamed *et al.*, 1995). The counts recorded during the present study were well comparable to those reported by Surendran *et al.* (1995, 2000) for more or less similar brackishwater farm conditions at Cochin. The significantly higher bacterial load in water and sediment of all the four semi-intensive farms studied suggests that increased nutrients and availability of substrate as a result of the accumulation of feed derived wastes and other organic and inorganic matter on pond bottom has enhanced the microbial load in the farm environment. In the traditional farms, on the contrary, only the native nutrients are present and hence it may not have promoted growth of bacteria. The increased bacterial load in water and sediment of semi-intensive farms could be an indication of environmental quality deterioration.

The mean microbial counts of whole shrimp (10^4 to 10^6/g) and shrimp surface (10^4 to 10^5/cm^2) were comparable to the counts reported by earlier workers from tropical countries including India (Fonseka, 1990; Llobrerra *et al.*, 1990; Putro *et al.*, 1990, Peranginangin *et al.*, 1992; Nayyarahamed *et al.*, 1995; Surendran *et al.*, 1995; Sharmila *et al.*, 1996; Lalitha, 1998). According to Peranginangin *et al.* (1992), the higher the bacterial load of the habitat (i.e. water and sediment) the higher the bacterial load found in the prawns. In all the four semi-intensive farms investigated, however, the bacterial load of whole shrimp samples remained higher than in water and sediment. A similar pattern of microbial abundance in shrimp and farm environment was observed by Vanderzant *et al.* (1971), Fonseka (1990) and Nayyarahamed *et al.* (1995). On

comparing between the traditional and semi-intensive farms, the bacterial load in shrimp and farm environment did not indicate any definite trend. A comparison of the bacterial counts of the shrimp body between traditional farms and semi-intensive systems would show no definite trend of variation according to culture practice except that the bacterial population habouring whole-shrimp was significantly higher in Farm-C1 than in Farm-C. This may probably be due to the most favourable ranges of environmental factors like pH (7.5-7.9), temperature (31.3-32.1°C) and salinity (10.25-15.80 ppt) of the surrounding water for bacterial growth and availability of an abundant mesophilic flora in the culture environment of the semi-intensive farms (Tables-6 & 9). It has been generally considered that a pH of around 7-8, temperature 30-32 °C and salinity of 10-30 ppt as optimum for maximum growth of a vast majority of bacterial isolates in brackish water pond ecosystems (Ivy Thomas, 1982; Singh,1986). Fonseka (1990), however, could not establish any clear relationship between the variation of bacterial load of prawns and pond water characteristics such as salinity, temperature and pH in Sri Lankan farm.

4.4 Qualitative studies of bacteria from shrimp culture systems

4.4.1 Traditional farms

The generic / group-wise distribution of bacterial flora associated with water, sediment, whole shrimp, shrimp surface and shrimp gut from the traditional shrimp farms (Tables-11, 12,13,14) was studied based on 1670 isolates. A total of 9 genera (*Vibrio, Aeromonas, Moraxella, Acinetobacter, Pseudomonas, Alcaligenes, Bacillus,*

Lactobacillus and *Arthrobacter)* and 2 groups (Enterobactereaceae and Micrococcaceae) were distinguished from this environment. The temporal variations in abundance of bacterial types are depicted in Figs. 4, 5, 6, 7. In general, gram-negative isolates dominated (61-82 %) over gram-positive forms in traditional culture systems. In water, the gram-positive form *Arthrobacter*, formerly grouped as 'coryneforms' (Surendran and Gopakumar, 1981), and gram-negative forms *Acinetobacter, Vibrio, Alcaligenes* and *Pseudomonas* constituted over 85% of the microbial population. While *Arthrobacter* was abundant in all the four farms studied in varying densities (20-38 %), *Vibrio* (18-31 %) and *Alcaligenes (26-27 %)* in Farms A & B, *Acinetobacter* (23-43 %) in Farms-C & D, *Pseudomonas* (12-16 %) also formed major constituent of the flora.

Table 10: Average composition of heterotrophic bacterial flora associated with different sample types from traditional shrimp farms

Bacterial genera / groups (Abbreviations in parenthesis)	Percentage occurrence			
	Water	Sediment	Whole shrimp	Shrimp surface
Vibrio (Vb)	15.42	10.42	21.21	39.40
Aeromonas (Ae)				
Enterobactereaceae (En)	3.49	10.37	16.47	5.42
Moraxella (Mo)	2.42	5.73		
Acinetobacter (Ac)	17.56	6.43	0.67	5.55
Pseudomonas (Ps)	10.69	9.45	21.00	14.52
Flavobacterium (Fl)				
Alcaligenes (Al)	13.24	20.65	1.67	17.52
Cytophaga (Cy)				
Micrococcaceae (Mi)	5.81	0.57	6.39	3.92
Bacillus (Ba)	0.79	23.75	5.17	0.43
Lactobacillus (Lb)		0.13	23.26	0.07
Arthrobacter (Ar)	30.71	12.44	4.23	13.16
Gram negative	62.82	63.05	61.02	82.41
Gram positive	37.31	36.94	39.05	17.58
Total No. of isolates	408	440	420	402

Among other items, Enterobactereaceae occurred in significant levels (13.95 %) in Farm-D and *Moraxella* (8.3 %) in Farm-A. In sediment, the predominant microbial forms associated were *Bacillus*, *Alcaligenes*, *Arthrobacter*, *Vibrio* and Enterobactereaceae, which together accounted 78%. While *Bacillus* (18-38 %) formed a major constituent in all the four farms, *Alcaligenes* (37-43 %) and Vbrio (14-15 %) appeared as other dominant items in Farms-A & B, *Arthrobacter* (17-33 %) in Farms- C & D and Enterobactereaceae (14-20 %) in Farms-C & D. *Pseudomonas* and *Moraxella* were encountered in considerable numbers (17-18 %) in Farm-C. The whole-shrimp samples showed predominance of *Lactobacillus*, *Vibrio*, *Pseudomonas* and Enterobactereaceae, which together constituted 82 %. Among these the occurrence was fairly high in all the farms for *Lactobacillus* (19-30 %) and *Vibrio* (14-28 %), while *Pseudomonas* (14-37 %)and Enterobactereaceae (16-25 %) were present in significant numbers in Farms-B, C & D and Farms-A, B & C respectively. Among other bacterial types the abundance of *Bacillus* (21 %) and Micrococcaceae (16 %) in Farm-D was noteworthy. The bacterial flora isolated from shrimp surface showed predominance of *Vibrio*, *Alcaligenes*, *Pseudomonas* and *Arthrobacter* which together constituted (85 %). Among these, *Vibrio* was the most abundant genus (35-65 %) in three of the farms (Farms-A, B & D). *Alcaligenes* was observed in maximum abundance in Farms-A & B (34-35 %), *Pseudomonas* in Farms-C & D (16-21 %) and *Arthrobacter* in Farm-C (38 %). Among other forms, *Acinetobacter* occurred fairly in abundance (20%) in Farm-C and Enterobactereaceae (13.2 %) in Farm-B. Qualitative study based on 62 isolates from 4

gut samples revealed a bacterial population consisting of *Vibrio* (54 %), *Aeromonas* (28 %), *Arthrobacter* (15 %) and *Bacillus* (3 %) in the order of their dominance.

The generic / groupwise analysis of bacterial isolates from the traditional shrimp culture systems have shown that 67 % of them were Gram negative and the rest Gram positive. This predominance of Gram negative forms was almost uniform at a level of 61-63 % in water, sediment and whole shrimp, while in shrimp surface slightly higher percentage (82 %) prevailed. However, examining the relative abundance of the two groups in total hetrotrophic population of individual farms (Tables-15,16,17,18) or habitats (Tables-11,12,13,14) this pattern of dominance was occasionally disturbed.

Table 11: Average composition of heterotrophic bacterial flora associated with water in different traditional shrimp farms

Bacterial genera / groups	Percentage Occurrence			
	Farm-A	Farm-B	Farm-C	Farm-D
Vibrio	31.80	18.07	6.43	5.78
Aeromonas				
Enterobactereaceae				13.95
Moraxella	8.30	1.37		
Acinetobacter	0.32	3.33	23.18	43.39
Pseudomonas	2.31	12.38	15.55	12.50
Flavobacterium				
Alcaligenes	26.20	26.75		
Cytophaga				
Micrococcaceae	30.50	0.43	15.35	4.39
Bacillus			3.17	
Lactobacillus				
Arthrobacter	28.59	37.67	36.32	20.26
Gram negative	68.51	61.90	45.16	75.62
Gram positive	31.64	38.10	54.84	24.65
Total No. of isolates	98	96	90	124

Table 12: Average composition of heterotrophic bacterial flora associated with sediment in different traditional shrimp farms

Bacterial genera / groups	Percentage Occurrence			
	Farm-A	Farm-B	Farm-C	Farm-D
Vibrio	14.63	14.36	4.47	8.21
Aeromonas				
Enterobactereaceae	0.37	7.01	20.16	13.93
Moraxella	4.13	1.83	16.94	
Acinetobacter	3.63	4.03	1.50	16.55
Pseudomonas	8.80	1.99	18.07	8.93
Flavobacterium				
Alcaligenes	42.95	37.28	2.37	
Cytophaga				
Micrococcaceae	0.83		1.15	0.28
Bacillus	24.17	38.48	18.26	19.10
Lactobacillus	0.50			
Arthrobacter			16.96	32.98
Gram negative	74.51	66.50	63.51	47.62
Gram positive	25.50	33.48	36.37	52.36
Total No. of isolates	90	100	110	140

Table 13: Average composition of heterotrophic bacterial flora associated with whole shrimp in different traditional shrimp farms

Bacterial genera / groups	Percentage occurrence			
	Farm-A	Farm-B	Farm-C	Farm-D
Vibrio	28.22	27.27	15.58	13.76
Aeromonas				
Enterobactereaceae	23.92	24.52	16.24	1.20
Moraxella				
Acinetobacter		1.71	0.70	0.25
Pseudomonas	5.40	13.87	37.69	27.04
Flavobacterium				
Alcaligenes	2.26	4.43		
Cytophaga				
Micrococcaceae	3.94	3.17	2.85	15.60
Bacillus				20.67
Lactobacillus	29.73	21.64	23.13	18.55
Arthrobacter	6.71	3.45	3.82	2.92
Gram negative	59.80	71.74	70.21	42.25
Gram positive	40.38	28.26	29.80	57.79
Total No. of isolates	96	100	96	128

Table 14: Average composition of heterotrophic bacterial flora associated with shrimp surface in different traditional shrimp farms

Bacterial genera / groups	Percentage occurrence			
	Farm-A	Farm-B	Farm-C	Farm-D
Vibrio	47.98	34.60	9.53	65.47
Aeromonas				
Enterobactereaceae	3.10	13.20		5.39
Moraxella				
Acinetobacter			19.57	2.64
Pseudomonas	11.20	10.02	20.51	16.35
Flavobacterium				
Alcaligenes	33.50	34.92	1.67	
Cytophaga				
Micrococcaceae	2.03	2.37	10.67	0.59
Bacillus	1.03	0.67		
Lactobacillus				0.29
Arthrobacter	1.05	4.23	38.07	9.28
Gram negative	95.78	92.74	51.28	89.85
Gram positive	4.11	7.27	48.74	10.86
Total No. of isolates	92	92	92	126

Table 15: Average composition of heterotrophic bacterial flora associated with Water , sediment, whole shrimp and shrimp surface in Farm-A

Bacterial genera / groups	Percentage occurrence			
	Water	Sediment	Whole shrimp	Shrimp surface
Vibrio	31.38	14.63	28.22	47.98
Aeromonas				
Enterobactereaceae		0.37	23.92	3.10
Moraxella	8.30	4.13		
Acinetobacter	0.32	3.36		
Pseudomonas	2.31	8.80	5.40	11.20
Flavobacterium				
Alcaligenes	26.20	42.95	2.26	33.50
Cytophaga				
Micrococcaceae	3.05	0.83	3.94	2.03
Bacillus		24.17		1.03
Lactobacillus		0.50	29.73	
Arthrobacter	28.59		6.89	1.05
Gram negative	68.51	74.51	59.80	95.78
Gram positive	31.60	25.50	40.38	4.11
Total No. of isolates	98	90	96	92

Table 16: Average composition of heterotrophic bacterial flora associated with water, sediment, whole shrimp and shrimp surface in Farm-B

Bacterial genera / groups	Percentage occurrence			
	Water	Sediment	Whole shrimp	Shrimp surface
Vibrio	18.07	14.36	27.27	34.60
Aeromonas				
Enterobactereaceae		7.01	24.52	13.20
Moraxella	1.37	1.83		
Acinetobacter	3.33	4.03	1.71	
Pseudomonas	12.38	1.99	13.87	10.02
Flavobacterium				
Alcaligenes	26.75	37.28	4.43	34.92
Cytophaga				
Micrococcaceae	0.43		3.17	2.37
Bacillus		33.48		0.67
Lactobacillus			21.64	
Arthrobacter	37.67		3.45	4.23
Gram negative	61.90	66.50	71.74	92.74
Gram positive	38.10	33.48	28.26	7.27
Total No. of isolates	96	100	100	92

Table 17: Average composition of heterotrophic bacterial flora associated with water, sediment, whole shrimp and shrimp surface in Farm-C

Bacterial genera / groups	Percentage occurrence			
	Water	Sediment	Whole shrimp	Shrimp surface
Vibrio	6.43	4.47	15.58	9.53
Aeromonas				
Enterobactereaceae		20.16	16.24	
Moraxella		16.94		
Acinetobacter	23.18	1.50	0.70	19.57
Pseudomonas	15.55	18.07	37.69	20.51
Flavobacterium				
Alcaligenes		2.37		1.67
Cytophaga				
Micrococcaceae	15.35	1.15	2.85	10.67
Bacillus	3.17	18.26		
Lactobacillus			23.13	
Arthrobacter	36.32	16.96	3.82	38.07
Gram negative	45.16	63.51	70.21	51.28
Gram positive	54.84	36.37	29.80	48.74
Total No. of isolates	90	110	96	92

Table 18: Average composition of heterotrophic bacterial flora associated with water, sediment, whole shrimp and shrimp surface in Farm-D

Bacterial genera / groups	Percentage occurrence			
	Water	Sediment	Whole shrimp	Shrimp surface
Vibrio	5.78	8.21	13.76	65.47
Aeromonas				
Enterobactereaceae	13.95	13.93	1.20	5.39
Moraxella				
Acinetobacter	43.39	16.55	0.25	2.64
Pseudomonas	12.50	8.93	27.04	16.35
Flavobacterium				
Alcaligenes				
Cytophaga				
Micrococcaceae	4.39	0.28	15.60	0.59
Bacillus		19.10	20.67	
Lactobacillus			18.55	0.29
Arthrobacter	20.26	32.98	2.92	9.28
Gram negative	75.62	47.62	42.25	89.85
Gram positive	24.65	52.36	57.74	10.86
Total No. of isolates	124	140	128	126

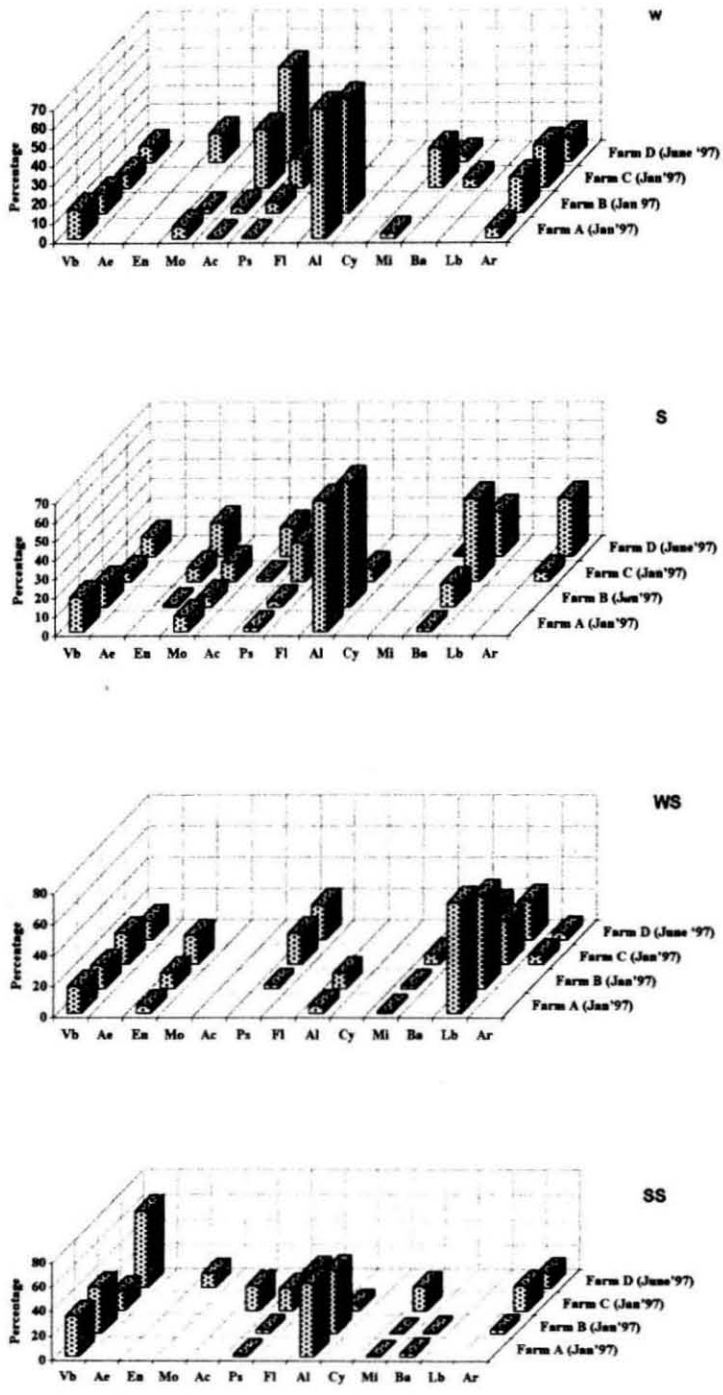

Fig. 4 Generic distribution of heterotrophic bacteria in traditional shrimp farms in January/June (Legend to bacteria given in Table 10)
W-water, S-sediment, WS-whole shrimp, SS-shrimp surface

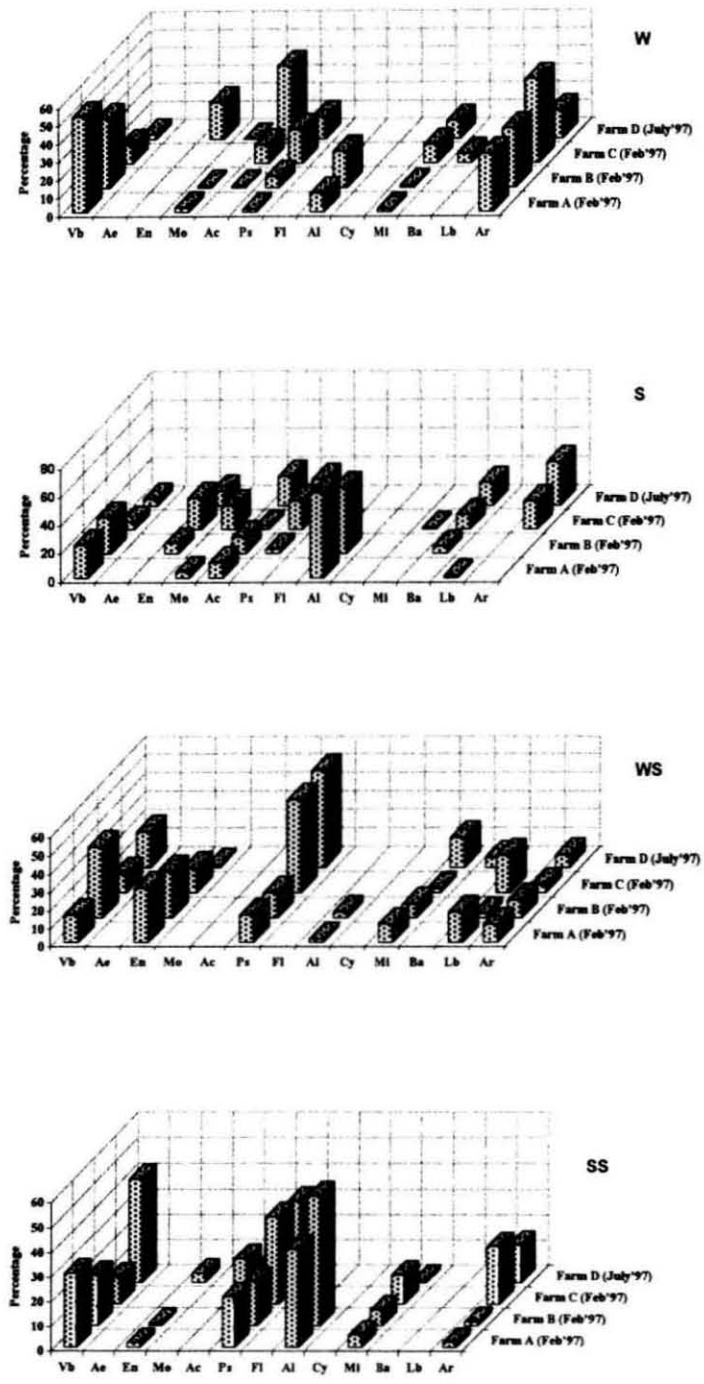

Fig. 5 Generic distribution of heterotrophic bacteria in traditional shrimp farms in February/July

W-water, S-sediment, WS-whole shrimp, SS-shrimp surface

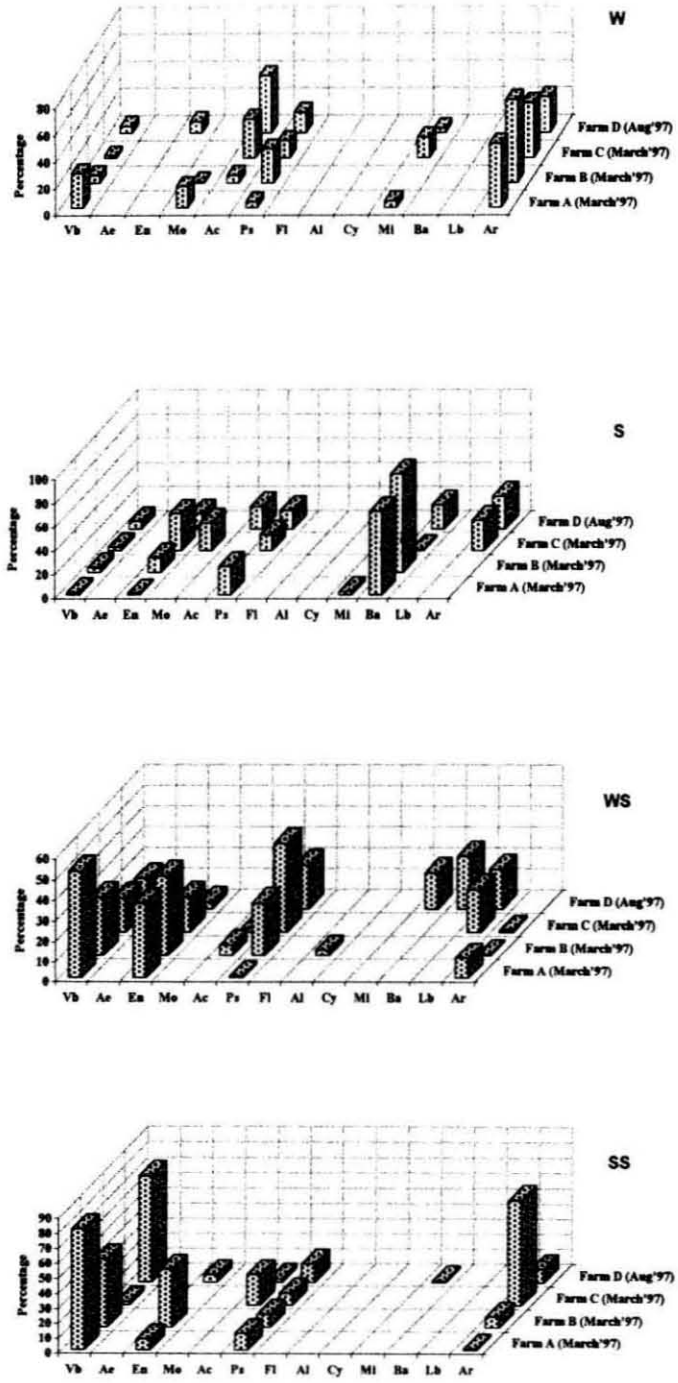

Fig. 6 Generic distribution of heterotrophic bacteria in traditional shrimp farms in March/August

W-water, S-sediment, WS-whole shrimp, SS-shrimp surface

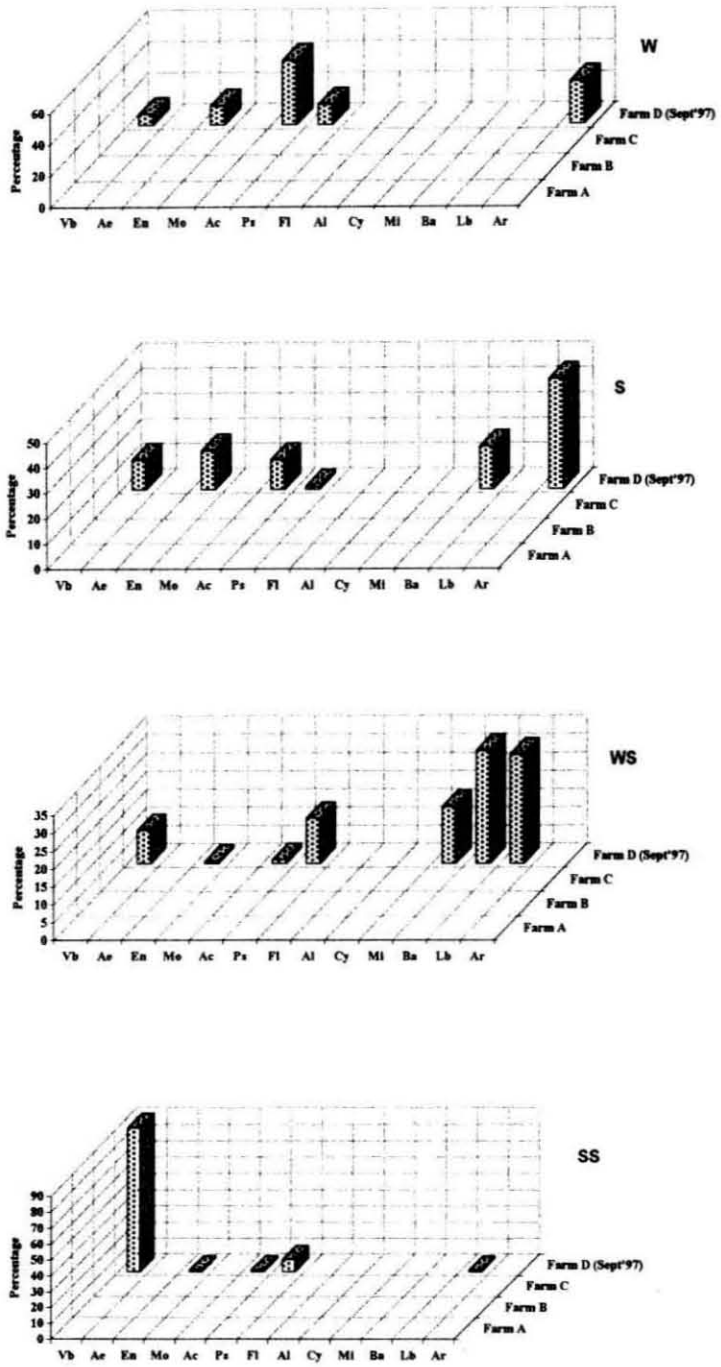

Fig. 7 Generic distribution of heterotrophic bacteria in traditional shrimp farm in September

W-water, S-sediment, WS-whole shrimp, SS-shrimp skin

As a general rule, Gram negative bacteria dominated over Gram positive organisms in the environment as well as shrimp body in Farms-A & B probably due to the fact that higher salinity of the farms might have favoured the proliferation of halophilic strains. Dominance of Gram negative bacteria in water, sediment and shrimp was also reported by Singh (1986) from Cochin backwaters, and in coastal waters off Cochin by Alavandi (1989). Singh (1986) also noticed maximum percentage of Gram negative bacteria on body surface of *P.indicus* (68.4 %) collected from Cochin Backwater.

Although a large number of bacterial genera were commonly represented in the microbial population of water, sediment and shrimp in traditional culture systems, striking variations were noticed in the major generic groups associated with the different habitats. When the flora of all the four sample types were treated together, *Vibrio* (21.6%), *Arthrobacter*(15.1%), *Pseudomonas* (13.9%), *Alcaligenes*(13.3%), Enterobactereaceae(8.9%), *Bacillus*(7.6%), *Acinetobacter*(7.6%) and *actobacillus*(5.9%) formed the important groups. In water, the microflora was dominated by *Arthrobacter*, *Acinetobacter*, *Vibrio*, *Alcaligenes* and *Pseudomonas*. When the bacterial types in high salinity (Farms-A & B) and low salinity (Farms- C & D) waters were compared, *Arthrobacter* emerged as a common item of dominance in all salinity gradients and found associated with large populations of *Alcaligenes* and *Vibrio* in high salinity farms and with *Acinetobacter*, *Pseudomonas* and Micrococcaceae in low salinity farms. Pond sediments showed dominance of *Bacillus*, *Alcaligenes*, *Arthrobacter*, *Vibrio* and *Pseudomonas* although the percentage of most of them in the total population was

relatively less than in the water column. The occurrence of Enterobactereaceae in sizeable level was also a noteworthy feature. As in water, significant changes in bacterial types could also be noticed in the sediment flora in farms of varying salinity conditions. While *Alcaligenes*, *Bacillus* and *Vibrio* constituted over 80 % of the sediment flora in Farms-A & B their total contribution in Farms-C & D remained less than 30 %. *Arthrobacter* and *Pseudomonas*, which constituted a major part of the flora of the overlying water, apparently dominated in the sediment of low salinity farms together with abundant population of *Bacillus*, Enterobactereaceae and *Moraxella*.

It would thus appear from the above generic distribution pattern of bacteria in water and sediment that during the non-monsoon months when the salinity remains high and reasonably stable the environment of traditional shrimp farm is characterized by a native flora dominated by *Arthrobacter*, *Alcaligenes*, *Pseudomonas*, *Acinetobacter*, *Vibrio* and *Bacillus*. However, in low salinity condition / monsoon months the farms harbour some what a different type of flora consisting of *Arthrobacter*, *Acinetobacter*, *Pseudomonas*, Micrococcaceae, Enterobactereaceae, *Moraxella* and *Bacillus*, majority of which are less halophilic in nature. A significantly low percentage of *Vibrio* in Farms-C & D and its increased occurrence in Farms- A&B may probably be due to the fact that when all other environmental factors like temperature, dissolved oxygen, pH and nutrients are optimum high salinity preferring *Vibrio*s would multiply and gain dominance over other micro-organisms. Seasonal changes in environmental flora of prawn filtration ponds have been reported by Surendran and Chandrika (1993), who

noticed *Alcaligenes* as the predominant heterotroph of sediment flora in association with *Flavobacterium*, *Cytophaga* and *Pseudomonas* in lesser proportions during premonsoon period, and *Alcaligenes* together with *Cytophaga*, *Flavobacterium*, *Pseudomonas*, *Vibrio* and *Micrococcus* during monsoon period. In Cochin backwater Singh (1986) observed *Vibrio*, *Pseudomonas*, *Micrococcus* and *Bacillus* as the dominant flora of water and sediment, while Alavandi (1989) recorded *Pseudomonas*, *Aeromonas*, *Moraxella*, *Flexibacter* and *Micrococcus* as the genera frequently encountered in the coastal waters of Cochin. All these observations suggest that the qualitative distribution of microflora in the marine and estuarine environments and the adjoining prawn filtration fields at Cochin is highly variable which may be due to the spatial and seasonal changes in physico-chemical conditions and productivity of the ecosystems .

Dominance of *Lactobacillus* and a larger population of *Vibrio*, Enterobactereaceae and *Pseudomonas* than in the surrounding environment appeared to be characteristic of the microflora of *P.indicus* in the traditional culture systems. While no significant relationship could be established between shrimp and its environment with regard to the distribution of major bacterial genera in Farms-A & B, the abundance in shrimp body of Enterobactereaceae and *Pseudomonas* in Farm-C and that of *Pseudomonas* and *Bacillus* in Farm-D appears to be a reflection of the sediment flora of the respective farms (Tables- 17 & 18). A similar relationship was also evident between the major generic groups of shrimp gut (*Vibrio*, *Arthrobacter* and *Bacillus*) and those of the sediment flora of Farm-D from where samples were examined. The significantly

higher levels of *Vibrio* (54 %) in shrimp gut as compared to its availability in the pond sediment may be either due to the animals' selective feeding habit (Dall, 1968) or due to the remarkable ability of the bacteria to multiply in the alimentary canal of the host animal (Singh *et al.*, 1998).

Studies on the bacteriology of wild shrimp from Cochin backwaters have shown predominance of *Vibrio* and *Micrococcus* with lesser abundance of *Pseudomonas* and *Acinetobacter* in *P.indicus* (Singh, 1986). A wide variety of microorganisms have been found to constitute the native flora of prawns caught from the inshore waters off Cochin. The most common among them were members of *Moraxella*, *Vibrio*, *Pseudomonas*, *Acinetobacter*, *Flavobacterium*, *Micrococcus*, *Bacillus* and Enterobactereaceae in *M.dobsoni* and *P.indicus* (Surendran and Gopakumar, 1981; Nirmala Thampuran and Gopakumar, 1990; Rosamma Philip and Perumalsamy, 1995).

During the present study, exceptionally large populations of *Vibrio* were found associated with the shrimp surface along with *Alcaligenes*, *Pseudomonas* and *Arthrobacter* in significant levels. In most cases availability of sizeable populations of these organisms on the surrounding water had reflected on the body surface of shrimp, which corroborate the observation of Singh (1986) in Cochin backwater.

4.4.2 Semi-intensive farms

A total of 1726 isolates were examined for the generic/groupwise distribution of microflora in semi-intensive farms (Tables-20,21,22,23). The microbial population was represented by 11 genera (*Vibrio, Aeromonas, Moraxella, Acinetobacter, Pseudomonas, Flavobacterium, Alcaligenes, Cytophaga, Bacillus, Lactobacillus* and *Arthrobacter)* and 2 groups (Enterobactereaceae and Micrococcaceae). Their temporal variations in percentage composition are shown in Figs. 8, 9, 10, 11. The water, sediment, whole shrimp and shrimp surface depicted predominance of Gram-negative organisms which constituted 59-82% of the total population (Table-19).

Table 19: Average composition of heterotrophic bacterial flora associated with different sample types from semi-intensive shrimp farms

Bacterial genera / groups (Abbreviations in parenthesis)	Percentage occurrence			
	Water	Sediment	Whole shrimp	Shrimp surface
Vibrio (Vb)	26.63	24.58	35.11	43.30
Aeromonas (Ae)	9.38	1.66	4.86	6.24
Enterobactereaceae (En)	3.23	2.02	28.61	7.74
Moraxella (Mo)	1.40	1.79	_	0.74
Acinetobacter (Ac)	0.55	0.62	8.94	1.41
Pseudomonas (Ps)	21.16	18.41	2.45	10.19
Flavobacterium (Fl)	4.63	_	1.66	6.44
Alcaligenes (Al)	1.48	9.64	_	0.85
Cytophaga (Cy)	0.93	_	0.03	_
Micrococcaceae (Mi)	10.14	5.23	7.87	8.84
Bacillus (Ba)	10.51	24.45	1.44	5.98
Lactobacillus (Lb)	0.87	4.53	5.77	2.59
Arthrobacter (Ar)	9.77	6.57	3.25	5.67
Gram negative	69.69	58.72	81.66	76.91
Gram positive	31.29	41.28	18.33	23.08
Total No. of isolates	474	444	408	400

In the environment as well as shrimp body *Vibrio* dominated showing relatively higher levels in shrimp body (35-43%) than in the environment (25-27%). In water it occurred at maximum level in Farm-C1 followed by Farms-B1, A1 and D1. Among the other bacterial isolates, which occurred in significant numbers, were *Pseudomonas*, Micrococcaceae, *Bacillus* and *Arthrobacter*. The occurrence of *Pseudomonas* was relatively high in Farms-C1 & D1 (28-50%). Maximum occurrence of *Bacillus* (20-22%), Micrococcaceae (13-15%) and *Arthrobacter* (15-20%) was noticed in Farms-A1 & B1 where the salinity was high indicating high salinity favours growth of them. In sediment, besides *Vibrio*, significant levels of *Bacillus* (24%), *Pseudomonas* (18%) and *Alcaligenes* (10%) were observed. While *Bacillus* was found maximum in Farm-C1 (54%), peak occurrence of *Pseudomonas* was noticed in Farm-D1 (50%) and that of *Alcaligenes* in Farms-A1 and B1 (18-20%). *Arthrobacter* showed maximum (12%) in Farm-A1. In whole shrimp samples *Vibrio* (35 %) was closely followed by Enterobactereaceae (29%) which occurred at maximum levels in Farm -A1 & B1 followed by Farms-D1 and C1. Among other important bacterial types recorded were *Acinetobacter* and Micrococcaceae. In shrimp surface vibrios contributed 43% on an average, maximum occurring in Farms-A1 (67%) and B1 (55%). The other important bacteria seen were *Pseudomonas*, Micrococcaceae and Enterobactereaceae. Significant levels of *Flavobacterium* were also detected in the shrimp surface of animals examined from Farms-A1 & B1 during non-monsoon months. The bacterial population of shrimp gut samples examined from Farm-D1 indicated an exclusively Gram negative flora

consisting of *Vibrio* (77.5%), *Aeromonas* (14.9%), *Pseudomonas* (4.8%) and Enterobactereaceae (3.1%) in the order of dominance.

Qualitative analysis of bacterial isolates from the semi-intensive systems also demonstrated predominance of Gram negative organisms in the environment and cultured shrimp as observed in traditional farms. The dominance of Gram negative bacteria was relatively higher in semi-intensive farms (72 %) than in traditional farms (67 %). In general, the shrimp harboured a larger population of Gram negative types (77-82%) than water and sediment (59-70%). Predominance of Gram negative bacteria in pond reared *P.indicus* and culture environment (water and sediment) of more or less similar type of semi-intensive farming was also reported by Sharmila *et al.* (1996) from the southeast coast of India.

The microflora of semi-intensive farms consisted of all the bacterial types recorded from the traditional farms. This would imply that all these organisms are normal inhabitants of the shrimp culture systems of the area. The two additional genera recorded, namely, *Flavobacterium* and *Cytophaga*, have also been reported by other workers from shrimp farming systems (Surendran and Chandrika, 1993) and adjoining estuarine/coastal waters of Cochin (Singh, 1986; Alavandi, 1989; Rosamma Philip and Perumalsamy, 1995)

The overall distribution of microorganisms in the environment and cultured shrimp of semi-intensive farms showed *Vibrio*s (32.4 %) to be the most predominant group followed by *Pseudomonas* (13.7 %), *Bacillus* (10.6 %), Enterobactereaceae (10.3 %), Micrococcaceae (8 %), *Arthrobacter* (6.3 %) and *Aeromonas* (5.4 %) among major isolates. *Vibrio* maintained dominance over all coexisting bacterial types in water, sediment, whole shrimp, shrimp surface and shrimp gut and recorded uniformly higher percentages in all these microbial habitats compared to traditional farms. As in the case of traditional farms, significant variations were evident between semi-intensive farms in the distribution of bacterial types, but with marked differences in the type of organisms that predominated in their biotic and abiotic habitats.

Table 20: Average composition of heterotrophic bacterial flora associated with water in different semi-intensive shrimp farms

Bacterial genera / groups	Percentage occurrence			
	Farm-A1	Farm-B1	Farm-C1	Farm-D1
Vibrio	22.63	26.30	37.87	19.72
Aeromonas	–	–	–	37.50
Enterobactereaceae	5.28	3.39	1.37	2.87
Moraxella	3.82	2.55	–	0.62
Acinetobacter	0.54	–	1.63	–
Pseudomonas	2.78	3.77	49.59	28.48
Flavobacterium	7.38	11.13	–	–
Alcaligenes	4.24	1.67	–	–
Cytophaga	–	–	3.70	–
Micrococcaceae	12.56	15.30	5.37	7.32
Bacillus	20.81	21.23	–	–
Lactobacillus	–	–	–	3.47
Arthrobacter	20.26	14.66	4.17	–
Gram negative	47.12	48.81	90.46	89..20
Gram positive	53.63	51.19	9.54	10.79
Total No. of isolates	122	122	92	138

Table 21: Average composition of heterotrophic bacterial flora associated with sediment in different semi-intensive shrimp farms

Bacterial genera / groups	Percentage occurrence			
	Farm-A1	Farm-B1	Farm-C1	Farm-D1
Vibrio	40.92	45.93	4.78	6.68
Aeromonas	–	–	–	6.32
Enterobactereaceae	–	0.89	1.00	6.20
Moraxella	1.47	3.83	3.33	–
Acinetobacter	–	–	2.47	–
Pseudomonas	3.50	1.99	21.30	50.35
Flavobacterium	–	–	–	–
Alcaligenes	20.33	18.24	–	–
Cytophaga	–	–	–	–
Micrococcaceae	1.84	6.22	3.17	9.69
Bacillus	20.03	18.79	53.56	2.61
Lactobacillus	–	–	–	18.12
Arthrobacter	11.82	4.09	10.35	–
Gram negative	66.25	70.88	32.89	69.55
Gram positive	33.69	29.10	67.08	30.43
Total No. of isolates	98	114	102	130

Table 22: Average composition of heterotrophic bacterial flora associated with whole shrimp in different semi-intensive shrimp farms

Bacterial genera / groups	Percentage occurrence			
	Farm-A1	Farm-B1	Farm-C1	Farm-D1
Vibrio	38.40	33.30	34.70	34.02
Aeromonas	–	–	–	19.43
Enterobactereaceae	35.00	35.30	17.16	26.96
Moraxella	–	–	–	–
Acinetobacter	18.38	17.37	–	–
Pseudomonas	–	–	5.84	3.98
Flavobacterium	1.65	2.57	1.73	0.33
Alcaligenes	–	–	–	–
Cytophaga	–	–	–	0.10
Micrococcaceae	–	7.13	10.66	13.67
Bacillus	–	–	5.75	–
Lactobacillus	–	–	23.06	–
Arthrobacter	6.51	4.38	1.03	1.44
Gram negative	93.43	88.55	59.44	84.81
Gram positive	6.51	11.52	40.50	15.11
Total No. of isolates	90	108	108	102

Table 23: Average composition of heterotrophic bacterial flora associated with shrimp surface in different semi-intensive shrimp farms

Bacterial genera / groups	Percentage occurrence			
	Farm-A1	Farm-B1	Farm-C1	Farm-D1
Vibrio	66.73	54.91	20.02	31.53
Aeromonas	–	–	–	24.97
Enterobactereaceae	4.28	3.17	12.25	11.26
Moraxella	0.18	0.70	2.08	–
Acinetobacter	–	1.67	2.08	1.87
Pseudomonas	–	–	28.97	11.77
Flavobacterium	12.94	12.81	–	–
Alcaligenes	–	3.41	–	–
Cytophaga	–	–	–	–
Micrococcaceae	–	4.58	22.65	8.12
Bacillus	7.40	14.42	1.67	0.43
Lactobacillus	7.40	1.50	–	1.46
Arthrobacter	1.00	2.83	10.27	8.58
Gram negative	84.13	76.60	65.41	81.40
Gram positive	15.81	23.33	34.59	18.59
Total No. of isolates	102	106	90	102

Table 24: Average composition of heterotrophic bacterial flora associated with water, sediment, whole shrimp and shrimp surface in Farm-A1

Bacterial genera / groups	Percentage occurrence			
	Water	Sediment	Whole shrimp	Shrimp surface
Vibrio	22.63	40.92	38.40	66.73
Aeromonas	–	–	–	–
Enterobactereaceae	5.28	–	35.00	4.28
Moraxella	3.82	1.47	–	0.18
Acinetobacter	0.54	–	18.38	–
Pseudomonas	2.78	3.50	–	–
Flavobacterium	7.38	–	1.65	12.94
Alcaligenes	4.24	20.33	–	–
Cytophaga	–	–	–	–
Micrococcaceae	12.56	1.84	–	–
Bacillus	20.81	20.03	–	7.40
Lactobacillus	–	–	–	7.40
Arthrobacter	20.26	11.82	6.15	1.00
Gram negative	47.12	66.25	93.43	84.13
Gram positive	53.63	33.69	6.51	15.81
Total No. of isolates	122	98	90	102

Table 25: Average composition of heterotrophic bacterial flora associated with water, sediment, whole shrimp and shrimp surface in Farm-B1

Bacterial genera / groups	Percentage occurrence			
	Water	Sediment	Whole shrimp	Shrimp surface
Vibrio	26.30	45.93	33.30	54.91
Aeromonas	–	–	–	–
Enterobactereaceae	3.39	0.89	35.31	3.17
Moraxella	2.55	3.83	–	0.70
Acinetobacter	–	–	17.37	1.67
Pseudomonas	3.77	1.99	–	–
Flavobacterium	11.13	–	2.57	12.81
Alcaligenes	1.67	18.24	–	3.41
Cytophaga	–	–	–	–
Micrococcaceae	15.30	6.22	7.13	4.58
Bacillus	21.23	18.79	–	14.42
Lactobacillus	–	–	–	1.50
Arthrobacter	14.66	4.09	4.38	2.83
Gram negative	48.81	70.88	88.55	76.66
Gram positive	51.19	29.10	11.52	23.33
Total No. of isolates	122	114	108	106

Table 26: Average composition of heterotrophic bacterial flora associated with water, sediment, whole shrimp and shrimp surface in Farm-C1

Bacterial genera / groups	Percentage occurrence			
	Water	Sediment	Whole shrimp	Shrimp surface
Vibrio	37.87	4.78	34.70	20.02
Aeromonas	–	–	–	–
Enterobactereaceae	1.37	1.00	17.16	12.25
Moraxella	–	3.33	–	2.08
Acinetobacter	1.63	2.48	–	2.08
Pseudomonas	49.59	21.30	5.84	28.97
Flavobacterium	–	–	1.73	–
Alcaligenes	–	–	–	–
Cytophaga	3.70	–	–	–
Micrococcaceae	5.37	3.17	10.66	22.65
Bacillus	–	53.56	5.75	1.67
Lactobacillus	–	–	23.06	–
Arthrobacter	4.17	10.35	1.03	10.27
Gram negative	90.46	32.89	59.44	65.41
Gram positive	9.54	67.08	40.50	34.59
Total No. of isolates	92	102	108	90

Table 27: Average composition of heterotrophic bacterial flora associated with water, sediment, whole shrimp and shrimp surface in Farm-D1

Bacterial genera / groups	Percentage occurrence			
	Water	Sediment	Whole shrimp	Shrimp surface
Vibrio	19.73	6.68	34.02	31.53
Aeromonas	37.50	6.32	19.43	24.97
Enterobactereaceae	2.88	6.20	26.96	11.26
Moraxella	0.62	–	–	–
Acinetobacter	–	–	–	
Pseudomonas	28.48	50.35	3.98	
Flavobacterium	–	–	0.33	–
Alcaligenes	–	–	–	–
Cytophaga	–	–	0.10	–
Micrococcaceae	7.32	9.70	13.67	8.12
Bacillus	–	2.61	–	0.43
Lactobacillus	3.47	18.13	–	1.46
Arthrobacter	–	–	1.44	8.58
Gram negative	8.20	69.55	84.82	81.40
Gram positive	10.79	30.43	15.11	1.87
Total No. of isolates	138	130	102	102

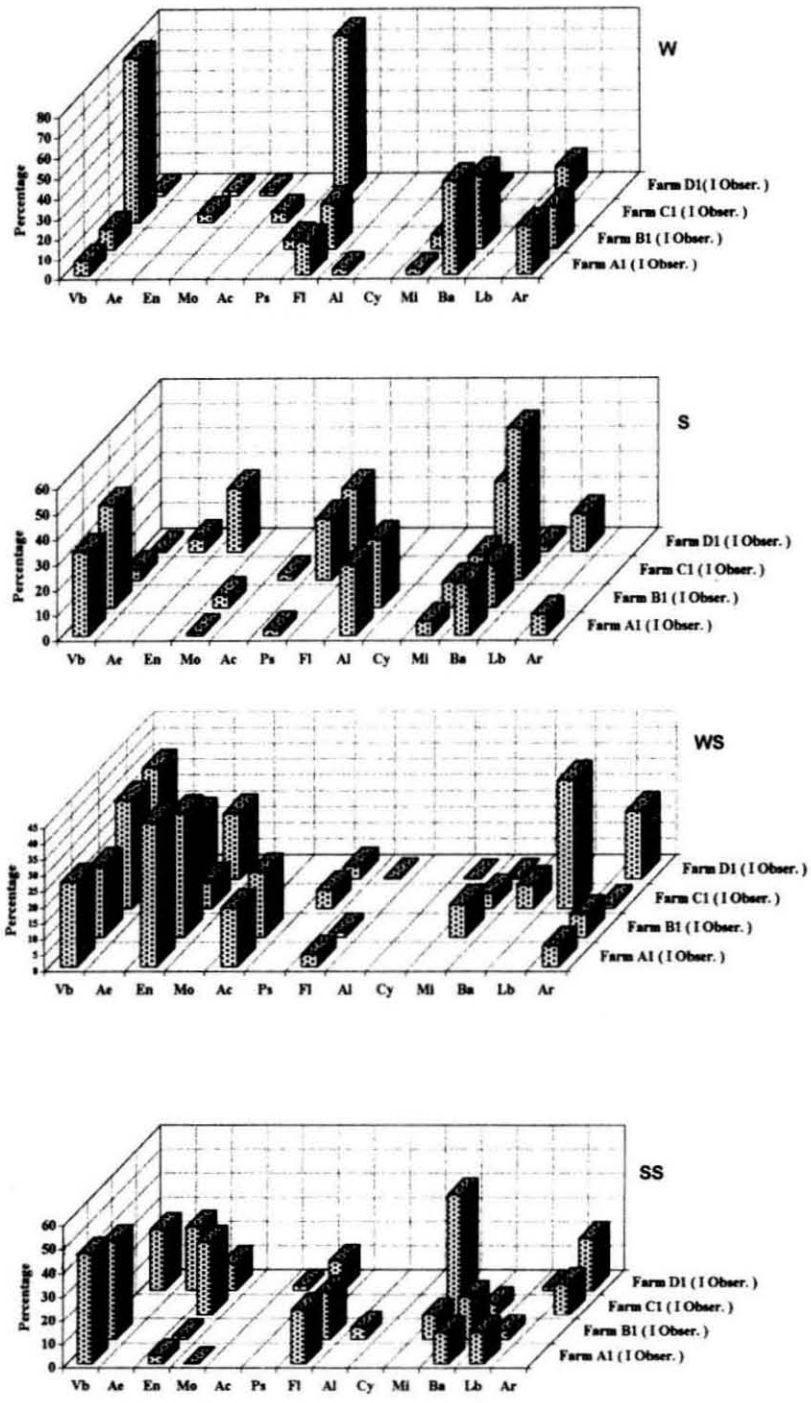

Fig. 8 Generic distribution of heterotrophic bacteria in semi-intensive farms during I observation (Legend to bacteria given in Table 19)
W-water, S-sediment, WS-whole shrimp, SS-shrimp surface

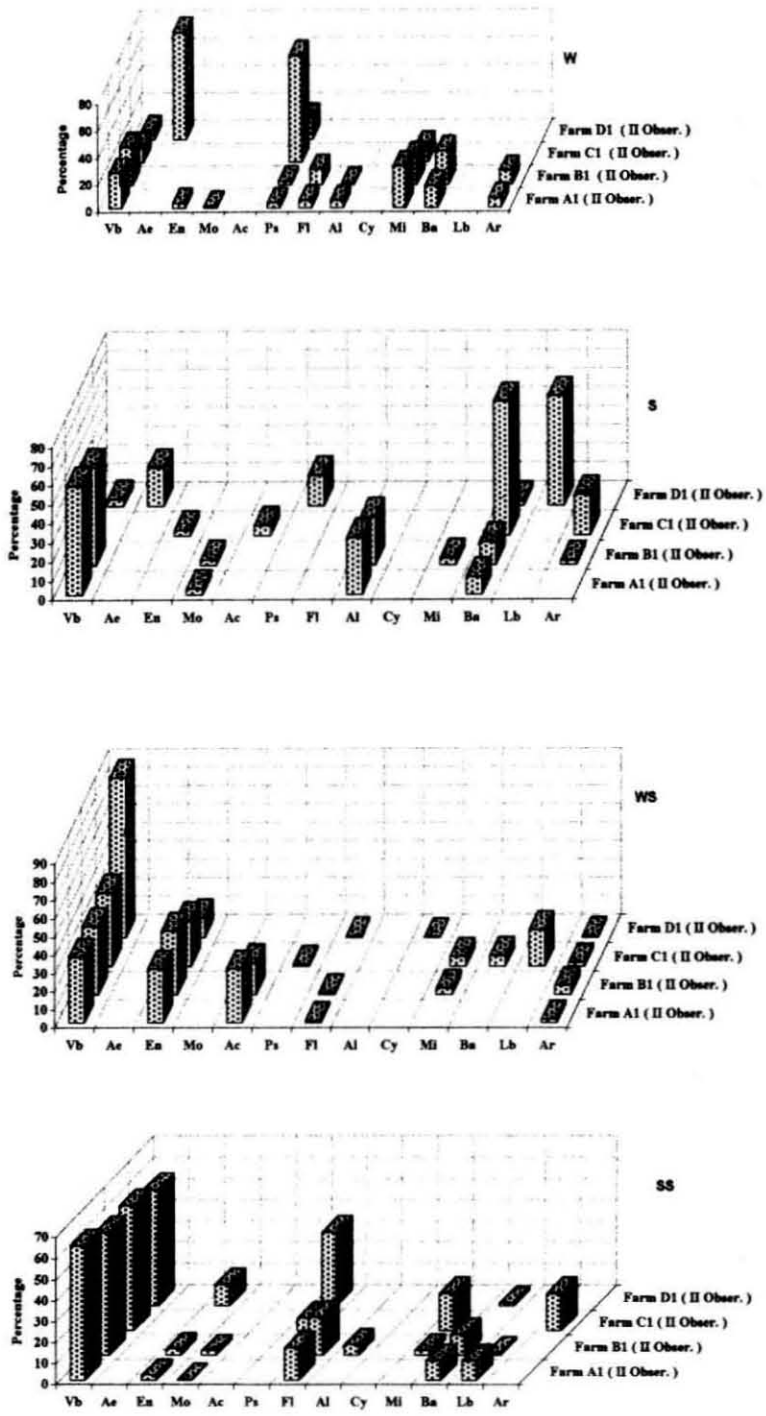

Fig. 9 Generic distribution of heterotrophic bacteria in semi-intensive farms during II observation

W-water, WS-whole shrimp, SS-shrimp surface

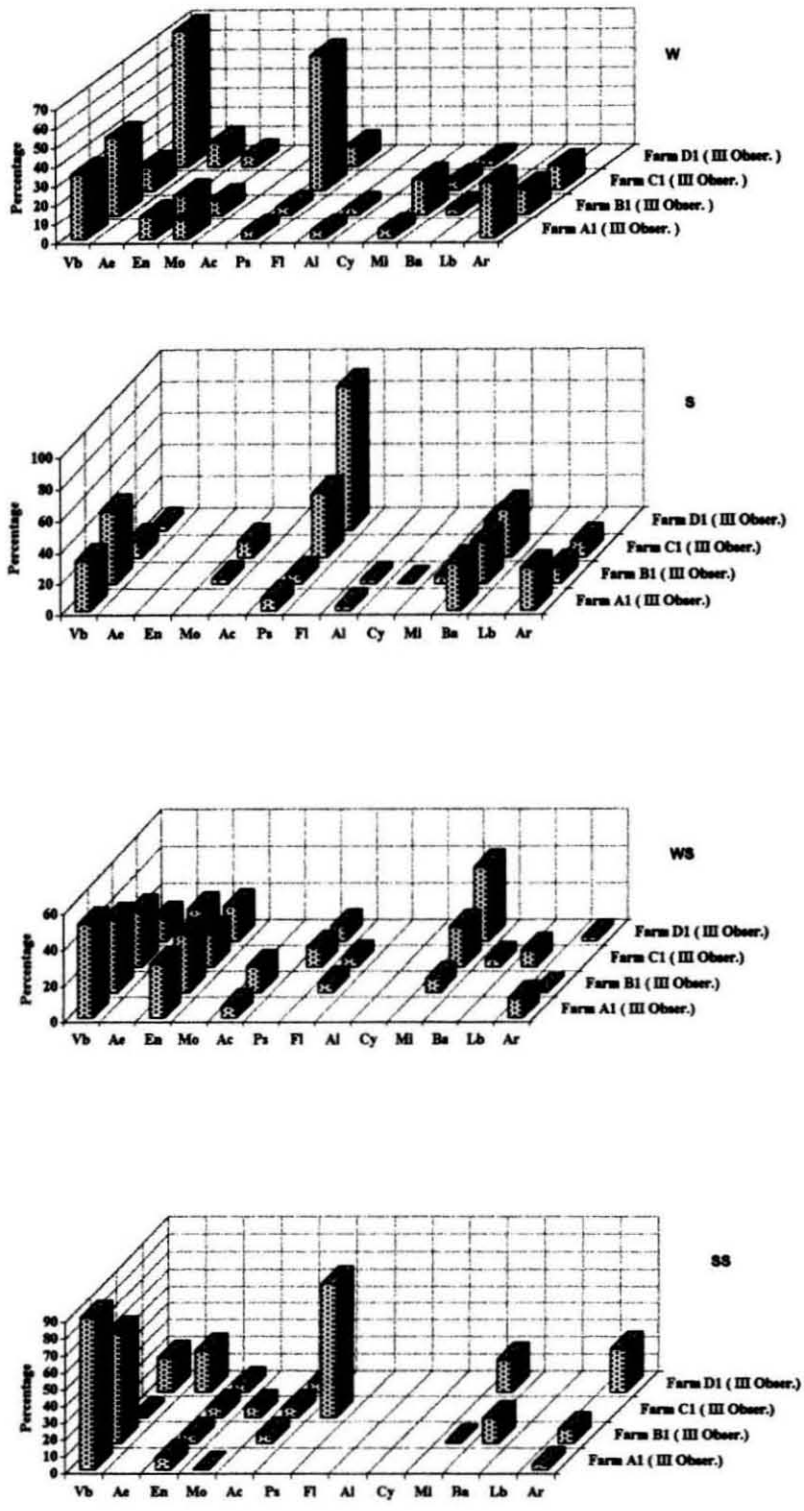

Fig.10 Generic distribution of heterotrophic bacteria in semi-intensive farms during III obsevation
W-water, WS-Wholeshrimp, SS-shrimp surface

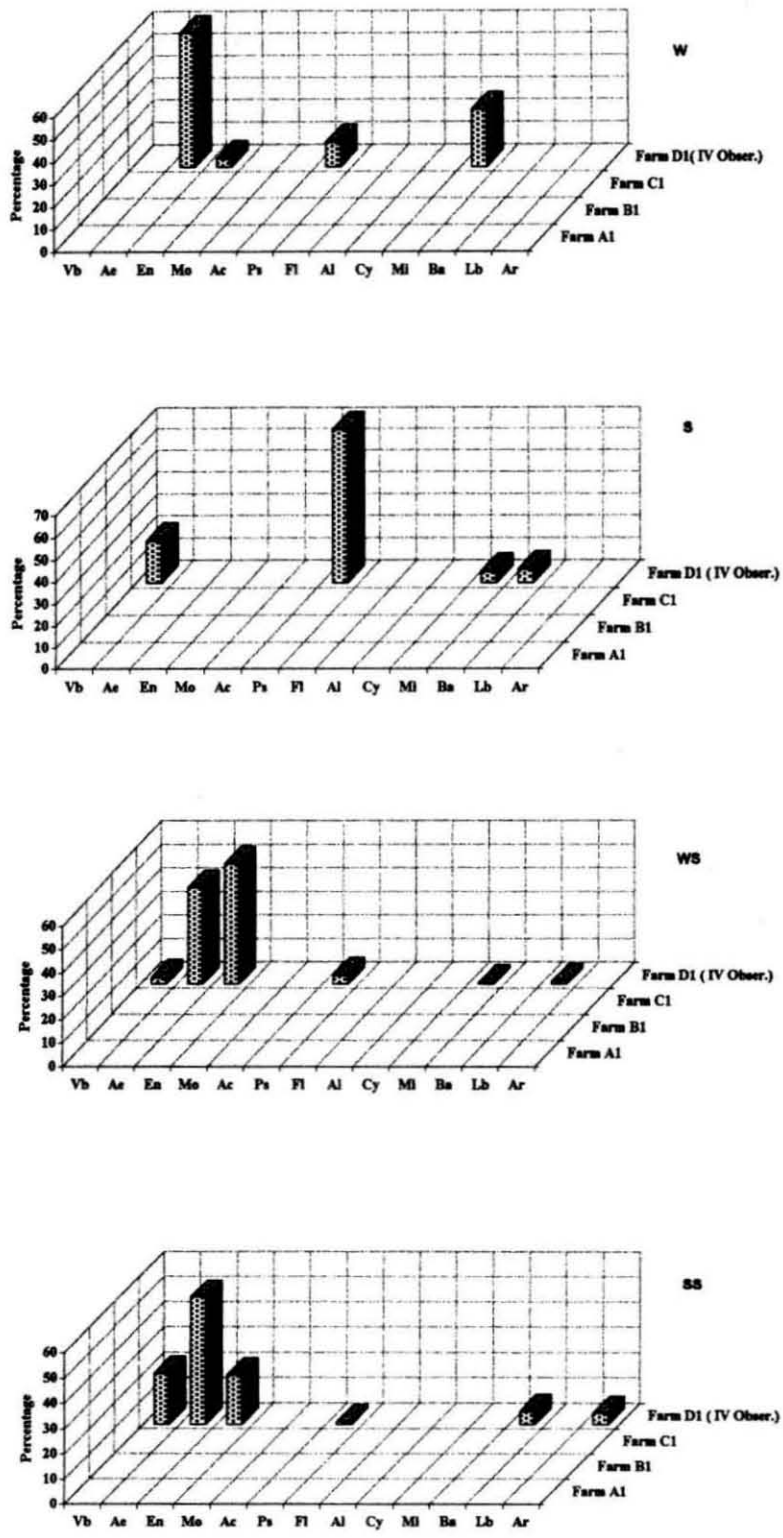

Fig. 11 Generic distribution of heterotrophic bacteria in semi-intensive farm during IV observation

W-water, S-sediment, WS-whole shrimp, SS-shrimp surface

In water, while *Vibrio*, *Bacillus*, *Arthrobacter*, Micrococcaceae and *Flavobacterium* constituted over 80 % of the flora in Farms-A1 & B1, *Vibrio* and *Pseudomonas* alone constituted the same percentage in Farm-C1 and *Vibrio*, *Aeromonas* and *Pseudomonas* in Farm-D1. The flora of sediment consisted mainly of *Vibrio*, *Alcaligenes*, *Bacillus* and *Arthrobacter* in Farms-A1 & B1, *Bacillus*, *Pseudomonas* and *Arthrobacter* in Farm-C1 and *Pseudomonas*, *Lactobacillus* and Micrococcaceae in Farm-D1. In whole shrimp samples the flora was mainly composed of *Vibrio*, Enterobactereaceae and *Acinetobacter* in Farms-A1 & B1, *Vibrio*, *Lactobacillus*, Enterobactereaceae and Micrococcaceae in Farm-C1 and *Vibrio*, Enterobactereaceae, *Aeromonas* and Micrococcaceae in Farm-D1. The microflora associated with shrimp surface mainly included *Vibrio*, *Flavobacterium* and *Bacillus* in Farms-A1 & B1, *Vibrio*, *Pseudomonas*, Micrococcaceae, Enterobactereaceae and *Arthrobacter* in Farm-C1 and *Vibrio*, *Aeromonas*, Enterobactereaceae and *Pseudomonas* in Farm-D1. In all the four farms the microflora associated with cultured shrimp was found to be more or less a reflection of the flora of water, sediment or both.

Microbiological studies conducted in semi-intensive shrimp culture systems from different parts of the world yielded varying results as regards distribution of microbial types in shrimp and culture environment. Vanderzant *et al.*,.(1971), working on pond-reared brown shrimp (*P.aztecus*) along the Texas Gulf Coast, noticed coryneform bacteria, now established as *Arthrobacter* spp. (Surendran and Gopakumar, 1981), and to a lesser extent *Vibrio* to be the predominant isolates in shrimp, and coryneform bacteria

and species of *Flavobacterium*, *Moraxella* and *Bacillus* in pond water. They also noticed lower incidence of coryneform bacteria when the salinity and temperature of the pond water were high (approx. 8-25 ppt and 26-31 °C respectively). Fonseka (1990) reported that over 75 % of the flora of farmed *P.monodon* in Sri Lanka consisted of Gram positive organisms such as *Micrococcus* spp., coryneform bacteria and *Bacillus* spp., while *Vibrio* formed the most common Gram negative organism when the shrimp were cultured in backishwater of 14-18 ppt salinity, 28-32 °C temperature and 7.4 – 8.2 pH. He further noted slight variation in type and levels of microflora over the culture period which was attributed to environmental conditions, feed type, rate of feeding, fertilization of pond and aeration. In India, Sharmila *et al.*,(1996) observed *Vibrio* spp. to be the most dominant flora of pond water (20 %), sediment (30 %) and cultured *P.indicus* (32 %). The other important groups which showed up by over 10 % of the total population were *Staphylococcus* spp., members of Enterobactereaceae, *Micrococcus* spp. and *Bacillus* spp. in water, *Micrococcus* spp, *Bacillus* spp. and Staphylococcus spp. in sediment and Enterobactereaceae, *Micrococcus* spp and *Staphylococcus* spp. in shrimp. It was also noticed by them that the concentration of *Vibrio* spp. in pond water was on the higher level from about the middle of the culture period till harvest and postulated that it might be due to the selective enrichment of these organisms by the nutrient rich pond environment (presumably due to feed application) or the dispersion of sediment flora. A similar increase in population of *Vibrio* spp., reaching up to 42 %, was also observed in shrimp after middle of the culture period, which they considered to be a reflection of the environment. At Cochin, Surendaran *et al.*,. (1995) observed members of *Vibrio* and

Pseudomonas to be the predominant bacteria together with lesser proportion of *Acinetobacter*, *Aeromonas*, *Micrococcus* and Enterobactereaceae in *P.momodon* grown in intensive backishwater farms. Occurrence of vibrios as an important constituent of the microflora of semi-intensive shrimp farms was also reported by Sini (1993).

As may be seen from earlier discussion, the microbiological features of traditional shrimp culture systems are to a large extent similar to those of the surrounding backwater, which may be due to the similar environmental conditions prevailing in both. Although similarly located, the semi-intensive farms exhibited visible changes in qualitative distribution of bacterial flora, as in the case of total plate counts levels in the environment as well as cultured *P.indicus*. It is generally considered that culture practices could greatly influence the microflora of semi-intensive farms (Nayyarahamed *et al.*, 1995; Surendran *et al.*, 1995). Various factors such as changes in salinity, temperature, dissolved oxygen and pH of water, stocking density, feed type, rate of feeding, fertilization of pond, aeration etc. have been reported to cause changes in the microflora of semi-intensive farms (Vanderzant *et al.*, 1971; Fonseka, 1990; Nayyarahamed *et al.*, 1995; Sunarya *et al.*, 1995; Surendran *et al.*, 2000). In the present study, all the bacterial types isolated from water, sediment and cultures shrimp appear to be normal inhabitants of both types of culture systems as well as the estuarine and coastal waters of Cochin. However, in semi-intensive farms it is possible that some of the organisms which are normally less abundant in traditional farms may proliferate and gain dominance over others with the enrichment of nutrients as a result of feed input and

availability of favourable environmental conditions like salinity, temperature, pH and substrates (shrimp stocks and detritus formed from feed wastes) for their attachment, growth, and multiplication. Shrimp feed also could be a source for increase of *Enterobactereace*, *Alcaligenes*, *Aeromonas*, and Gram positive organisms that dominated in the microflora of the feed used in these farms

The present observations also suggest that salinity could be a limiting factor for the dominance of various physiological groups of bacteria in the semi-intensive farms. Under high salinity conditions (28.2 – 35.2 ppt), as was the case in Farms-A1 and B1, the most dominant groups of bacteria in decreasing order of preponderance are vibrios, *Alcaligenes*, *Bacillus*, *Arthrobacter*, Micrococcaceae and *Flavobacterium* in the environment and vibrios, *Enterobactereaceae*, *Acinetobacter*, *Flavobacterium* and *Bacillus* in shrimp. However, in medium to very low salinity conditions (0.1-15.8 ppt), as in Farms – C1 and D1, the microflora is mainly dominated by vibrios, *Pseudomonas*, *Aeromonas*, *Bacillus*, *Arthrobacter*, *Lactobacillus* and Micrococcaceae in the environment and vibrios, *Pseudomonas*, Micrococcaceae, Enterobactereaceae, *Aeromonas*, and *Arthrobacter* in shrimp. Variations in diversity and abundance of microflora in culture environment and pond reared shrimp due to fluctuation in salinity and other water quality parameters have been pointed out by Vanderzant *et al.*, (1971). Straub and Dixon (1993) have established direct correlation between changes in salinity and diversity of bacterial flora associated with brine shrimp.

The uniformly higher incidence of vibrios in all salinity conditions prevailing in Farms-A1 - D1 and the incidence of *Aeromonas* in significant levels only in very low salinity condition prevailing in Farm -D1 are noteworthy. Although both these bacterial types form part of the natural flora of shrimp culture systems (Vanderzant *et al.*, 1971; Sini, 1993; Sharmila *et al.*, 1996; Surendran *et al.*, 2000), the present results suggest that enrichment of nutrients and organic substrates as a result of the application of large amounts of shrimp feed favours their multiplication. In the case of *Aeromonas*, though the shrimp feed used was a possible contaminant of the organism in the culture systems, it was not isolated from any of the high-salinity farms (Farms-A1 to C1) indicating that higher saline environment is not conducive for proliferation of the bacteria even under nutrient-laden conditions. Twiddy and Reilly (1995) recorded high counts of *Aeromonas* in water, sediment and fish flesh in integrated semi-intensive freshwater aquaculture ponds of Indonesia, while Surendran *et al.*, (2000) made similar observations in freshwater and low-salinity (8.65ppt) brakishwater farms and cultured prawns in India.

4.5 Detection of faecal indicators and human pathogens from shrimp culture systems

4.5.1 Incidence and quantitative abundance of faecal indicators

Coliform organisms, faecal coliforms and *Escherichia coli* are primarily used to indicate some degree of potentially hazardous contamination based on the assumption that the natural habitat of the family Enterobactereaceae to which these bacteria belong is the faeces of man and other mammals, thereby indicating faecal contamination. Similarly

faecal streptococci is also tried as bacterial indicators of fecal pollution. *E. coli* is considered as the most positive indicator of human faecal contamination. Presence of *E. coli* in a food implies the possibilities of finding one or more of a wide diversity of enteric pathogens which may also have gained assess to the food and hence introduce human health hazards (Fonseka, 1990). During the present study analyses of samples of water, sediment, whole shrimp and shrimp surface were made for the detection and enumeration of faecal indicator organisms such as total coliforms, faecal coliforms, *E. coli* and faecal streptococci, and the results are presented in Tables-28 & 29.

In the traditional farms, total coliforms, faecal coliforms, and faecal streptococci were encountered in the environment as well as shrimp body at varying levels (Table-28). In water total coliforms and faecal coliforms were positive in 23% (3/13) of the samples each, while faecal streptococci occurred in 15% (2/13) of the samples. In sediment total coliforms and faecal coliforms were detected in 15 %(2/13) of the samples each, whereas faecal streptococci was absent. In whole shrimp, total coliforms and faecal coliforms were positive in 7.7% (1/13) of samples while faecal streptococci was detected in 31% (4/13) of the samples. In shrimp surface the incidence of coliforms was same as in shrimp body while faecal streptococci was positive in 23% (3/13) of the samples. MPN value of total coliforms positive samples was 9.5-25/ml in water, 2-4/g in sediment, 250/g in shrimp body and 2.17/cm^2 in shrimp surface. Faecal coliforms counts were 1.5-25/ml in water, 1.1-4/g in sediment, 250/g in whole shrimp and 2.17/cm^2 in shrimp surface. *E. coli* was not detected in any of the samples tested from these farms. The count of faecal

streptococci was 20-30/ml in water, 180/g - $1.5x10^3$/g in whole shrimp and 27-80/cm^2 in shrimp surface, while it was not detected in any of the sediment samples.

In the semi-intensive farms, total coliforms, faecal coliforms and *E. coli* were detected in 25% (3/13) of samples each in water, 7.7% (1/13) each in whole shrimp and 25% (3/12) each in shrimp surface. Faecal streptococci was positive in 15% (2/13) of samples in water, 17% (2/12) each in whole shrimp and shrimp surface. In sediment samples faecal streptococci was significantly absent. The presence of *E. coli* in semi-intensive farms and farmed shrimp was noteworthy. All Coliform bacteria were detected only during the monsoon season (Farm-D1) in these farms. Faecal streptococci were detected in the pond water during non-monsoon season and in shrimp body during monsoon season. Quantitatively, coliforms showed higher levels in the farm environment and shrimp during monsoon period (Farm-D1) and faecal streptococci in the environment (water) during non-monsoon period (Farms-A1 & C1) and in shrimp samples during monsoon period as compared to their wider distribution in the traditional farms. MPN of total coliforms of positive samples was 9-95/ml in water, 450/g in sediment, 9-95/g in whole shrimp and 0.3-9.6/cm^2 in shrimp surface. The corresponding MPN values for the four types of samples for faecal coliforms were respectively 6-20/ml, 250/g, 9-95/g and 0.3-1.8/cm^2.

Table 28: Incidence and quantitative abundance of faecal bacteria indicators in traditional shrimp farms and cultured shrimp
(TPC / gm/ml/cm^2 : MPN / g / ml /cm^2 : Count / g / ml /cm^2)

Details of positive samples Source / Type / Month	TPC (CFU)	Total coliforms (MPN)	Faecal coliforms (MPN)	*E. coli* (MPN)	Faecal streptococci (Count)
Farm C (W) Jan (/ml)	$3.9x10^3$	ND	ND	ND	30
Farm A (W) March (/ml)	$3.7x10^3$	ND	ND	ND	20
Farm D (W) June (/ml)	$1.0x10^3$	9.5	1.5	ND	ND
Farm D (W) July (/ml)	$1.0x10^3$	16	11.5	ND	ND
Farm D (W) Sept (/ml)	$1.1x10^3$	25	25	ND	ND
Farm D (S) Aug (/g)	$2.9x10^4$	2	1.1	ND	ND
Farm D (S) Sept (/g)	$3.1x10^4$	4	4	ND	ND
Farm C (WS) Jan (/g)	$2.5x10^5$	ND	ND	ND	180
Farm A (WS) Feb (/g)	$6.3x10^5$	ND	ND	ND	$1.2x10^3$
Farm B (WS) Feb (/g)	$4.6x10^5$	ND	ND	ND	180
Farm D (WS) Sept (/g)	$2.5x10^5$	250	250	ND	$1.5x10^3$
Farm C (SS) Jan (/cm^2)	$6.0x10^5$	ND	ND	ND	80
Farm B (SS) Feb (/cm^2)	$6.2x10^5$	ND	ND	ND	4
Farm D (SS) Aug (/cm^2)	$2.4x10^5$	2.17	2.17	ND	27

(W) = Water, (S) = Sediment, (WS) = Whole shrimp, (SS) = Shrimp surface

ND = not detected

Table 29: Incidence and quantitative distribution of faecal indicators in semi-intensive culture systems and farmed shrimp (TPC per gm/ml/cm^2 , MPN per g/ml/cm^2 , Count per g/ml/cm^2)

Details of positive samples Source / Type / Month	TPC (CFU)	Total coliforms (MPN)	Faecal coliforms (MPN)	*E. coli* (MPN)	Faecal streptococci (Count)
Farm A1 (W) Jan (/ml)	1.2×10^3	ND	ND	ND	60
Farm C1 (W) Feb (/ml)	2.6×10^5	ND	ND	ND	150
Farm D1 (W) June (/ml)	5.8×10^3	25	6	1.1	ND
Farm D1 (W) July (/ml)	3.1×10^3	ND	ND	ND	ND
Farm D1 (W) Aug (/ml)	1.1×10^3	9	9	9	ND
Farm D1 (W) Sept (/ml)	5.7×10^3	95	20	9	ND
Farm D1 (S) June (/g)	4.3×10^5	450	250	95	ND
Farm D1 (WS) July (/g)	3.0×10^6	95	95	20	ND
Farm D1 (WS) Aug (/g)	9.7×10^4	20	20	11	5.7×10^4
Farm D1 (WS) Sept (/g)	3.6×10^5	9	9	9	4.1×10^4
Farm D1 (SS) July (/cm^2)	6.0×10^5	9.6	1.8	0.13	ND
Farm D1 (SS) Aug (/cm^2)	5.8×10^5	0.3	0.3	0.3	430
Farm D1 (SS) Sept (/cm^2)	1.8×10^5	0.8	0.8	0.13	18

(W) = Water , (S) = Sediment , (WS) = Whole shrimp , (SS) = Shrimp surface
ND = not detected

The MPN of *E. coli* ranged 1.1-9/ml in water, 95/g in sediment, 9-20/g in whole shrimp and 0.1-0.3/cm^2 in shrimp surface. The count of faecal streptococci in water during non-monsoon crop ranged 60-150/ml. In shrimp body significantly higher levels of this indicator bacteria were observed during the monsoon period, the counts ranging 4.1×10^4 to 5.7×10^4/g in whole shrimp and 18-430/cm^2 in shrimp surface.

The occurrence of bacteria of faecal origin in significant levels both in traditional and semi-intensive shrimp farms observed during the present study indicates insanitary condition of the farming. This shows that the water used in the culture systems is unclean or contains effluents from unhygienic sources. High incidence of coliforms, *E. coli* and faecal streptococci due to sewage discharge and faecal contamination of both human and non-human origin has been reported to be a common feature in Cochin backwater (Gore *et al.*, 1979; Pradeep and Lakshanaperumalsamy, 1986) and connected mangroves (Kumaran *et al.*, 1996) and beach waters (Raveendran *et al.*, 1978). Since Cochin backwater is the only source of water for shrimp farming in both types of culture systems around it, the presence of indicator organisms in significant numbers in the farm environment and cultured shrimp is quite natural. According to Surendran *et at.* (1995) bacteria of faecal origin are natural contaminants of cultured prawns in Cochin. Presence of coliform organisms, faecal coliforms and *E. coli* in shrimp farms and cultutred shrimp have been reported in varying levels by many other workers also (Fonseka, 1990 ; Putro *et al.*, 1990; Rattagool *et al.*, 1990; Peranginangin *et al.*, 1992; Sunarya, 1992 ; Nayyarahamed *et al.*, 1995) from different Asian countries

A common feature observed in both types of culture systems was the incidence of faecal coliforms only in those farms that operated during the monsoon period (Farms-D & D1). This may be due to the fact that relatively higher densities of coliforms and *E. coli* occurred in water and sediment in Cochin backwater during monsoon period as a result of land drainage and consequent faecal contamination following monsoon rains

(Gore *et al.*, 1979; Pradeep amd Lakshanaperumalsamy, 1986). A comparison of the present data between traditional farms and semi-intensive farms (Tables-28 & 29) reveals significant variations in qualitative and quantitative abundance of the indicator organisms, Farm-D1 showing much higher incidence and counts of the organisms than Farm-D. Total coliforms, faecal coliforms and *E. coli*, were maximum abundant in sediment followed by whole shrimp samples in Farm-D1, which may be due to the availability of high nutrients and accumulation of organic substrates in pond bottom as a result of supplementary feeding and the shrimp's bottom feeding habit (Dall, 1968). Putro *et al.*, (1990) reported detection of faecal coliforms in sediments even when samples were negative for this group of organisms. Similar observations were also made by Nayyarahamed *et al.*, (1995) who concluded that *E. coli* was able to colonize in the sediments of semi-intensive farms efficiently.

4.5.2 Incidence of human pathogens

The occurrence of human pathogens *Vibrio cholerae, V. parahaemolyticus, V. vulnificus* and *Salmonella* and the occurrence and quantitative abundance of *Staphylococcus aureus* in the farm environment and shrimp have been studied and the results are presented in Tables-30 & 31.

Table 30: Incidence of human pathogens in water, sediment and shrimp from traditional culture systems

Sample	*Vibrio* spp.			*Salmonella*	*Staphylococcus aureus*
	v.c	*v.p*	*v.v*		
Farm A					
• Water	+	+	+	-	-
• Sediment	+	-	-	-	-
• Whole Shrimp	-	+	+	-	-
• Shrimp surface	-	-	-	-	-
Farm B					
• Water	-	-	-	-	-
• Sediment	-	-	-	-	-
• Whole Shrimp	-	-	-	-	-
• Shrimp surface	-	-	-	-	-
Farm C					
• Water	-	-	-	-	-
• Sediment	-	-	-	-	-
• Whole Shrimp	-	-	-	-	-
• Shrimp surface	-	-	-	-	-
Farm D					
• Water	-	-	-	-	-
• Sediment	-	-	-	-	-
• Whole Shrimp	-	+	-	-	-
• Shrimp surface	-	-	-	-	-
• Shrimp gut	+	-	-	-	-

+ = Present
- = Absent

v.c. = *V. cholerae*
v.p. = *V. parahaemolyticus*
v.v = *V.vulnificus*

Table 31 : Incidence of human pathogens in water,sediment and shrimp from semi-intensive culture systems

Sample type	*Vibrio* spp.			*Salmonella*	*Staphylococcus aureus*
	v.c	*v.p*	*v.v*		
Farm A1					
• **Water**	-	-	-	-	-
• **Sediment**	-	-	-	-	-
• **Whole Shrimp**	-	-	-	-	-
• **Shrimp surface**	-	+	-	-	-
Farm B1					
• **Water**	-	-	-	-	-
• **Sediment**	-	-	-	-	-
• **Whole Shrimp**	-	-	-	-	-
• **Shrimp surface**	-	-	-	-	-
Farm C1					
• **Water**	-	+	-	-	-
• **Sediment**	-	+	-	-	-
• **Whole Shrimp**	-	+	-	-	-
• **Shrimp surface**	-	-	-	-	-
Farm D1					
• **Water**	+	+	+	-	+
• **Sediment**	+	-	-	-	+
• **Whole Shrimp**	-	+	-	-	-
• **Shrimp surface**	+	+	-	+	-
• **Shrimp gut**	-	-	+	-	-

+ = present
- = absent

v.c. = *V. cholerae*
v.p. = *V.parahaemolyticus*
v.v = *V.vulnificus*

Contamination of seafoods by these organisms have led to many outbreaks of food-borne diseases in man (Inglis *et al.*, 1993). *V. cholerae* is known to cause cholera epidemics and sporadic diarrhoea, *V. parahaemolyticus* food poisoning/gastro-enteritis, *V. vulnificus* primary septicemia and wound infections and *Salmonella* and *S. aureus* food poisoning

(Datta *et al.*, 1984; Fonseka, 1990; Karunasagar *et al.*, 1990; Inglis *et al.*, 1993; Reilly, 1998; Sanjeev, 1999).

In traditional farms *V. cholerae* was detected in 12.5% of the samples (7/56) of water, sediment, whole shrimp, shrimp surface and shrimp gut. Of the 7 positive samples 4 were recorded in water and sediment samples (4/18) during non-monsoon period and 3 from shrimp gut samples (3/4) during monsoon period. All strains of *V. cholerae* belonged to the non-01 serogroup. *V. parahaemolyticus* was detected in 16% of all types of samples (9/56) of which 2 positive samples were from water and 3 from whole shrimp during non-monsoon period and 4 from whole shrimp during monsoon period. The incidence of this pathogen was 100% in whole shrimp samples (4/4) during monsoon period. All the positive strains of this pathogen were found to be Kanagawa negative. *V. vulnificus* occurred in 9% of the samples (5/56) of which 2 were isolated from water (2/13) and 3 from whole shrimp (3/13). The occurrence of this pathogen was noticed only during non-monsoon season. None of the samples tested proved positive for *Salmonella* or *S. aureus* in the traditional culture systems.

In semi-intensive farms *V. cholerae* was noticed in 5.8% of samples (3/52) of which one incidence each was from water, sediment and shrimp surface. All the three positive samples were during monsoon season and all the isolates belonged to the non-01 serogroup. *V. parahaemolyticus* was positive in 19.2% of samples (10/52) and it was

detected from the environment as well as shrimp body during both seasons. Four of the positive isolates were from water, one from sediment, three from whole shrimp and two from shrimp surface. During monsoon season one of the positive strains isolated from water and the one isolated from whole shrimp were found to be Kanagawa positive. *V. vulnificus* occurred in 5.8% of all samples (3/52) which was recorded only during the monsoon season. Two of the positive samples were detected from water and one from shrimp gut. *Salmonella* was isolated from only one samples of shrimp surface during monsoon crop. *S. aureus* was also encountered only during the monsoon crop when it was detected from two samples, one each from water and sediment, the counts being $0.4x10^2$/ml in the former and $1.4x10^2$/g in the latter samples.

The presence of *V. cholerae* non-01 in both traditional and semi-intensive farms, with no seasonal pattern, indicates that this organism could be normal inhabitants of the shrimp culture systems and also probably the Cochin Backwater. Presence of this organism, which has been recognized as a causative agent of sporadic outbreaks of gastro-enteritis in the country (Karunasagar *et al.*, 1990), was also noticed in the mangrove ecosystems adjoining Cochin backwater during premonsoon and post monsoon months by Kumaran *et al.*(1996), who drew attention to the threat of pathogenicity prevailing in the area throughout the year. Non-01 *V. cholerae* has also been isolated from shrimp samples (shrimp surface) collected from semi-intensive farms of southwest and southeast coasts of India by Nayyarahamed *et al.* (1995) and from freshly harvested seafoods and water samples from Karnataka coasts by Mathew *et al.* (1988) and

Karunasagar *et al.*(1990). Studying the incidence of *V. cholerae* and *Salmonella* in selected shrimp farms of Southeast Asia, Reilly and Twiddy (1992) noticed that brakishwater ponds and cultured prawns were inherently contaminated with *Salmonella* spp. and *V. cholerae* non-01 serotype, the latter occurring during the wet season in semi-intensive and intensive farms using only commercial feeds. They observed that the feeds given would not have been the source of pathogens because of the high temperature involved in their production. A more likely explanation given by them for the microbial contamination was that high organic loading in ponds due to the accumulation of residual or waste feeds, animal feaces etc. and land drainage during wet season would have favoured an increase of the pathogens. In the present case also the above reasons may be assigned to the occurrence of *V. cholerae* in the environment and shrimp surface and *Salmonella* in the environment of semi-intensive farms during monsoon period.

V. parahaemolyticus was the first of the non-cholera vibrios to be widely recognized as a serious human pathogen causing food poisoning from contaminated seafoods (Karunasagar *et al.*, 1990). Essentially an inhabitant of coastal and estuarine environments, this organism is found more commonly in waters rich in organic nutrients such as those present in sewage (Liston, 1973). In India it occurs throughout the west and east coasts (Karunasagar and Mohankumar, 1980; Bandekar *et al.*, 1982; Venkateswaran and Natarajan, 1987) and has been found in significant levels in the backwater (Pradeep and Lakshmanaperumalsamy, 1984) and mangrove environments (Kumaran *et al.*, 1996) at Cochin. *V. parahaemolyticus* has been reported from shrimp

aquaculture systems of many Asian countries including India (Fonseka, 1991; Nayyarahamed *et al.*, 1995; Sunarya *et al.*,1995; Sanjeev, 1999; Surendran *et al.*, 2000). During the present investigation, though this organism was present in both traditional and semi-intensive farms its occurrence was more in semi-intensive farms particularly during monsoon season (Farm-D1). Datta *et al.* (1984) reported that a distinsintion has been made between those strains of *V. parahaemolyticus* isolated from human disease and those strains widely distributed in the marine and estuarine environments. This distinsion is based on the ability of some strains to produce a heat-stable hemolysin termed the 'Kanagawa Phenomenon (KP) hemolysin'. Almost all the environmental isolates of the species are KP^-, whereas more than 96 % of clinical isolates produce hemolysin and are KP^+ (Miyamoto *et al.*, 1969; Joseph *et al.*, 1983). During the present study two of the strains isolated from Farm-D1 were Kanagawa-positive. Very high counts of Kanagawa-positive strains of *V. parahaemolyticus* have been reported in water (18 %), sediment (12%) and shrimp (2%) of brakishwater culture pond at Cochin by Sanjeev (!999)

The occurrence of other pathogens such as *V. vulnificus*, *Salmonella* and *S. aureus* in the shrimp culture systems, more particularly *Salmonella* and *S. aureus* in semi-intensive farm (Farm-D1), shows that the environment tends to be more conducive for the growth of the microorganisms during monsoon period due to organic enrichment through feed application and other farm management practices and also through heavy sewage discharge into the source water.

4.6 Antibiotic resistance of bacteria

4.6.1 Resistance of general flora in shrimp culture systems

A total of 252 bacterial cultures isolated from water (56), sediment (52), and shrimp (144) from the traditional farms were screened for the development of resistance against 21 antibacterials, including 13 antibiotics (amoxycillin, ampicillin, chloramphenicol, chlortetracycline, erythromycin, gentamycin, kanamycin, novobiocin, neomycin, oxytetracycline, penicillin-G, streptomycin and tetracycline) and 8 antimicrobials (bacitracin, furazolidone, nalidixic acid, nitrofurantoin, oxacillin, polymyxin-B, sulphadiazine and trimethoprim) as classified by Weston (1996). These include those antibacterials which are commonly used in aquaculture practices (Twiddy and Reilly, 1995; GESAMP, 1997; Surendran and Nirmala Thampuran, 1999) and those used for treatment of bacterial infections in man (Geddes, 1982; Modr, 1982; Sabath, 1982; Williams, 1982; Yeoman, 1982). All the 252 cultures tested showed resistance to one or more individual antibiotics*. Details of antibiotic resistance pattern of bacterial cultures isolated from water, sediment and shrimp from traditional farms are shown in Table-32, and that of major microbial groups in Table-34. About 75% of the total isolates tested showed resistance to oxacillin, 60-65 % to penicillin-G, and sulphadiazine, 45-60 % to ampicillin and novobiocin, 30-35% to amoxcycillin, erythromycin, bacitracin, furazolidone, and trimethoprim, and 2-30 % to the rest of the antibiotics.

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* The term 'antibiotics' is used hereafter as common for all the 21 antibacterials for sake of brevity.

Among the important microbial groups *Vibrio* showed resistance to 95% of the antibiotics and *Pseudomonas* and Enterobactereaceae to over 50 % of the antibiotics tested.

In semi-intensive farms, a total of 270 bacterial cultures isolated from water (64), sediment (52) and shrimp (154) were screened for the development of resistance to the same 21 antibiotics (Tables-33 & 35). In these farms also all the cultures tested showed resistance to one or more individual antibiotics. About 73% of the cultures showed resistance to oxacillin, 50-65 % to penicillin-G and ampicillin, 30-50 % to novobiocin , bacitracin and erythromycin and 2-30 % to the rest of the antibiotics. While *Vibrio* showed resistance to all the antibiotics, Enterobactereaceae and *Pseudomonas* recorded resistance to 80-95 %, *Aeromonas* and *Arthrobacter* to 60-75 % and *Bacillus*, *Cytophaga* and Micrococcaceae to 40-60 % of the antibiotics tested. Organisms such as *Vibrio*, Enterobactereaceae, *Pseudomonas*, Micrococcaceae, *Bacillus* and *Arthrobacter* showed resistance to larger number of antibiotics in semi-intensive farms than in traditional farms.

There was no significant difference in the pattern of antibiotic resistance between the traditional and semi-intensive farms, especially with regard to the intensity of resistance of the microorganisms to antibiotics of clinical origin. Almost all the antibiotics to which over 50% of bacterial cultures showed resistance are not normally used in aquaculture systems, and on the contrary they are greatly in clinical application

Table 32: Antibiotic resistance pattern of bacterial cultures isolated from traditional shrimp farms

Antibiotics	Water (n=56)	Sediment (n=52)	Shrimp (n=144)	All cultures (n=252)
Amoxcycillin	21.4	46.1	36.1	34.9
Ampicillin	57.1	61.5	55.5	57.1
Chloramphenicol	7.1	7.7	2.8	4.8
Chlortetracycline	7.1	15.4	2.8	6.3
Erythromycin	14.3	53.8	27.7	30.2
Gentamycin	7.1	30.7	13.8	15.8
Kanamycin	21.4	7.7	8.3	11.1
Novobiocin	50.0	61.5	38.8	46.0
Neomycin	14.3	30.7	11.1	15.8
Oxytetracycline	14.3	15.3	8.3	11.1
Penicillin-G	64.2	53.8	66.6	63.4
Streptomycin	7.1	0.0	0.0	1.6
Tetracyclin	0.0	23.1	2.8	6.3
Bacitracin	35.7	30.7	30.5	31.7
Furazolidone	28.6	46.1	30.5	33.3
Nalidixic acid	7.1	0.0	2.8	3.2
Nitrofurantoin	21.4	38.4	22.2	25.3
Oxacillin	64.2	84.6	75.0	74.6
Polymyxin-B	0.0	30.7	13.8	14.2
Sulphadiazine	57.1	53.8	63.8	60.3
Trimethoprim	28.5	53.8	22.2	30.2

n = Number of cultures tested

Note: Values given are percentage of cultures showing antibiotic resistance

Table 33: Antibiotic resistance pattern of bacterial cultures isolated from semi-intensive shrimp farms

Antibiotics	Water (n=64)	Sediment (n=52)	Shrimp (n=154)	All cultures (n=270)
Amoxcycillin	37.5	19.2	27.7	28.1
Ampicillin	56.2	38.4	57.1	53.3
Chloramphenicol	0.0	0.0	7.8	4.4
Chlortetracycline	0.0	0.0	1.3	0.0
Erythromycin	25.0	38.4	41.5	37.0
Gentamycin	31.2	26.9	24.6	26.7
Kanamycin	3.1	11.5	6.5	6.7
Neomycin	3.1	11.5	13.0	10.4
Novobiocin	37.5	34.6	46.7	42.2
Oxytetracycline	3.1	11.5	6.4	6.7
Penicillin-G	53.1	61.5	57.1	60.7
Streptomycin	3.1	3.8	6.5	5.2
Tetracyclin	3.1	0.0	2.6	2.2
Bacitracin	40.6	42.3	36.3	38.5
Furazolidone	28.1	7.7	22.1	20.7
Nalidixic acid	0.0	0.0	9.0	5.2
Nitrofurantoin	18.8	7.7	28.5	22.2
Oxacillin	68.7	69.2	75.3	72.6
Polymyxin-B	3.1	7.7	6.5	5.9
Sulphadiazine	59.3	57.6	64.9	62.2
Trimethoprim	9.4	26.9	13.0	14.8

n = Number of cultures tested

Note: Values given are percentage of cultures showing antibiotic resistance

Table 34: Antibiotic resistance pattern of microbial groups in traditional farms

Bacteria	Number of isolates tested	Number and percentage of antibiotics to which resistance was shown	
		(n)	(%)
Vibrio	112	20	95.2
Enterobactereaceae	32	12	57.1
Pseudomonas	20	13	61.9
Alcaligenes	32	10	47.6
Micrococcaceae	4	4	19.1
Bacillus	12	9	42.9
Lactobacillus	24	9	42.9
Arthrobacter	16	3	14.3

Table 35: Antibiotic resistance pattern of microbial groups in semi-intensive farms

Bacteria	Number of isolates tested	Number and percentage of antibiotics to which resistance was shown	
		(n)	(%)
Vibrio	132	21	100.0
Aeromonas	24	15	71.4
Enterobactereaceae	38	20	95.2
Acinetobacter	4	8	38.1
Pseudomonas	30	17	81.0
Flavobacterium	4	2	9.5
Alcaligenes	4	5	23.8
Cytophaga	2	10	47.6
Micrococcaceae	12	9	42.9
Bacillus	6	12	57.1
Lactobacillus	6	9	42.9
Arthrobacter	8	13	61.9

Development of antibiotic resistance among members of the native microbial populations in farm environment where antibiotics are routinely incorporated in feeds has been reported from India (Sanjeev, 1999; Surendran and Nirmala Thampuran, 1999; Surendran *et al.*, 2000) and other parts of the world (GESAMP, 1991, 1997; Twiddy and Reilly, 1995). Surendran and Nirmala Thampuran (1999) have also detected antibiotic residues in the tissues of farmed fish and shrimp (*P.monodon*) from Kerala and pointed out the health risks associated with the same. The present results suggest that antibiotic resistance of bacteria has become a common feature in both traditional and semi-intensive shrimp farms and the possibility of prevalence of same condition in the surrounding backwater cannot be ruled out considering the constant mixing taking place between the waters of the estuary and the shrimp farms during water exchange.

Studying the antibiotic resistance profile of bacterial isolates in integrated semi-intensive / intensive fish farms in the Southeast Asia, Twiddy and Reilly (1995) noticed that 74-75 % of the microorganisms tested by them were resistant to oxytetracycline and tetracycline, and 47 % to furazolidone, while resistance to the other antibiotics tested (nalidixic acid, oxolinic acid, chloramphenicol, neomycin and sulphamethoxazole combined with trimethoprim), which were also in common use in fish farms, were considerably low (6-25 %). The results obtained by Sanjeev (1999) from brackishwater culture pond at Cochin showed maximum resistance of bacterial strains to ampicillin, penicillin, polymyxin-B and erythromycin (68-97 %). All the strains, however, were found sensitive to chloramphenicol and as many as 68 % to gentamycin.

Surendran and Nirmala Thampuran (1999) and Surendran et al.(2000) also recorded maximum resistance of bacterial isolates towards erythromycin followed by ampicillin and cloxacillin and least resistance towards gentamycin, chloramphenicol, and chlortetracycline in semi-intensive shrimp farms. During the present study when the bacterial cultures were exposed to a large number of antibiotics including those commonly used in aquaculture farms (Primavera, 1993; GESAMP, 1997; Weston, 1997) as well as those predominantly used in hospitals to treat bacterial diseases in man (Geddes, 1982; Williams, 1982), the maximum frequency of resistance was observed towards antibiotics of clinical use rather than those used in aquaculture. This points to the possibility that either the shrimp farms receive considerable quantities of antibiotics of clinical origin through the source water and, as a consequence, the native flora of farm environment develops resistance to those antibiotics, or the farms receive bacterial strains which have already become resistant to such antibiotics in the backwater or elsewhere through the intake water during water exchange. Although data are lacking to substantiate the possibility of contamination of Cochin backwater system with antibiotic drugs of clinical origin, the numerous hospitals working in and around Cochin and the large-scale discharge of poorly-treated hospital sewages into this environment (Kumaran *et al.*, 1996)/ It has been reported that hospital environment is one of the major sources of antibiotic resistant bacteria and the sewage from hospitals serves as reservoirs of bacterial strains resistant to clinically used antibiotics (Linton *et al.*, 1974; Williams, 1982). According to Hatha and Lakshmanaperumalsamy (1995), the wrong use and abuse of antibiotics in human therapy has produced multiple antibiotic resistant (MAR)

/ would suggest that such contamination may be taking place in significant levels.

pathogenic microorganisms in the faeces of human beings. In many countries, release of pathogenic bacteria in faeces resulted in dispersal into aquatic systems were they contaminated the fish and shellfish harvested from such waters. These authors have opined that once these organisms are in the aquatic environment, plasmid exchange between the bacteria readily facilitated and results in higher frequency of MAR forms. During the present study, the antibiotic-resistance pattern was more or less similar in both types of culture systems. However, the level of antibiotic-resistance expressed by the organisms was relatively higher in semi-intensive farms than in traditional farms, which may be due to the more favourable environmental conditions prevailing there as a result of nutrient enrichment due to feed application.

4.6.2 Antibiotic resistance of human pathogens

The antibiotic resistance potential of human pathogens isolated from the environment and farmed shrimp such as *V. cholerae, V. parahaemolyticus, V. vulnificus, Salmonella* and *Staphylococcus aureus* was studied and the resistance pattern shown by the organisms in the traditional culture systems and semi-intensive farms are shown in Figs. 12 & 13. From traditional farms a total of 15 isolates of *V. cholerae*, 18 isolates of *V. parahaemolyticus* and 10 isolates of *V. vulnificus* from water, sediment and shrimp gut samples were tested for 21 antibiotics.

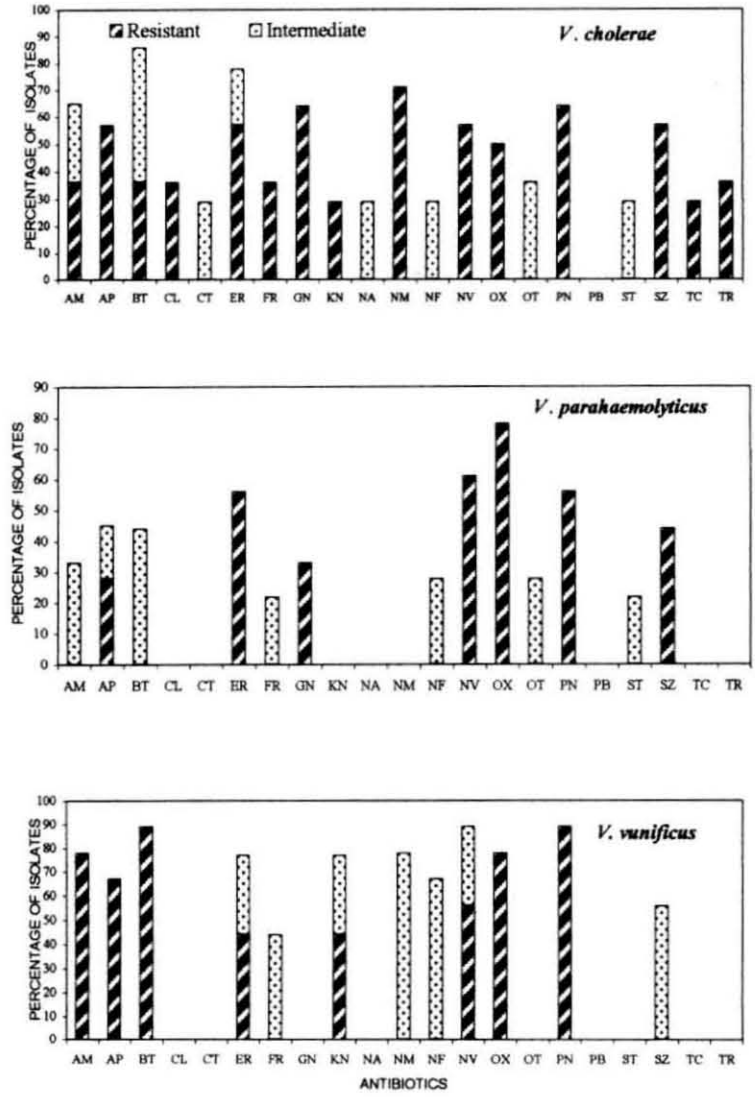

Fig. 12: Antibiotic resistance profiles of human pathogens isolated from traditional shrimp farms

Legend to antibiotics : AM - Amoxcycillin, AP - Ampicillin, BT - Bacitracin CL - Chloramphenicol, CT - Chlortetracycline, ER - Erythromycin, FR - Furazolidone, GN - Gentamycin, KN - Kanamycin, NA - Nalidixic acid, NM - Neomycin, NF - Nitrofurantoin, NV - Novobiocin, OX - Oxacillin, OT- Oxytetracycline, PN - Penicillin-G, PB - Polymyxin-B, ST - Streptomycin, SZ - Sulphadiazine, TC -Tetracycline, TR - Trimethoprim

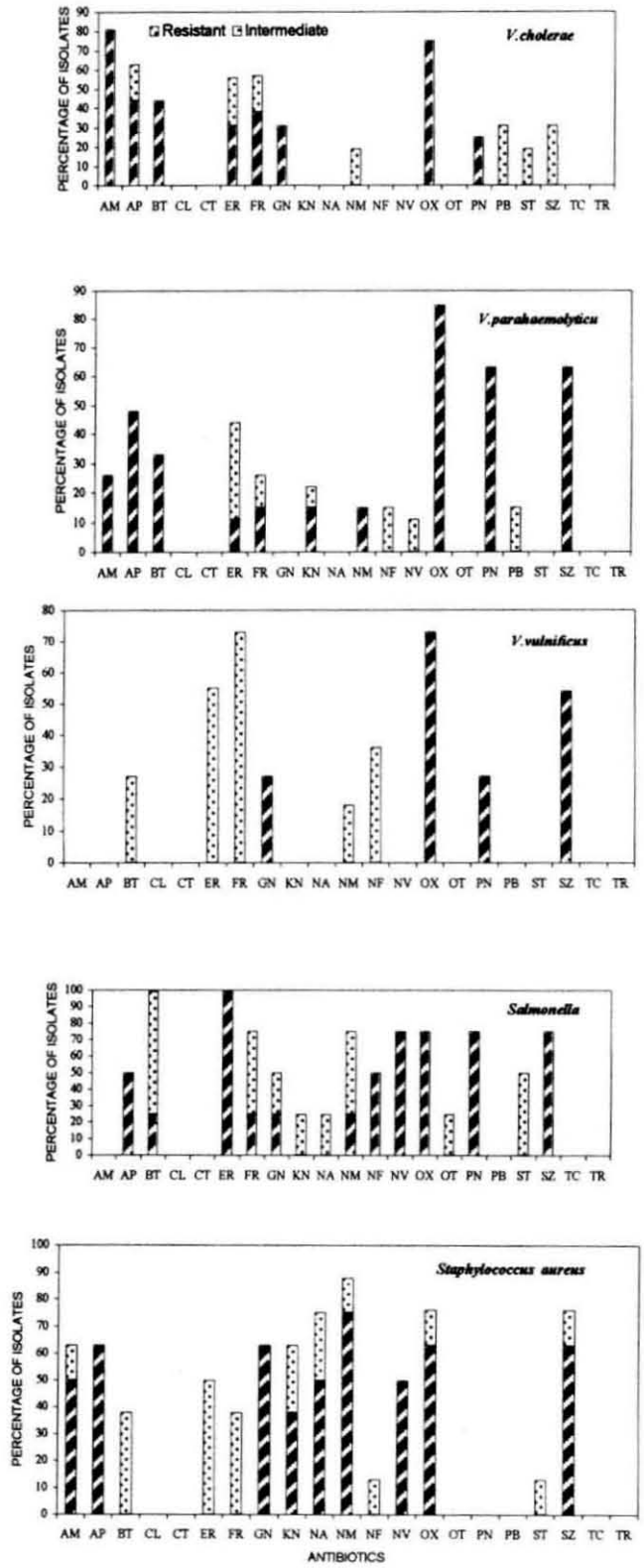

Fig. 13 : Antibiotic resistance profiles of human pathogens isolated from semi-intensive shrimp farms

In *V. cholerae*, 67 % of the isolates showed multiple antibiotic resistance (MAR) and the rest single drug resistance. A number of them also showed intermediate resistance to many antibiotics. While 70 % of the isolates were resistant to neomycin, 50-65 % showed resistance to ampicillin, erythromycin, gentamycin, novobiocin, penicillin-G, sulphadiazine and oxacillin. About 30 % of the isolates exhibited intermediate resistance to chlortetracycline, nalidixic acid, nitrofurantoin, oxytetracyline and streptomycin, while none of the isolates showed resistance to polymyxin-B. Isolates of *V. parahaemolyticus* showed resistance / intermediate resistance to 13/21 antibiotics and were sensitive to the rest of the antibiotics. All the strains, which were positive to the tests, were resistant to two or more antibiotics. The level of resistance to oxacillin was more than 80% and to erythromycin, novobiocin and penicillin-G it was between 55 and 65 %. In *V. vulnificus*, all the isolates examined proved to be MAR and showed high levels of resistance to bacitracin, penicillin-G, amoxcycillin and oxacillin (70-90 %). Significantly higher numbers of strains also showed resistance / intermediate resistance to ampicillin, erythromycin, kanamycin, neomycin, nitrofurantoin and novobiocin. MAR index values of the isolates (Krumperman, 1983) were 0.365 for *V. cholerae*, 0.206 for *V. parahaemolyticus* and 0.310 for *V. vulnificus*.

From semi-intensive farms, a total of 15 isolates of *V. cholerae*, 30 isolates of *V. parahaemolyticus*, 12 isolates of *V. vulnificus*, 4 isolates of *Salmonella* and 8 isolates of *S. aureus* were tested for antibiotic resistance (Fig. 13). *V. cholerae* strains showed resistance to 8 / 21 antibiotics and all of them were MAR. Resistance to amoxcycillin and oxacillin was above 70 % and a significant number of isolates (40-65 %) showed

resistance and intermediate resistance to ampicillin, erythromycin and furazolidone. About 20-45 % of the isolates were resistant to bacitracin, gentamycin and penicillin-G. In *V. parahaemolyticus* the isolates showed resistance to 10 of the antibiotics tested and all of them were MAR. About 80 % of the positive strains were resistant to oxacillin, 60-65 % to penicillin-G and sulphadiazine, 48 % to ampicillin and 35 % to bacitracin. The strains of *V. vulnificus* showed resistance to oxacillin (75 %), sulphadiazine (58 %), penicillin-G (25 %) and gentamycin (25 %), and intermediate resistance to five other antibiotics in varying levels. All the positive isolates were resistant to two or more antibiotics. The results of antibiotic resistance tests of 4 *Salmonella* strains isolated from shrimp surface showed resistance to 11 individual antibiotics. Three of the strains (75 %) showed MAR and one strain single drug resistance. All the strains were resistant to erythromycin, three to novobiocin, oxacillin, penicillin-G and sulphadiazine, two to ampicillin and nitrofurantoin and one each to bacitracin, furazolidone, gentamycin and neomycin. All the 8 strains of *S. aureus* tested were resistant to more than one antibiotic and majority of them showed MAR. The isolates showed resistance to 9 / 21 antibiotics of which resistance to neomycin was more than 70 % and to amoxcycillin, ampicillin, gentamycin, nalidixic acid, novobiocin, oxacillin and sulphadiazine between 50 – 65 %. MAR index values of the isolates of different pathogens were 0.222 for *V. cholerae*, 0.230 for *V. parahaemolyticus*, 0.143 for *V. vulnificus*, 0.286 for *Salmonella* and 0.262 for *S. aureus*

Development of antibiotic resistance among human pathogens in aquaculture systems is considered as a serious problem not only because it can cause fish-

treatment failures, but the resistant bacterial strains can enter the wider food chain and cause human health hazards. Resistant organisms present in an animal population may be transferred to humans through association with these animals or by contact with the animal products (Linton, 1977). Occurrence of antibiotic-resistant human pathogens such as *Salmonella*, *Aeromonas hydrophila*, *Plesiomonas shilgelloides* and *V. parahaemolyticus* in aquaculture systems and farmed animals as a consequence of drugs entering the farm environment through feed or other routes has been reported from different parts of the world including India (Twiddy and Reilly, 1995; GESAMP, 1997; Sanjeev, 1999; Surendran and Nirmala Thampuran, 1999; Surendran *et al.*., 2000).

Resistance of bacteria to antibiotics depends on the amount and kind of antibiotics used in the farms. All the reported cases of antibiotic-resistance of human pathogens from aquaculture environment were towards those drugs which were most frequently used such as oxytetracycline, chloramphenicol, chlortetracycline, tetracycline, furazolidone, oxolinic acid, nalidixic acid etc. (Twiddy and Reilly, 1995; GESAMP, 1997; Weston, 1997). The antibiotic-resistance pattern of human pathogens observed during the present study, however, was almost a reflection of the resistance pattern of the general flora of the culture systems, in which resistance to clinically used antibiotics such as oxacillin, erythromycin, penicillin-G, novobiocin, sulphadiazine, bacitracin and gentamycin was relatively high. Among *V. parahaemolyticus* strains isolated from semi-intensive shrimp farms at Cochin, Sanjeev (1999) noticed very high levels of resistance (68-92 %) to ampicillin, erythromycin, penicillin and polymyxin-B and least resistance (1-29 %) to

chloramphenicol, and tetracycline. During the present study it was also noticed that there was no significant variation in the pattern of antibiotic-resistance of bacterial strains between the two types of culture systems. This would indicate that development of antibiotic-resistance among the pathogens is unrelated to application of feed in the culture systems.

MAR indexing of bacterial isolates is attempted to find out the possible sources of contamination (Krumperman, 1983). According to this method, MAR index values higher than 0.2 are considered to have originated from high-risk sources of contamination like humans, commercial poultry farms, swine and dairy cattle where antibiotics are often used. MAR index values of less than or equal to 0.2 indicate a strain originated from animals in which antibiotics are seldom or never used. The MAR indexes determined in this study were above the arbitrary value of risk contamination (0.2) for all the human pathogens isolated from both types of culture systems except for *V. vulnificus* from semi-intensive farms. This points to the possibility that antibiotic - resistance in pathogenic strains also would have occurred from a common source of contamination and the most probable source could be hospital sewage that empties into the backwater. According to Kaspar *et al.* (1990), isolates with an identical MAR index and the same resistance profile may have a common origin. Another contributory factor for the development of antibiotic resistance among human pathogens detected during this study may be the heavy faecal pollution in Cochin backwater (Leela Menon, 1999), the only source of water for brackishwater shrimp farming in the area.

4.7 Detection of plasmid DNA in human pathogens

Ten strains of *V. parahaemolyticus* isolated from the shrimp culture systems were screened for the presence of plasmid. Of the ten strains (*Vp* 1 to *Vp* 10) analyzed for plasmid DNA profiling (Table-36), three strains recovered from traditional farms (*Vp* 1 to *Vp* 3) and four from semi-intensive farms (*Vp* 4, *Vp* 7, *Vp* 9 & *Vp* 10) proved negative for the presence of plasmids. The remaining strains from semi-intensive farms (*Vp* 5, *Vp* 6 & *Vp* 8), two of which were recovered from water and sediment during non-monsoon period and one from water during monsoon period, harboured plasmids. The plasmid DNA profiles of these strains resolved by agarose gel electrophoresis are shown in Plate-I. The plasmid profile varied significantly in terms of number and size of plasmids. While the strains isolated from water and sediment samples during non-monsoon period (*Vp* 5 & *Vp* 6) carried multiple plasmids, the one isolated from water sample during monsoon period (*Vp* 8) was found to harbour a single plasmid. Among the plasmid-positive strains with multiple plasmids, *Vp* 5 contained two plasmids (Plate-I, Lane 1), one smaller of 23 kb and the other bigger of about 33 kb sizes. The strain *Vp* 6 carried three plasmids (Plate-I, Lane 4), one smaller of approximately 23 kb and the others bigger of about 32 and 33.5 kb sizes. The size of the single plasmid detected in *Vp* 8 (Plate-I, Lane 2) was determined to be about 24 kb.

The presence of plasmid DNA in *V. parahaemolyticus* strains from shrimp culture systems observed during the present study is significant in the context of fairly higher levels of antibiotic resistance seen among the bacterial population of shrimp culture

systems including human pathogens, and the remarkable role of plasmids in conferring antibiotic resistance to the host organism. Plasmids have been found in wide variety of bacteria including pathogenic forms known to be associated with seafoods such as *E. coli*, *Salmonella* spp., *Vibrio* spp. and *Aeromonas* spp. and fish-spoilage bacteria such as *Pseudomonas* spp. (Twiddy and Reilly, 1995). Analyzing ten strains of *Salmonella* isolated from farmed shrimp (*P. monodon*) in Sri Lanka, Fonseka *et al.*, (1995) obtained a low plasmid carriage rate (10%) with a single plasmid (2 kb) for the positive strain. Information on plasmid DNA profile of *V. parahaemolyticus* strains from cultured fish / shrimp or pond environment is scanty. The limited studies conducted on this aspect by a few workers in recent times (Tushara, 1998; Li *et al.*, 1999) have shown strains carrying single or multiple plasmids with molecular weights ranging from 3 – 68 kb. A strain with a single plasmid of 68 kb size was also found to be resistant to ampicillin, cefuroxime and trimethoprim (Li *et al.*, 1999).

During the present investigation plasmids were detected in three strains out of the total ten strains examined, thus showing a plasmid carriage rate of 30 %. Although this level of plasmid carriage among *V. parahaemolyticus* strains does not signify any environmental hazard, the occurrence of multiple plasmid and invariably large sizes of them is indicative of the potential for serious consequence through transference and eventual spread of antibiotic resistance among the bacterial pathogens.

Table- 36 : Results of plasmid profiling of *V. parahaemolyticus* strains from shrimp culture systems

Strain number	Source of strain	Plasmid profile	
		No. of plasmids	Size of plasmids
V.p. 1	Traditional farm, non-monsoon, water	Plasmid free	-
V.p. 2	Traditional farm, non-monsoon, shrimp body	Plasmid free	-
V.p. 3	Traditional farm, monsoon, shrimp body	Plasmid free	-
V.p. 4	Semi-intensive farm, non-monsoon, shrimp surface	Plasmid free	-
V.p. 5	Semi-intensive farm, non-monsoon, water	Two	23 kb 33 kb
V.p. 6	Semi-intensive farm, non-monsoon, sediment	Three	23 kb, 32 kb 33.5 kb
V.p. 7	Semi-intensive farm, non-monsoon, shrimp body	Plasmid free	-
V.p. 8	Semi-intensive farm, monsoon, water	One	24 kb
V.p. 9	Semi-intensive farm, monsoon, shrimp body	Plasmid free	-
V.p. 10	Semi-intensive farm, monsoon, shrimp surface	Plasmid free	-

It is noteworthy that during the present study plasmids could be detected in bacterial strains only from semi-intensive farms, while none of the isolates examined from traditional farms showed the presence of plasmids. Tushara (1998), however, detected single and multiple plasmids in *V. parahaemolyticus* strains obtained from pokkali fields (traditional shrimp farms) at Cochin, and based on size variations of these plasmids she inferred that plasmids in the size range of 19-23 kb were R-plasmids encoding resistance to antibiotics. In the light of this report of plasmid-bearing strains of *V. parahaemolyticus* from traditional shrimp culture systems (Tushara, 1998) it may be concluded that development / acquisition of plasmid DNA in the human pathogen can not be related to application of feed in the culture system.

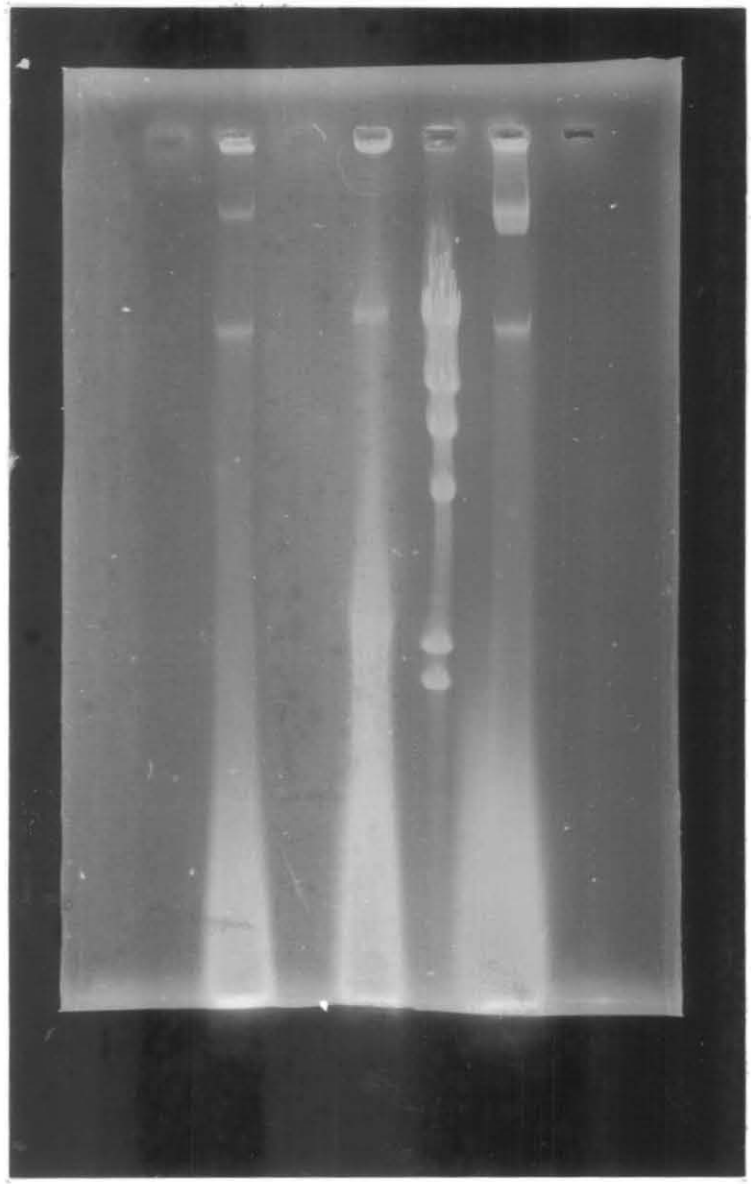

Plate: I - Agarose gel electrophoresis of plasmid DNA from *V. parahaemolyticus* strains. Lanes from left to right: Lane 1 – *Vp* 5, Lane 2 – *Vp* 8, Lane 3 – Standard DNA marker, Lane 4 – *Vp* 6.

5. SUMMARY

1. The thesis embodies the results of a study of the impact of supplementary feeding on environmental conditions and microbiological features of shrimp culture systems and farmed shrimp (*Penaeus indicus*) by a comparative evaluation of the physico-chemical and microbiological parameters of semi-intensive farms with those of traditional farms located around the Cochin backwater system during December '96 to April '98. The physico-chemical parameters examined included salinity, temperature, pH, dissolved oxygen, turbidity, ammonia, nitrate, nitrite, and total phosphorus and hydrogen sulphide content of pond water.

2. In traditional farms there was no supplementary feeding, and the prawn filtration process yielded monthly production of 35-100 kg / ha of shrimp during non-monsoon period and 20-70 kg/ha of shrimp during monsoon period, of which *P.indicus* constituted 30-40%. The semi-intensive farms carried out monoculture of *P.indicus* using hatchery-reared seed (PL-20) at stocking densities ranging 11-15 nos /m^2. Supplementary feeding was done using "HIGASHI" pelletised commercial feed, and the growout period extended 100-110 days during non-monsoon season and 120 days during monsoon season. The total feed input in a farm during a crop period varied from 2640 kg to 3570 kg/ha and the harvested shrimp production ranged from 2100 to 2500 kg/ha during non-monsoon season and 1300 kg/ha during monsoon season. The FCR for the nutritional supplement ranged from 1.5: 1 to 2:1 during the dry season and wet season respectively.

3. The mean values of environmental parameters of traditional farms were within the normal ranges (salinity: 1.05-32.17 ppt, temperature: 28.63-31.0 °C, pH: 7.45-8.83, dissolved oxygen: 5.79-7.93 mg/l, ammonia: 14.54-38.57 µg.at N/l, nitrite: 2.61-6.88 µg.at N/l, nitrate: 5.22-15.79 µg.at N/l, total phosphorus: 6.65-18.09 µg.at P/l, H_2S: 0.01-0.02 ppm), and exhibited the characteristic seasonal pattern reported for Cochin backwater system.

4. In semi-intensive farms, though salinity, temperature, and pH did not vary significantly from those of traditional farms, the dissolved oxygen concentration (4.14-5.09 mg/l) in all the farms monitored remained remarkably lower than in traditional farms which would suggest that a greater amount of dissolved oxygen available in the farm water was consumed disproportionate to the amount of oxygen that might have been produced within the system. It is possible that accumulation of organic matter from uneaten feed, faecal wastes and phytoplankton (as evident from secchi disc readings) in the semi-intensive farms, and its decomposition and successive oxidation processes mediated by bacteria would have accounted for the oxygen depletion.

5. Among the nitrogenous nutrients present in semi-intensive farms, ammonia formed the principal constituent followed by nitrate and nitrite in the order of concentration. In some of the farms it was also noticed that ammonia and nitrate contents in water were relatively higher than those in traditional farms, which may be due to the

application of feed and fertilizers during farming. Since the stocking density of shrimp seed and quantity of feed applied are of moderate level at present, the nutrient values recorded in the farms are not significantly high when compared to the same reported for intensive shrimp farming operations or experimental high-density shrimp farming trials reported from elsewhere in the country or abroad.

6. The distribution of temporal values of ammonia, nitrite and nitrate contents in semi-intensive farms has shown that the increase and decrease of nutrients in pond water occurred in sequential manner indicating that the two major steps involved in the nitrogen cycle, namely, ammonification and nitrification processes, were actively operative in these farms. Since the level of concentration of the three chemical species of nitrogen in the ponds examined was only moderate (Mxm. ammonia-51.42 µg.at N/l, nitrite-10.71 µg.at N/l and nitrate- 35.23 µg.at N/l) and within the acceptable limits, it is presumed that the supplementary feeding as practiced at present does not pose the problem of nutrient loading in the environment.

7. The commercial feed used in the semi-intensive farms contained a fairly large population of heterotrophic bacteria (10^3-10^5/g) which was dominated by Gram positive organisms (46-71%). Members of *Bacillus*, Enterobactereaceae and Micrococcaceae formed the major constituents of the microflora, and no pathogenic and indicator organisms were encountered.

8. Microbiological changes in pond environment and cultured shrimp were studied by examining samples of water, sediment, whole shrimp, shrimp surface and shrimp gut from the two types of culture system. Data on total plate counts (TPC) indicated that both traditional and semi-intensive farms harboured fairly large populations of heterotrophic aerobes throughout the culture period. In traditional farms, the abiotic samples showed mean bacterial counts ranging from $1.4x10^{3}$ to $4.1x10^{3}$/ml for water and $6.8x10^{3}$ to $3.2x10^{4}$/g for sediment while in semi-intensive farms the counts ranged from $3.9x10^{3}$ to $2.3x10^{5}$/ml for water and $6.9x10^{4}$ to $2.2x10^{5}$/g for sediment. In both types of farms, the bacterial load was invariably higher in sediment than in water. Though the total bacterial counts of water in shrimp farms did not show statistically significant correlation with any of the environmental parameters, the TPC of sediment had a significant ($P<0.05$) negative correlation with pH and dissolved oxygen content of water in traditional farms and with salinity, pH and dissolved oxygen content of water in semi-intensive farms.

A comparison of the total bacterial counts of water and sediment samples from semi-intensive farms with those of traditional farms has shown that the microbial load in all the semi-intensive farms examined was significantly higher. This suggests that increased nutrients and availability of substrates as a result of accumulation of feed derived wastes and other organic and inorganic matter on pond bottom had enhanced the multiplication of microorganisms in the farm environment. The increased bacterial load

in water and sediment of semi-intensive farms could be an indication of environmental quality deterioration.

9. The total bacterial counts of cultured shrimp remained generally higher than those of water and sediment in both types of culture system. The mean bacterial counts ranged from $4.9x10^5$ to $8.6x10^6$/g in whole shrimp, $3.4x10^5$ to $3.4x10^6$/cm^2 in shrimp surface and $2.7x10^7$/g in shrimp gut in traditional farms. In semi-intensive farms, the mean bacterial counts ranged from $8.7x10^4$ to $1.6x10^6$/g in whole shrimp and $1.8x10^4$ to $4.5x10^5$/cm^2 in shrimp surface and $1.2x10^6$/g in shrimp gut. In semi-intensive farms, there existed statistically significant ($P=<0.05$) positive correlation between TPC of whole shrimp and TPC of shrimp surface. From the results of this study it is evident that, when compared to shrimp harvested from traditional farms, the bacterial load of shrimp raised in semi-intensive farms has not changed significantly causing shrimp quality problems due to microbial abundance, and that it remains within the international standard of 10^6/g.

10. Qualitatively, Gram-negative organisms dominated over Gram-positive types in water, sediment, and shrimp in both traditional (67%) as well as semi-intensive (72%) farms. In traditional farms, the bacterial population comprised of members of the genera *Vibrio, Aeromonas, Moraxella, Acinetobacter, Pseudomonas, Alcaligenes, Bacillus, Lactobacillus* and *Arthrobacter* and the groups Enterobactereaceae and Micrococcaceae. Of these, members of *Vibrio* (21.6%), *Arthrobacter* (15.1%), *Pseudomonas* (13.9%), *Alcaligenes* (13.3%), Enterobactereaceae (8.9%), *Bacillus* (7.6%), *Acinetobacter* (7.6%)

and *Lactobacillus* (5.9%) formed the major constituents of the microflora. Considerable variations existed in the composition of the microflora among sample types and also between seasons. While in water the microflora was dominated by *Arthrobacter*, *Acinetobacter*, *Vibrio*, *Alcaligenes* and *Pseudomonas*, pond sediments showed dominance of *Bacillus, Alcaligenes, Arthrobacter, Vibrio* and *Pseudomonas* in the order of abundance. Predominance of *Lactobacillus*, *Vibrio*, Enterobactereaceae and *Pseudomonas* appeared to be characteristic of the microflora of *P. indicus* in these farms. Examination of shrimp gut flora during monsoon season showed predominance of *Vibrio*, *Aeromonas* and *Arthrobacter*. While no significant relationship could be established between shrimp and its pond environment with regard to the distribution of major bacterial genera in high-salinity farms during non-monsoon season, the abundance in shrimp of Enterobactereaceae, *Pseudomonas* and *Bacillus* in low-salinity farms during monsoon and non-monsoon seasons appeared to be a reflection of the sediment flora.

11. In semi-intensive farms the microflora consisted of all the bacterial types encountered in traditional farms, and in addition two more genera, namely, *Flavobacterium* and *Cytophaga* occurred sporadically. This suggests that most of these organisms are normal inhabitants of the shrimp culture systems of the area. The overall distribution of microorganisms in the pond environment and cultured shrimp of semi-intensive farms showed vibrios (32.4%) to be the most dominant group followed by *Pseudomonas* (13.7%), *Bacillus* (10.6%), Enterobactereaceae (10.3%), Micrococcaceae (8%), *Arthrobacter* (6.3%) and *Aeromonas* (5.4%) among major isolates. Vibrios

maintained dominance over all other bacterial types in water sediment, whole shrimp, shrimp surface and shrimp gut. Among the other important groups of microorganisms, considerable variations occurred in their composition among sample types and also between seasons. The distribution pattern and abundance of major bacterial genera/groups in different sample types examined has further revealed that the microflora associated with shrimp was more or less a reflection of the flora of water, sediment or both.

12. The results of the present study demonstrated that unlike in traditional shrimp farms in which the microbiological features were, to a large extent, similar to those of the surrounding backwater, the semi-intensive farms exhibited visible changes in qualitative distribution of bacterial flora as in the case of total plate counts in the farm environment as well as cultured *P.indicus*. It is therefore inferred that in semi-intensive farms some of the organisms (like vibrios) which are normally less abundant in traditional farms may proliferate and gain dominance over others with the enrichment of nutrients as a result of feed input and availability of favourable environmental conditions like salinity, temperature, pH and substrates (shrimp stocks and detritus) for their attachment, growth and multiplication. Shrimp feed also could have contributed to the increase in microbial density in the case of organisms like Enterobactereaceae, *Alcaligenes*, *Aeromonas* and Gram-positive organisms that dominated in the microflora of feed used in these farms.

13. The present observations further suggest that salinity exert a strong influence on the dominance of various physiological groups of bacteria in semi-intensive farms. Under high salinity conditions (28.2-35.2 ppt) the most dominant bacteria in the decreasing order of abundance are vibrios, *Alcaligenes, Bacillus, Arthrobacter*, Micrococcaceae and *Flavobacterium* in the environment, and vibrios, Enterobactereaceae, *Acinetobacter, Flavobacterium* and *Bacillus* in shrimp. In medium to very low salinity conditions (0.1-15.8 ppt), on the other hand, the flora is mainly dominated by vibrios, *Pseudomonas, Aeromonas, Bacillus, Arthrobacter, Lactobacillus* and Micrococcaceae in the environment and vibrios, *Pseudomonas*, Micrococcaceae, Enterobactereaceae, *Aeromonas* and *Arthrobacter* in shrimp.

14. The uniformly higher incidence of vibrios in all salinity conditions prevailing in semi-intensive farms during monsoon as well as non-monsoon seasons, and the incidence of *Aeromonas* in significant levels only in very low salinity condition prevailing during monsoon season are noteworthy. Although both these organisms form part of the natural flora of the shrimp farms, enrichment of nutrients and organic substrates favours their multiplication. In the case of *Aeromonas*, though the shrimp feed used was a possible contaminant of the organism in the semi-intensive culture systems, it was not isolated from any of the high-salinity farms even under nutrient-laden conditions. It is therefore possible that higher saline environment is not favourable for proliferation of these bacteria and on the contrary they thrive well and multiply in low salinity farms where

allochthonous organic substrates and nutrients have become plentiful as a result of application of feed and fertilizers.

15. Vibrios have caused a great deal of damage to the shrimp farming industry in Asia over the last several years. Therefore, emergence of vibrios as the most dominant group of the microbial flora of semi-intensive farms is a matter of great concern. As majority of the members of this genus are opportunistic pathogens, there are greater chances of them becoming pathogenic to shrimp in stressed environmental conditions associated with improper feed management practices. This can also result in microbiological quality problems for the cultured shrimp and shrimp products.

16. Analyses of samples for indicator organisms have shown that total coliforms, faecal coliforms and faecal streptococci occurred in varying levels in both traditional and semi-intensive farms. These organisms were detected in pond water, sediment and shrimp, the semi-intensive farms showing much higher incidence and counts of the organisms than traditional farms. In semi-intensive systems, *E. coli* was detected in water, sediment, whole shrimp and shrimp surface with the maximum MPN of 95/g in sediment. A common feature observed in both types of culture systems was the incidence of faecal coliforms only in those farms which operated during monsoon season. This may be due to the fact that relatively higher densities of the organisms would have occurred in the source water (Cochin backwater) following monsoon rains. Total coliforms, faecal coliforms and *E. coli* were maximum abundant in sediment followed by

whole shrimp in semi-intensive farms, which may be due to the availability of high nutrients and accumulation of organic substrates at pond bottom as a result of supplementary feeding and shrimp's bottom feeding habit.

17. The occurrence of bacteria of faecal origin in significant levels in both traditional and semi-intensive farms observed during the present study indicates insanitary condition of the farming. This shows that the water used in the culture systems is unclean or contains effluents from unhygienic sources. It is therefore essential that adoption of appropriate sanitation and hygiene principles is needed during processing of the cultured shrimp in order to ensure microbiologically safe frozen shrimp.

18. Analyses of samples were made for human pathogens such as *V. cholerae, V. parahaemolyticus, V. vulnificus, Salmonella* and *Staphylococcus aureus*. Samples from traditional farms showed the presence of all the three pathogenic vibrios in water, sediment and shrimp, while they proved negative for *Salmonella* and *S. aureus*. *Vibrio cholerae* and *V. parahaemolyticus* were isolated during monsoon and non-monsoons seasons, whereas *V. vulnificus* was observed during non-monsoon season. All the strains of *V. cholerae* isolated from these farms belonged to the non 01-serogroup and those of *V. parahaemolyticus* were Kanagawa negative. In semi-intensive farms, the samples showed the presence of all the five pathogenic forms, of which *V. cholerae, V. vulnificus, Salmonella* and *S. aureus* were encountered only during monsoon season, while *V. parahaemolyticus* occurred during monsoon as well as non-monsoon seasons in all

salinity gradients. All the isolates of *V. cholerae* belonged to the non-01 serogroup. In the case of *V. parahaemolyticus*, two of the ten positive strains (one each from water and whole shrimp), which were isolated during monsoon season, were Kanagawa positive.

19. The occurrence of *V. cholerae, V. parahaemolyticus*, and *V. vulnificus* in both types of culture systems, with no definite seasonal pattern, would imply that the brackish water shrimp farms and cultured shrimp at Cochin are inherently contaminated with these pathogenic organisms. In the case of *V. parahaemolyticus*, however, the incidence was relatively more in semi-intensive farms during monsoon season. This, coupled with the fact that strains of *Salmonella* and *S. aureus* could be isolated only from semi-intensive farm during monsoon period, would suggest that organic enrichment in the farm from feed wastes and also possibly from land drainage and heavy sewage discharge in the source water during monsoon season favours an increase of the pathogens. The occurrence of Kanagawa positive strains of *V. parahaemolyticus* during monsoon season further reveals the possibility of contamination of the pathogens from clinical sources.

20. Development of antibiotic resistance among general flora of the farm environment and cultured shrimp was studied by screening 252 bacterial isolates from traditional farms and 270 isolates from semi-intensive farms to 21 antibiotics (= antibacterials for sake of clarity) including those commonly used in shrimp aquaculture. In both types of culture systems, all the bacterial isolates tested showed resistance to one or more individual antibiotics. In semi-intensive farms, about 73 % of the cultures showed

resistance to oxacillin, 50-65 % to penicillin-G and ampicillin, 30-50% to novobiocin, bacitracin and erythromycin and 2-30 % to the rest of the antibiotics. *Vibrio* showed resistance to 95 % of the antibiotics in the traditional farms and to all the 21 antibiotics in semi-intensive farms. Almost all the antibiotics to which over 50 % of the bacterial isolates showed resistance are not normally used in aquaculture systems, and on the contrary, they are greatly in clinical application. The study has also shown that there was no significant difference in the pattern of antibiotic resistance of the general flora between traditional and semi-intensive farms, especially with regard to the intensity of resistance of the microorganisms to antibiotics of clinical origin. The present finding points to the possibility that either the shrimp farms (both types) receive considerable quantities of antibiotics of clinical origin through the source water and, as a consequence, the native flora of farm environment develops resistance to those antibiotics, or the farms receive bacterial strains, which have already become resistant to such antibiotics in the backwater or elsewhere, through the incoming water during water exchange.

21. Antibiotic resistance potential of human pathogens isolated from the culture systems was examined by screening the isolates to the same 21 antibiotics. Significant levels of antibiotic resistance were noticed among the microorganisms in both types of shrimp farms. Invariably all the organisms showed multiple drug-resistance (MAR). In traditional farms, maximum number of isolates were found resistant to neomycin (70 %) in *V. cholerae*, oxacillin (80 %) in *V. parahaemolyticus*, and to bacitracin, penicillin-G, amoxcycillin and oxacillin (70-90 %) in *V. vulnificus*, whereas in semi-intensive farms

the same were to amoxcycillin and oxacillin (70 %) in *V. cholerae*, oxacillin, penicillin-G and sulphadiazine (60-80 %) in *V. parahaemolyticus*, oxacillin (75 %) in *V. vulnificus*, erythromycin, novobiocin, oxacillin, penicillin-G and sulphadiazine (75-100 %) in *Salmonella* and to neomycin (70 %) in *S. aureus*. The antibiotic resistance pattern of human pathogens observed during the present study was almost a reflection of the resistance pattern of the general flora of the culture systems in which resistance to clinically used antibiotics such as oxacillin, erythromycin, penicillin-G, novobiocin, sulphadiazine, bacitracin and gentamycin was relatively high. It was also noticed that there was no significant variation in the pattern of antibiotic resistance of bacterial strains between the two types of culture systems. This would indicate that development of antibiotic resistance among the pathogens is unrelated to application of feed in the culture systems. The MAR indexes determined in this study were above the arbitrary value of risk contamination (0.2) for all the human pathogens isolated from both types of culture systems except for *V. vulnificus* from semi-intensive farms. This points to the possibility that antibiotic resistance in the pathogenic strains would have occurred from a common source of contamination and the most probable source could be hospital sewage that empties into the backwater.

22. Considering the remarkable roll of plasmid DNA in the transference of drug-resistance among microorganisms, ten strains of *V. parahaemolyticus* isolated from the shrimp farms were screened for the presence of plasmids by agarose gel electrophoresis. Three of the strains isolated from water, sediment and shrimp from semi-intensive farms

harboured single and multiple plasmids of the sizes 23 kb to 33.5 kb. The occurrence of multiple plasmids and invariably large sizes of them indicate the potential for transference and eventual spread of antibiotic-resistance among the bacterial pathogens. In the light of previous report of occurrence of plasmid-bearing strains of *V. parahaemolyticus* in traditional shrimp farms from the area, it is inferred that development / acquisition of plasmid DNA in the bacterium is not related to application of feed in the shrimp culture systems.

6. REFERENCES

Abraham, T.J. and R. Manley 1995. Luminous and non-luminous *Vibrio harveyi* associated with shell disease in cultured *Penaeus indicus*. J. Aqua. Trop., **10**: 273-276.

ADB/NACA 1991. Report on a regional study and workshop on fish diseases and fish health management. ADB Agric. Dept. Rep. Ser. No.1. Bangkok, Thailand.

Akiyama, D.M. 1991. Future considerations for the aquaculture feed industry, pp. 5-9. *In*: D. M. Akiyama and R. K. H. Tan (Eds.) Proceedings of the aquaculture feed processing and nutrition workshop, Thailand and Indonesia, September 19-25, 1991.

Akiyama, D.M. 1992. Nutrition of penaeid prawn. *In:* A.W. Fast and L.J. Lester (Eds.) Marine shrimp culture: Principles and Practices. Elsevier Science Publishers, Amsterdam, Holland.

Akiyama, D. M. and W.G. Dominy 1989. Penaeid shrimp nutrition for the commercial feed industry. *In*: Texas shrimp farming manual, vol. 1: Growout technology. Technical Report of Texas Agricultural Extension Service and Texas A and M. University Sea Grant College Programme, Corpus Christy, Texas, USA.

Akiyama, D.M. and N.L.M. Chwang 1995. Shrimp feed requirements and feed management. Aqua. International, **3**(4-5): 14-25, August 1995.

Akiyama, D.M., W.G. Dominy and A.L. Lawrence 1992. Penaeid shrimp nutrition. *In*: A.W. Fast and L.J. Lester (Eds.) Marine shrimp culture: Principles and Practices, Elsevier Science Publishers B.V., Chapter 25, pp. 535-567.

Alagarswami, K. 1995 a. Report on a Regional study and Workshop on the Environmental Assessment and Management of Aquaculture Development (TCP/RAS/2253). FAO, NACA, pp.141-186.

Alagarswami, K. 1995 b. Environmental issues of shrimp farming - Indian Experience. *In*: Indaqua'95, Madras, Jan-27-30, 1995, The MPEDA.

Alapide-Tendencia, E.V. and L.A. Dureza 1997. Isolation of *Vibrio* spp. from *Penaeus monodon* (Fabricius) with red disease syndrome. Aquaculture, **154**: 107-114.

Alavandi, S.V. 1989. Hetrotrophic bacteria in the coastal waters of Cochin. Ind. J. Mar. Sci., 18: 174-176.

Alderman, D.J. 1998. Fisheries chemotherapy: a review. *In*: J.F. Muir and R.J. Roberts(Eds.) Recent Advances in Aquaculture, Vol. 3: pp. 1-61. Croom Helm, London.

Ali, S.A. 1997. Shrimp feed industry's development in India. Aqua International, **4**(9): 18-26 p. January 1997.

Anderson, J.M. 1987. Production and decomposition in aquatic ecosystems and implications for aquaculture, p. 123-147. *In*: D.J.W. Moriarty and R.S.V. Pullin (Eds.) Detritus and microbial ecology in aquaculture. ICLARM Conference

Proceedings 14, 420 p. International Centre for Living Aquatic Resources Management, Manila, Philippines.

Anderson, I.G., M. Shamsudin, M. Shariff and G. Nash 1988. Bacterial septicemia in juvenile tiger shrimp, *Penaeus monodon*, cultured in Malaysian brackishwater ponds. Asian Fisheries Science.**2**: 93-108.

Andrews W.H. V. R. Bruce, G. June, F. Satchell, P. Sherrod 1992. *Salmonella.* Chap. 5 *In*: FDA Bacteriological Analytical Manual. 7th edition. p. 51-70. AOAC International , USA

Anonymous 1994. Aquaculture and environment. Fisheries World, September 1994, pp. 57-67.

Anonymous 1995. Nutrition and feeding of shrimp. Waterbase Infoshare, **2**(1): 1-6.

Anonymous 1997. International trade in aquaculture products. FAO Aquaculture Newsletter, No.17, pp. 2-6.

AOAC (ASSOCIATION OF OFFICIAL ANALYTICAL CHEMISTS) 1990. Official methods of analysis, 15th edition (AOAC). Arlington, VA.

Aoki, T. 1988. Drug-resistant plasmids from fish pathogens. Microbiological Sciences, **5**: 219-223.

Aoki, T. 1992. Present and future problems concerning the development of resistance in aquaculture. *In*: C.M. Michel and D.J. Alderman (Eds.) Chemotherapy in Aqiaculture: from theory to reality. Paris, Office International des Epizooties, pp. 254-262.

Aoki, Y., S. Hsu and D. Chun 1967. Distribution of *Vibrio parahaemolyticus* in the sea and harbours in Southeast Asia and Central Pacific. Endemic Diseases Bull.,Nagasaki University, **8**: 191-192.

Aoki, T., S. Egusa, Y. Ogata And T. Watanabe 1971. Detection of resistance factors in fish pathogen *Aeromonas liquefaciens*. Journal of General Microbiology, **65**: 343-349.

Aoki, T., T. Kanazawa And T. Kitao 1985. Epidemiological surveillance of resistant *Vibrio anguillarum* strains. Fish Pathol., **20**: 199-208.

Aoki, T., Y. Mitoma and J.H. Crosa. 1986. The characterization of a conjugative R-plasmid isolated from *Aeromonas salmonicida*. Plasmid, **16**: 213-218.

Apud, F., J.H. Primavera and P.L. Tores, Jr. 1989. Farming of prawns and shrimps. Aquaculture Extension Manual No.5, Third Edition. SEAFDEC, Aquaculture Department, Tigbauan, Iloilo, Philippines.

Armstrong, D.A. 1979. Nitrogen toxicity to crustacea and aspects of its dynamic in culture system. Second Biennial Crustacean Health Workshop, Texas, pp.329-360.

Austin, B. 1988 . Marine microbiology. Cambridge University Press, Cambridge, 222 p.

Austin, B. and D.A. Austin 1987. Bacterial fish pathogens: disease in farmed and wild fish. Ellis Horwood Ltd. John Wiley & Sons, New York, 317 p.

Avault, Jr. J.W. 1995. Quality assurance and HACCP, What they mean to you. Aquaculture Magazine, January/February 1995, pp. 65-71.

Ayyappan, S. 1998. Microbiological studies for sustained aquafarming practices. Proceedings of the Workshop, National aquaculture week, Jan-Feb, 1997, held at Vijayawada, Chennai, Mandapam Camp and Tuticorin, pp. 44-49.

Bailey-Brock, J.H. and S.M. Moss. 1992. Penaeid Taxonomy, Biology and Zoogeography. *In*: A.W. Fast and L.J. Lester (Eds.)1. Marine Shrimp Culture, pp. 9-27.

Baird, D.J., M.C.M. Beveridge, L.A. Kelly and J.F. Muir (Eds.) 1996. *Aquaculture and Water Resource Management.* Blackwell Science Ltd., UK.

Banchong, T. 1995. Environmental issues of shrimp farming South East Asian experience. *In*: Indaqua'95, Madras January 27-30, 1995, The Marine Products Export Development Authority.

Bandekar, J.R., R.Chander, D.P. Nerkar and N.F.Lewis 1982. Occurrence of Kanagawa positive *Vibrio parahaemolyticus* strains in shrimp (*Penaeus indicus*). Indian J. Microbiol., **22** : 247-248.

Banerjea, S.M. 1967. Some observations on water quality and soil condition of fish ponds in some states of India in relation to fish production. Indian J. Fish., **14**(1&2): 115-142.

Banerjee, R.K. and P.B. Pakrasi 1986. Physico-chemical nature and biomass production of newly constructed brackishwater impoundments (Nona Gheri) in the Lower Sunderbans.. Proc. Symp. Coastal Aquaculture, 4: 1103-1106.

Bang, U. and C.R. Lavilla-Pitogo 1996. The use of chemicals in aquaculture: A summary Brief of Two International Expert Meetings. FAO Aquaculture Newsletter No.14, December 12-14.

Baticados, M.C.L. 1988. Chapter 6: Diseases. *In*: Biology and Culture of *Penaeus monodon*. SEAFDEC Aquaculture Dept. Iloilo, Philippines.

Baticados, M.C.L., R.M. Coloso and R.C. Duremdez 1986. Studies on the chronic soft-shell syndrome in the tiger prawn, *Penaeus monodon* Fabricius, from brackishwater ponds. Aquaculture, **56**: 271-285.

Baticados, M.C.L., C.R. Lavilla-Pitogo, E.R.Cruz-Lacierda, L.D.De La Pena and N.A. Sunaz 1991. Studies on the chemical control of luminous bacteria *Vibrio harveyi* and *V. splendidus* isolated from diseased *Penaeud monodon* larvae and rearing water. Dis. Aquat. Org., **9**: 133-139.

Bauer, A.W., W.M.M. Kirby, J.C. Sherris and M. Turck 1966. Antibiotic susceptibility testing by standardized single disk method. American Journal of Clinical Pathology, **45**: 493-496

Beer, J.D. and R.E. Mclachlan 1998. HACCP implementation in US and developing countries. Aqua International, August-September 1988, pp. 7-11.

Bell, T.A. 1991. Overview of diseases and drug needs for major aquaculture species: Shrimp. Vet. Hum. Toxicol. 33 (Supplement 1): 19-23.

Bennett, R.W. and G. A. Lancette. 1992. *Staphylococcus aureus* Chap. 12. *In*: FDA Bacteriological Analytical Manual. 7th edition. p. 161-166. AOAC International , USA.

Bergheim, A. and T. Asgard 1996. Waste production from aquaculture. *In*: D.J. Baird, M.C.M. Beveridge, L.A. Kelly and J.F. Muir (Eds.) Aquaculture and Water Resource Management, Institute of Aquaculture, Blackwell Science Ltd., U.K.

Beveridge, M.C.M. and M.J. Phillips 1993. Environmental impact of tropical inland aquaculture, p. 213-236. *In*: R.S.V. Pullin, H. Rosenthal and J.L. Maclean (Eds.) Environment and aquaculture in developing countries. ICLARM Conf. Proc. 31, 359 p.

Beveridge, M.C.M., M.J. Phillips and R.M. Clarke 1991. A quantitative and qualitative assessment of wastes from aquatic animal production. *In*: D.W. Brune and J.R. Tomasso (Eds.) Aquaculture and Water Quality, pp. 506-533. The World Aquaculture Society, Baton Rouge, LA.

Blackburn, T.H. 1987. Role and impact of anaerobic microbial processes in aquatic systems, p. 32-53. *In*: D.J.W. Moriarty and R.S.V. Pullin (Eds.) Detritus and microbial ecology in aquaculture. ICLARM Conference Proceedings 14, 420 p. International Culture for Living Aquatic Resources Management, Manila, Philippines.

***Boyd, C.E.** 1981. Water quality in warm water fish ponds. Agricultural Experiment Station, Auburn, 359 p.

Boyd, C.E. 1982. Water quality management for pond fish culture. Elsevier, Amsterdam. p. 1-318.

Boyd, C.E. 1989. Water quality management and aeration in shrimp farming. Fisheries and Allied Aquacultures Departmental Series No.2, 183 p. Auburn University, Alabama.

Boyd, C.E. 1990. Water quality in ponds for Aquaculture. Alabama Agricultural Experimental Station, Auburn University, Auburn, AL., 482 p.

Boyd, C.E. 1995. Water quality management in shrimp farming. Fisheries World, India, October, 1995, pp. 33-38.

Boyd, C.E. 1997. Practical aspects of chemistry in pond aquaculture. The Progressive Fish-Culturist, **59**: 85-93.

Boyd, C.E. 1999. Codes of practice for responsible shrimp farming. Global Aquaculture Alliance, St. Louis, MO USA. 48p.

Boyd, C.E. and V.K. Pillai 1985. Water quality management in aquaculture. CMFRI Special Publication, No. 22, 97p.

Boyd, C.E. and A.W. Fast 1992. Pond monitoring and management. *In*: A.W. Fast and L.J. Lester (Eds.) Marine Shrimp culture: principles and practices. Developments in Aquaculture and Fisheries Science, Vol. 23. Elsevier, Amsterdam. pp. 497-513.

Boyd, C.E. and J.W. Clay 1998. Shrimp aquaculture and the environment. Scientific American, June 1998, pp. 59-65.

***Boyd, C.E. and T.S. Tucker** 1998. Pond aquaculture water quality management. Kluwer Academic Publishers, Boston, MA.

Boyd, E.B., L. Massaut and L.J. Weddig 1998. Towards reducing environmental impacts of pond aquaculture. INFOFISH International, pp. 27-33.

***Braaten, B. and H. Hektoen** 1991. The environmental impact of aquaculture. *In*: Report on a Regional study and Workshop on Fish Diseases and Fish Health Management. Fish Health Management in Asia-Pacific. ADB Agricultural Department Report Series No.1, June, 1991.

Brazil, G., D. Curley, F. Gannon and P. Smith 1986. Persistence and acquisition of antibiotic resistance plasmids in *Aeromonas salmonicida*. *In*: S.B. Levy and P.P. Novick (Eds.) Antibiotic Resistance genes: Ecology, Transfer and Expression, pp. 107-112. Cold Springs Harbor Laboratory, Cold Springs Harbor, N.Y.

Brown, E.E., (Ed.) 1983. World shrimp farming: Cultivation and Economics. 2nd ed., AVI publishing Co., Inc., Westport Connecticut, p. 1.

Brown, J.H. 1989. Antibiotics: their use and abuse in aquaculture. World Aquaculture **20**(2): 34-43.

Brune, D.E. and J.R. Tomasso (Eds.) 1991. Aquaculture and water quality. Advances in World Aquaculture, Vol. 3, 606 p.

Buchanan, R.E and N. E. Gibbons 1974. Bergey's Manual of Determinative Bacteriology. 8th ed., Baltimore. The Williams and Wilkins Company, 1268p

Cato, J.C. and C.A. Lima Dos Santos 1998. Bangladesh: Shrimp processors invest in plant upgrades and HACCP to produce safe products. INFOFISH International 5/98, pp. 51-56.

Chakrabarti, T., A.N. Chakrabarty and S.G. Dastidar. 1994. Plasmid-determined degradative metabolism and halophilism of *Vibrio parahaemolyticus*. Indian J. Exp. Biol., **32**: 619-622.

Chen, D. 1992. An overview of the disease situation, diagnostic techniques, treatments and preventives used on shrimp farms in China. *In*: W. Fulks and K.L. Main (Eds.) Diseases of cultured shrimp in Asia and the United States. The Oceanic Institute, Honolulu, Hawaii. pp. 47-55.

Chen, J.C. and Y.Z. Kou 1993. Accumulation of ammonia in the haemolymph of *Penaeus monodon* exposed to ambient ammonia. Aquaculture, **109**: 177-185.

Chen, L. 1990. Aquaculture in Taiwan. Fishing News Books.

Chen, S.N., S.L. Huang and G.H. Kou 1992. Studies on the epizootiology and pathogenicity of bacterial infections in cultured giant tiger prawns, *Penaeus monodon*, in Taiwan. *In*: W. Fulks and K.L. Main (Eds.) Diseases of cultured shrimp in Asia and the United States. The Oceanic Institute, Honolulu, Hawaii. pp. 195-205.

Cherian, P.V. 1967. Hydrological studies in and around Cochin Harbour. Bull. Dept. Mar. biol. Oceanogr. Univ Kerala., 9-17.

Chong, V.C. and A. Sasekumar 1981. Food and feeding habits of the white prawn *Penaeus merguiensis*. Mar. Ecol. Prog. Ser., **5**: 185-191.

Chopra, I. 1985. Mode of action of the tetracyclines and the nature of bacterial resistance to them. *In*: J.J. Hlavka and J.H. Boothe (Eds.) The tetracyclines. Berlin, Springer, Handb. Exp. Pharmacol., 78: 317-392.

Chou, R. 1995. Aquafeeds and feeding strategies in Singapore. *In*: M.B. New, A. G. J. Tacon and I. Csavas (Eds.) Farm-made aquafeeds, FAO Fisheries Technical paper, No.343, Rome, FAO, 434 p.

Christopher, F.M., C. Vanderzant, J.D. Parker and F.S. Conte 1978. Microbial flora of pond reared shrimp (*Penaeus stylirostris, Penaeus vannamei* and *Penaeus setiferus*.) J. Fd. Protection, **41**: 20-23.

Chua, T.E. 1992. Coastal Aquaculture Development and the Environment: The Role of coastal area management. Marine Pollution Bulletin,. 25, 1-4, pp. 98-103.

Chua, T.E., J.N. Paw and E. Tech 1989. Coastal aquaculture development in ASEAN: the need for planning and environmental management, pp. 57-70. *In*: T.E. Chua and D. Pauly (Eds.) Coastal area management in Southeast Asia: policies, management strategies and case studies. ICLARM Conference Proceedings 19, 254 p.

Chua, T.E., J.N. Paw and F.Y. Guarin 1989. The environmental impact of aquaculture and the effects of pollution on coastal aquaculture development in Southeast Asia. Marine Pollution Bulletin, **20**(7). 335-343.

Claybrook, D.L. 1983. Nitrogen metabolism. *In*: L.H. Mantel and L.H. Farmer (Eds.) The Biology of Crustacea. Vol. 5. Internal Anatomy and Physiological Regulation. pp. 163-213, New York, Academic Press.

Colt, J. 1984. Computation of dissolved gas concentrations in water as functions of temprature, salinity and pressure. Amer. Fish. Soc.,Publ. No. 14, Bethesda, Maryland, 154 p.

Colwell, R.R. 1984. Vibrios in the environment. John Wiley & Sons, New York.

Couch, J. 1978. Diseases, parasites, and toxic responses of commercial shrimps of the Gulf of Mexico and South Atlantic Coasts of North America. Fish. Bull. 76: 1-44.

Cruz, P.S. 1991. Shrimp feed management: Principles and Practices. Kabukiran Enerprises, Inc. Davao City, Philippines, 460 p.

Csavas, I. 1995. Development of shrimp farming with special reference to Southeast Asia. Fishing Chimes, pp. 33-43

Dall, W. 1968. Food and feeding of some Australian penaeid shrimp. FAO Fish. Report, 57: 251-258.

Dall, W., B.J. Hill, P.C. Rothlisberg and D.J. Staples 1990. Morphology Vol. 27 p. 21-29. *In*: J.H. S. Blaxter and A.J. Southward (Eds.) The biology of Penaeid, Advances in Marine Biology, 489 p. Academic Press, London.

Dalmin, G., A. Purushothaman, E. Kannapiran, G. Sankar and K. Srinivasan. 1997. Microbiology of a shrimp pond with reference to total heterotrophic, coliform and beneficial bacteria. *In*: R. Santhanam, V. Ramadhas and P. Gopalakrishnan (Eds.) Proceedings of the National Seminar on Water Quality Issues in Aquqculture Systems, pp. 9-14.

Datta, N.C., S. Chaudhuri, M. Das and B. Ghosh 1983. Ecological characteristics of brackishwaer bheri of the Suderbans, West Bengal. Env. Ecol., 1(4): 235-239.

Datta, A.R., J.B. Kaper and A.M. Macquillan 1984. Shuttle cloning vectors for the marine bacterium *Vibrio parahaemolyticus*. J. bacteriol., **160**(2): 808-811.

Debevere, J. and K. Neyts 1998. Application of HACCP in processing raw tropical shrimp. INFOFISH *International* 3/98, pp. 49-55.

De La Cruz, M.C., G.Erazo and M.N. Bautista 1989. Effect of storage temperature on quality of diets for the prawn. *Penaeus monodon* (Fabricius). Aquaculture, **80**: 87-95.

Devapiriyan, R. 1990. Studies on the ecology and production of algae in prawn culture systems. Ph. D. Thesis, Cochin Univ. Sci. & Tech. 81 p. (Un pub.).

Devassy, V.P. and P.M.A. Bhattathiri 1974. Phytoplankton ecology of the Cochin backwater. Indian J. mar. Sci., **3**: 46-50.

De Voc, M.R. 1994. Aquaculture and the marine environment: Policy and management issues and opportunities in the United States. Bull. Natl. Res. Inst. Aquaculture, Suppl. 1: pp. 111-123.

Divakaran, S., B. R. Leamaster and W.A. Walsh 1994. Long-term storage stability of low-moisture shrimp feeds. J. Aqua. Trop., **9**: 55-66.

Djunaidah, I.S. 1995. Aquafeeds and feeding strategies in Indonesia. *In*: M.B. New, A.G.J. Tacon, and I.Csavas (Eds.) Farm-made aquafeeds, FAO Fisheries Technical paper, No.343, Rome, FAO, 434 p.

Elliot, E.L., C.A. Kaysner, M.L. Tamplin 1992. *V.cholerae, V. parahaemolyticus, V.vulnificus*, and other *Vibrio* spp Chap. 9. *In*: FDA Bacteriological Analytical Manual. 7th edition. p. 17-26. AOAC International , USA

FAO/NACA 1995. Regional study and workshop on the environment assessment and management of aquaculture development. NACA Envir. Aquacult. Dev. Ser., (1): 492 p.

Fast, A.W. 1992. An overview of Asian marine shrimp culture. *In*: A.W. Fast and L.J. Lester (Eds.) Marine Shrimp Culture: Principles and practices. Developments in Aquaculture and Fisheries Science, Vol. 23. Elsevier, Amsterdam. pp. 623-640.

FDA 1972. FDA Bacteriological Analytical Manual, Food and Drug Administration, Bureau of Foods, AOAC Washington, DC, USA.

FDS (Feed Development Section) 1994. Feed and feeding of Milkfish, Nile Tilapia, Asian Sea Bass, and Tiger shrimp. SEAFDEC Aquaculture Department, Tigbauan, Iloila, Philippines, 97 pp.

Fenchel, T. and T.H. Blackburn 1979. Bacteria and mineral cycling. Academic Press, London.

Folke, C. and N. Kautsky. 1992. Aquaculture with its Environment: prospects for sustainability. Ocean & coastal management, 17: 5-24.

Fonseka, T.S.G. 1990. Microbial flora of pond cultured prawn (*Penaeus monodon*). FAO Fish. Rep., 401: 24-31.

Fonseka, T.S.G. 1995. Ribotyping of *Salmonella* isolated from farm shrimp (*Penaeus monodon*). FAO Fisheries Report. No.514, Suppl. Rome, pp. 45-55.

Fry, J.C. 1987. Functional roles of the major groups of bacteria associated with detritus, p. 83-122. *In* : D.J.W. Moriarty and R.S.V. Pullin (Eds.) Detritus and microbial ecology in aquaculture. ICLARM Conference Proceedings 14, 420 p. International Centre for living Aquatic Resources Management, Manila, Philippines.

Fulks, W. and K.L. Main ((Eds.).) 1992. Diseases of cultured shrimp in Asia and the United States. The Oceanic Institute, Honolulu, Hawaii, 392 p.

Geddes, A.M. 1982. The impact of clinical practice of antibiotic-resistant micro-organisms. *In:* C.H. Stuart-Harris and D.M.Harris, (Eds.) The Control of Antibiotic- Resistant Bacteria, Academic Press, London, pp. 1-16.

George, M.J. 1958. Observations on the Plankton of Cochin Backwater. Indian J. Fish. **5**: 375-401.

George, K.V. 1974. Some aspects of prawn culture in the seasonal and perennial fields of Vypeen Island. Indian J. Fish, **21**(1): 1-19.

George, M.J. 1975. Observations on the growth in certain penaeid prawns studied in prawn culture experiments in paddy field. Bull. Dept. Mar. Sci. Univ. Cochin, **7**(1): 41-53.

George, M.J., K.H. Mohamed and N.N. Pillai 1968. Observations on the paddy field prawn filteration of Kerala, India. FAO. Fish Rep., **57**(2): 427-442.

GESAMP (IMO/FAO/UNESCO/WMO/WHO/IAEA/UN/UNEP) 1991. Joint group of experts on the scientific aspects of marine pollution) Reducing environmental impacts of coastal aquaculture. Rep. Stud. GESAMP, (47): 35 p.

GESAMP (IMO/FAO/UNESCO-IOC/WMO/WHO/IAEA/UN/UNEP Joint Group of Experts on the Scientific Aspects of Marine Environmental Protection) 1997. Towards safe and effective use of chemicals in coastal aquaculture. *Rep. Stud. GESAMP*,(65): 40 p.

Gibert, P.G. and V.K. Pillai 1986. Physico-chemical characteristics of soils in the aquaculture systems of Cochin estuarine area. J.aqua. Trop., **1**(2): 161-170.

Gopakumar, G. 1998. Risks associated with contaminated fish from aquaculture and control measures. Paper presented at the National Symposium on Sustainable Aquaculture organised by Society of Ocean Scientists and Technologists and Dept. of Botany, University of Delhi, February 20-21, 1998, New Delhi.

Gopalakrishnan, P., V. Ramadhas and V. Sundararaj 1997. Nitrification in a semi-intensive shrimp culture system. *In*: R. Santhanam, V. Ramadhas and P. Gopalakrishnan (Eds.) Proceedings of the National Seminar on Water Quality Issues in Aquaculture systems, pp. 23-27.

Gopalakrishnan, T.C., C.B. Lalithambika Devi, P.N. Aravindakshan, K.K.C. Nair, T. Balasubramanian and M. Krishnankutty. 1988. Phytoplankton and zooplankton of some of the paddy-cum prawn culture fields in and around Cochin. Mahasagar, Bull. Natn. Inst. Oceanogr., **21**(2): 85-94.

Gopalakrishnan, V. 1952. Food and feeding habits of *Penaeus indicus*. J. Madras Univ., **22**: 69-75.

Gopinath, K. 1956. Prawn culture in the rice fields of Travancore-Cochin India. Proc. Indo-Pacif. Fish. Counc., 6th Sess., 18: 419-424.

Gopinathan, C.P. 1972. Seasonal abundance of phytoplankton in the Cochin backwater. J. Mar. biol. Ass. India, **14**(2): 568-577.

Gopinathan, C.P., P.V.R.Nair and A.K.K. Nair 1974. Studies on the phytoplankton of the Cochin backwater, a tropical estuary. Indian J. Fish., **21**(2): 501-513.

Gopinathan, C.P., P.V.R. Nair, V.K. Pillai, P.P.Pillai, M. Vijayakumaran and V.K. Balachandran. 1982. Environmental characteristics of the seasonal and perennial prawn culture fields in the estuarine system of Cochin. *Proc. Symp. Coastal Aquaculture*, Marine Biological Association of India, Pt.1: 369-382.

Gore, P.S., O.Raveendran and R.V. Unnithan 1979. Pollution in Cochin backwaters with reference to indicator bacteria. Ind. J. Mar. Sci., Vol. **8** : 43-46.

***Hall, G.H. and C. Jeffries** 1984. The contribution of nitrification in the water column and profundal sediments to the total oxygen deficit of the hypolimniion of a mesotrophic lake (Grasmere, English Lake District). Micro. Ecol., **10** : 37-46.

Hameed, A.S.S. 1994. Experimental transmission and histopathology of brown spot disease in shrimp (*Penaeus indicus*) and lobster (*Panulirus homarus)*. J. Aqua. Trop., **9**: 311-322.

Hameed, A.S.S. and P.V.Rao 1994. Studies on the chemical control of a *Vibrio Campbellii*-like bacterium affecting hatchery-reared *Penaeus indicus* larvae. Aquaculture, **127**: 1-9.

Hansen, P.K., M.B.T. Lunestad and O.B. Samuelsen 1992. Effects of oxytetracycline, oxalinic acid, and flumequine on bacteria in an artificial marine fish farm sediment. Canadian Journal of Microbiology, 38, 1307-12.

Haridas, P., M. Madhupratap and T.S.S. Rao 1973. Salinity, temperature, oxygen and zooplankton biomass of the backwaters from Cochin to Alleppey. Indian J. Mar. Sci., **2**(2): 94-102.

Hatha, A.A.M and P. Lakshmanaperumalsami 1995. Antibiotic resistance of *Salmonella* strains isolated from fish and crustaceans. Letters in Applied Microbiology, **21**: 47-49.

Henry, D.M. II 1996. Aquaculture and the law: A primer Article Three of a series. Aquaculture Magazine, May/June 1996, pp. 72-79.

***Herbert, R. A.** 1982. Nitrate dissimilation in marine and estuarine sediments, pp.53-72. *In*: D.B. Nedwell and C.M. Brown (Eds.) Sediment microbiology. Academic Press, London.

Hirond Yosuke and Mark Leslie 1992. Shrimp culture industry in Ecuador. *In* A.G.Fast and L.J. Lester, (Eds.) Marine shrimp culture: Principles and practices. pp. 783 – 815, Amsterdam: Elsevier.

Hitchins, A.D., P. Feng, W.D. Watkins, S.R. Rippey and L.A. Chandler 1992 *Escherichia coli* and the colform bacteria. Chap. 4. *In*: FDA Bacteriological Analytical Manual. 7th edition. p. 27-49. AOAC International , USA

Hopkins S. J. 1992. Shrimp culture in the United States. *In*: A.W. Fast and L.J. Lester, (Eds.) Marine shrimp culture: Principles and practices. Amsterdam, Elsevier.

Hopkins, S. J., C.L. Browdy, D.R. Hamilton, and J.A. Heffernan J.A. 1995 a . The effect of low-rate sand filteration and modified feed management of effluent quality, pond water and production of intensive shrimp ponds. Estuaries, 18, 1A: 116-123.

Hopkins, S.J., P.A. Sandifer, M.R. De Voe, A. Frederick Holland, C.L. Browdy and A.D. Stokes 1995 b. Environmental impacts of shrimp farming with special reference to the situation in the continental United States. Estuaries, **18**, 1A, P. 25-42.

Huss, H.H. 1991. Public health aspects of seafood consumption. Infofish International 3/91. pp.27-32.

Indrani Karunasagar, S.K.Otta and I. Karunasagar 1997. Histopathological and bacteriological study of white spot syndrome of *Penaeus monodon* along the west coast of India. Aquaculture, **153**: 9-13.

Inglis, V., E. Yimer, E.J. Bacon and S. Ferguson 1993 a. Plasmid-mediated antibiotic resistance in *Aeromonas salmonicida* isolated from Atlantic Salmon, *Salmo salar* L., in Scotland. Journal of Fish Diseases, **16**: 593-597.

Inglis, V., S. D. Millar and R.H. Richards 1993 b. Resistance of *Aeromonas salnonicida* to amoxicillin. Journal of Fish diseases, **16**: 389-395.

Inglis, V., R. J. Roberts and N. R. Bromage 1993 c. Public health aspects of bacterial infections of fish. *In*: Bacterial diseases of fish. pp. 284 -303.

Ingole, B.S., A.Z. Ansari, and A.R. Sreepada 1994. Aquaculture practices and the coastal marine environment. Proceedings of Seminar on Ocean and industry: Realities and expectations, held on October 25, NIO: 93-99.

Ishimaru, K., M. Akagawa-Matsushita and K. Muroga 1995. *Vibrio penaeicida* sp. nov., a pathogen of Kuruma prawn (*Penaeus japonicus*). Int. J. Syst. Bacteriol., **45**: 134-138.

Ivy Thomas, 1982. Studies on chitinoclastic bacteria of the coastal zones of Cochin. Ph.D. Thesis, Cochin University, 215 p. (Un pub.)

Johnson, S.K. 1975. Handbook on shrimp diseases. Texas A & M Univ. Sea Grant Publ. No. SG-75-603, 19 p.

Johnson, S.K. 1989. Handbook of Shrimp Diseases. Texas A & M Sea Grant College Program, Texas A & M University, College Station, TX. 25 p.

Jory, D.E. 1995a. Shrimp aquafeeds in commercial production ponds. *Aquaculture Magazine*, Sep/Oct. 1995, pp. 89-92.

Jory, D.E. 1995b. Feed management practices for a healthy pond environment. Swimming through troubled water. Proceedings of the special session on shrimp farming : World Aquaculture Society: 118-143.

Jory, D.E. 1996. Management of commercial and farm-made feeds in marine shrimp ponds. Aquaculture Magazine, Jan/Feb. 1996, pp. 86-97.

Josanto, V. 1971. The bottom salinity characters and the factors that influence the salt water penetration in the Vembanad Lake. Bull. Dept. Mar. bio. Oceanogr., 5 : 1-16.

Josanto, V. 1975. Bottom salinity characteristics of the Cochin backwaters. Proc. Symp. Estuarine. Biol. Annamalai Univ.pp. 223-230.

Joseph, K.C., M. Muralidhar, B.P. Gupta, K.K. Krishnani, P. Ravichandran, K.K. Vijayan and M. Krishnan 1999. *In*: National Symposium on "Sustainable development of fisheries towards 2020 AD - Opportunities & Challenges", 21-23 April, 1999, School of Industrial Fisheries, Cochin University of Science and Technology, Cochin, India (Under publication).

Joseph, S.W., R.R. Colwell, and J.B. Kaper 1983. *Vibrio parahaemolyticus* and related halophilic vibrios. Crit. Rev. Microbiol. **10** : 77-124.

Joshi, K.K. 1990. Nature and ecological significance of nutrient regeneration in different prawn culture fields. Ph.D. Thesis, Cochin Univ. Sci & Tech., Cochin, 192 p (Un pub.).

Kanazawa, A. 1981. Penaeid Nutrition. *In*: G.D. Pruder, C.J. Langdon and D.E. Conklin (Eds.) Proc. Second Int. Conf. Aquacult. Nutrition: Biochemical and Physiological Approaches to shellfish nutrition, World Maricult. Soc., Special Pub. 2: 87-105.

Kanazawa, A. 1984. Nutrition of penaeid prawns and shrimps. *In*: Proceedings of the first International Conference on the culture of penaeid prawns/shrimps, Iloilo City, Philippines, SEAFDEC Aquaculture Department.

Kanazawa, A. 1985. Nutrition of penaeid prawns and shrimps. *In*: Y. Taki. J.H. Primavera and J.A. Llobrera, (Eds.) Proc. of the First Int. Conf. on the culture of Penaeid Prawns/Shrimps, pp. 123-30. 4-7 Dec. 1984, Aquaculture Dep. SEAFDEC, Iloilo City, Philippines.

Karunasagar, I. and K.C. Mohankumar 1980. Occurrence of Kanagawa positive *Vibrio parahaemolyticus* stains around Mangalore (South India). Indian J. Med. Res., 72: 619-621.

Karunasagar, I., M. Susheela, G.R. Malathi and I. Karunasagar 1990. Incidence of human pathogenic vibrios in seafoods harvested along the coast of Karnataka (India). FAO Fish. Rep., 401: 53-56.

Karunasagar, I., S.M. Ismail H.V. Amarnath and Indrani Karunasagar 1991. Bacteriology of tropical shrimp and marine sediments. FAO Fish. Rep., 470: 1-7.

Karunasagar, I., R. Pai, G.R. Malathi and I. Karunasagar 1994. Mass mortality of *Penaeus monodon* larvae due to antibiotic resistant *Vibrio harveyi* infection. Aquaculture, **128**: 203-209.

Karunasagar, I. and Indrani Karunasagar 1995. Shrimp disease and control. Fisheries World, September/October, 1995, pp. 15-19.

Karunasagar, I., S.K. Otta and Indrani Karunasagar 1998. Disease problems affecting cultured penaeid shrimp in India. Fish Pathology, **33**: 413-419.

Kaspar, W., J.I. Burgess, I.T. Knight and R.R.Colwell 1990. Antibiotic resistance indexing of *Escherichia coli* to identify the sources of faecal contamination in water. Canadian Journal of Microbiology, **36**: 891-894.

***Kinne, O.** 1976. Cultivation of marine organisms: water quality management and technology. *In*: O. Kinne (Ed.) Marine Ecology, Vol. III, Part 1. Wiley-Interscience, New York, NY, pp. 79-300.

Kirchman, D.L. and H.W. Ducklow 1987. Trophic dynamics of particle - bound bacteria in pelagic ecosystems: a review, p. 54-82. *In*: D.J.W. Moriarty and R.S.V. Pullin (Eds.) Detritus and microbial ecology in aquaculture ICLARM Conference Proceedings 14,420 p. International Centre for Living Aquatic Resources Management, Manila, Philippines.

Kissil, G. Won., I. Lupatsch & A. Neori 1992. Approaches to fish feed in Israeli Mariculture as a result of environmental constraints. *In*: G.Won. Kissil and L. Saar (Eds.) Proc. U.S.-Israel Workshop on Mariculture and the Environment, pp. 359-369.

Kompiang, I.P. 1990. Shrimp nutrition. *In*: M.B. New, H.D. Saram and T. Singh (Eds.) Technical and economic aspects of Shrimp Farming. Proc. Aquatech'90 Conference, Kaula Lumpur,Malaysia, 11-14 June 1990, pp. 69-73.

Kongkeo, H. 1990. Pond management and operation. *In*: M.B. New, H. de Saram and T. Singh (Eds.) Technical and economic aspects of shrimp farming. Proceedings of *Aquatech'90* Conference, Kuala Lumpur, Malaysia, 11-14 June 1990, pp. 56-65.

Krishnan, P. and V. Ramadhas 1997. Microbial oxidation of organic and inorganic substrates in aquaculture systems. *In*: R. Santhanam, V. Ramadhas and P. Gopalakrishnan (Eds.) Proceedings of the National Seminar on Water Quality Issues in Aquaculture Systems, pp. 123-130.

Krumbein, W.E. 1983. Microbial geochemistry. Blackwell Scientific Publications, Oxford.

Krumperman, P.H. 1983. Multiple antibiotic resistance indexing of *Escherichia coli* to identify high risk sources of faecal contamination of foods. Applied and Environmental Microbiology **46**, 165-170.

Kumaran, S. and T.S.S. Rao 1975. Phytoplankton distribution and abundance in the Cochin backwaters during 1971-72. Bull. Dept. Mar. Sci. Univ. Cochin, **7**(4): 791-799.

Kumaresan, S., K.K. Ashokan, O. Raveendran and V.N. Sankaranarayanan 1996. Bacteriological studies on the mangroves in and around Cochin. Proc. Eighth Kerala Science Congress, Jan., 1996, Kochi, pp.90-91.

Kumaresan, S., A. Sethuraman and A.N. Subramanian 1997. Some aspects of soil and water quality management in a modified extensive shrimp farm with special reference to iron and copper. *In*: R. Santhanam, V. Ramadhas and P. Gopalakrishnan (Eds.) Proceedings of the National Seminar on Water Quality

Issues in Aquaculture Systems, Dec. 18 & 19, 1996, Fisheries College and Research Institute, TNVASU, Tuticorin, pp. 37-42.

Lakshmanan, P.T., C.S. Shynamma, A.N. Balchand, P.G. Kurup and P.N.K. Nambisan 1982. Distribution and seasonal variation of temperature and salinity in Cochin Backwaters. Indian J. Mar. Sci., **11**(2): 170-172.

Lakshmanan, P.T., C.S. Shyanamma, A.N. Balachand and.N.K. Nambisan 1987. Distribution and variability of nutrients in Cochin backwater, Southwest coast of India. Indian J. Mar. Sci., **16**(2): 99-102.

Lalitha, K.V. 1998. Ecology and pathogenicity of the anaerobic pathogen *Clostridium botulinum* seen in farmed fish, shellfish and fisheries products. Ph.D. Thesis , Central Institute of Fisheries Education (Deemed University), Mumbai, 208 p. (Un pub.).

Lavilla-Pitogo, C.R. 1995. Bacterial diseases of penaeid shrimps: an Asian view. *In*: M. Shariff, J.R. Arthur and R.P. Subasinghe (Eds.) Diseases in Asian aquaculture II. Asian Fisheries Society, Manila, pp. 107-121.

Lavilla-Pitogo, C.R. and L.D.De La Pena 1998. Bacterial diseases in Shrimp (*Penaeus monodon*) culture in the Philippines. Fish Pathology, **33**(4): 405-411.

Lavilla-Pitogo, C.R., M.C.L. Baticados, E.R. Cruz-Lacierda and L.D.De La Pena 1990. Occurrence of luminous bacterial disease of *Penaeus monodon* larvae in the Philippines. Aquaculture, **91**: 1-13.

Leela Menon 1999. Vypeen Island to get environmental facelift. The New Express, Kochi: 4 April : 3.

Lester J. L. 1992. Overview of shrimp farming in the Western Hemisphere. *In*: Fast A.W. and L.J. Lester (Eds.) Marine shrimp culture: Principles and practices. pp. 771-782. Amsterdam: Elsevier.

Li, J., J. Yie, R.W.T.Foo, J.M.L. Ling, H. Xu and N.Y.S.Woo 1999. Antibiotic resistance and plasmid profiles of Vibrio isolates from cultured silver sea bream, *Sparus sarba*. Marine Pollution Bulletin, 39 (1-12): 245-249.

Liao, I.C. 1989. *Penaeus monodon* culture in Taiwan: Through two decades of growth. Int. J. Aq. Fish. Technol. **1**: 16-24.

Liao, I.C. 1990. The world's marine prawn culture industries: today and tomorrow. *In*: R. Hirano and I. Hanyu (Eds.) The Second Asian Fisheries Forum Asian Fisheries Society, Manila. p. 11-27.

Liao, I.C., M.S. Su and C.F. Chang 1992. Diseases of *Penaeus monodon* in Taiwan: A review from 1977 to 1991. *In*: W. Fulks and K.L. Main (Eds.) Diseases of cultured shrimp in Asia and the United States. The Oceanic Institute, Honolulu, Hawaii. pp. 113-137.

Liao, I .C. and Chao H.N. 1993. Hatchery and growout: penaeid prawns. CRC Handbook of Mariculture, CRC Press pp. 349-388.

Liao, I.C. and S.S. Sheen 1993. Prawn nutrition studies in Taiwan. *In*: Proc. of the FAO/AADCP Regional Expert consultation on Farm-Made Aquafeeds, 14-18 December 1992 Bangkok, Thailand. FAO-RAPA/AADCP, Bangkok, Thailand.

Liao, I. Chiu, Chung-Zen Shyu, and Nai-Hsien Chao (Eds.) 1992. Aquaculture in Asia: Proceedings of the 1990-APO Symposium on Aquaculture. TFRI

Conference Proceedings 1. Taiwan Fisheries Research Institute, Keelung, Taiwan, R.O.C. 312 p.

Lightner, D.V. 1977. Shrimp diseases. *In*: C.J. Sindermann (Ed.) Developments in aquaculture and fisheries science, Vol 6. Disease diagnosis and control in North American marine aquaculture, pp. 10-77 Elsevier, Amsterdam and New York.

Lightner, D.V. 1985. A review of the diseases of cultured penaeid shrimps and prawns with emphasis on recent discoveries and developments. *In*: Y. Taki, J.H. Primavera and J.A. Llobrera (Eds.) Proceedings of the First International Conference on the Culture of Penaeid Prawns/Shrimps, Iloilo City, Philippines. pp. 79-103.

Lightner, D.V. 1993. Diseases of cultured penaeid shrimp. *In*: J.P. McVey and B. Raton (Eds.) Crustacean Aquaculture. CRC Handbook of Mariculture, 2nd ed. Vol.I, CRD Press, Boca Raton, FL. pp. 393-486.

Lightner, D.V., T.A. Bell, R.M. Redman, L.K. Mohney, J.M. Natividad, A. Rukyani And A. Poernomo 1992. A review of some major diseases of economic significance in penaeid prawns/shrimps of the Americas and Indopacific. *In*: M. Shariff, R.P. Subasingle and J.R. Arthur (Eds.) Diseases in Asian Aquaculture I. Fish Health Section, Asian Fish. Soc., Manila, pp. 57-80.

Lin, C.K. 1989. Prawn culture in Taiwan. What went wrong? World Aquaculture. **20**(2): 19-20.

Ling, S.H. 1977. Aquaculture in southeast Asia: A historical overview. Univ. Washington Press. Seattle, Washington, 108 pp.

Linton, A.H. 1977. Animal to man transmission of Enterobactereaceae. Royal Society of Health Journal 1: 115-119.

Liston, J. 1980. Microbiology in fishery science, p. 138-155. *In*: J.J. Connel ((Eds.).) Advances in Fish Science and Technology, 138 p. Fishery News Books Ltd., Farnham.

Liu, K.C. 1989. Histopathological study of the bacterial induced hepatopancratitis of cultured shrimp (*Penaeus monodon* Fabricius). Fish Disease Research. 9: 34-41.

Liu, T., G. Su and W. Huang 1989. Pathogenic and biological research on the cause of "red leg disease" in *Penaeus penicillatus*. Fujian Fisheries. **2**: 1-3.

Llobrerra, T.A., L.M. Bulalacao and A. Tan 1990. Effect of farming phase and in-plant processing on microbiological quality of prawns (*Penaeus monodon*). FAO Fish. Rep., 401: 1-5.

Luu, L.T. 1995. Aquafeeds and feeding strategies in Vietnam. *In*: M.B. New, A.G.J. Tacon and I. Csavas (Eds.). Farm-made aquafeeds FAO Fisheries Technical paper, No. 343, Rome, FAO. 434 p.

Macintosh, D.J. and M.J. Phillips 1992. Environmental considerations in shrimp farming. Infofish International 6/92 p. 38-42.

Madhupratap, M. 1978. Studies on the ecology of zooplankton of Cochin backwaters. Mahasagar, Bull. Natn. Inst. Oceanogr., **11**: 45-56.

Maguire B. Greg and G. L. Allan 1992. Shrimp culture industry in Australia. *In*: Fast A.W, and L.J. Lester (Eds.) Marine Shrimp Culture: Principles and practices: pp. 757-769 Amsterdam: Elsevier.

Main, K.L. and W. Fulks, (Eds.) 1990. The culture of cold-tolerant shrimp. *In*: Proceedings of an Asian-US. Workshop on shrimp culture. Sponsored by the National Oceanic and Atmospheric Administration, United States Department of Commerce. The Oceanic Institute, Honolulu, Hawaii.

Mallik, T.K. and G.K. Suchindan 1984. Some sedimentological aspects of Vembanad lake, Kerala, West Coast of India. Indian J. Mar. Sci. **13**: 159-163.

Maniatis, T., E.T. Fritsch and J. Sambrook 1989. *In*: Molecular cloning: A laboratory manual. Cold Spring Harbor laboratory press, p. 1.25-1.27.

Mathew, S., I. Karunasagar, G.M. Rao and I. Karunasagar 1988. *Vibrio cholerae* in seafoods and environs., Mangalore, India. Asian Fisheries Science, **2** : 121-126.

Mc Vey, J.P. 1980. Current developments in the penaeid shrimp culture industry. Aquaculture Magazine, July-August/80, pp. 20-25.

Menasveta P. 1992. Shrimp culture industry in Thailand. *In*: Fast A.W and L.J. Lester (Eds.) Marine shrimp culture Principles and practices, pp. 691-699. Amsterdam: Elsevier.

Menon, M.K. 1954. On the paddy field prawn fishery of Travancore-Cochin and an experiment in prawn culture. Proc. Indo-Pacif. Fish Counc., 5th Sess., Sec. II: 1-5.

Menon, N.R., P. Venugopal and S.C. Goswami 1971. Total biomass and faunistic composition of the zooplankton in Cochin backwater. J. mar. biol. Ass. India, 13: 220-225.

Mevel, G. and S. Chamroux 1981. A study on nutrification in the presence of prawns (*Penaeus japonicus*) in marine closed systems. Aquaculture, **23**: 29-43.

Mistakidis, M.N. (Ed.) 1970. Proceedings of the world scientific conference on the biology and culture of shrimps and prawns. Mexico city. FAO Fish Rep., (57) Vol. 4: 1167-1627.

Miyamoto, Y., T.Kato, Y.Obara, A.Akiyama, K.Takizawa, and S.Yamai 1969. In vitro hemolytic characteristic of *Vibrio parahaemolyticus*: its close relation with human pathogenicity. J.Bacteriol., **100**: 1147-1149.

Moats, W.A. 1988. Inactivation of antibiotics by heating in foods and other substrates: a review. Journal of Food Protection, **51**, 491-7.

Modr, Z. 1982. Statutory control of antibiotic use in man versus voluntary restriction. . *In*: C.H. Stuart-Harris and D.M Harris (Eds.) The Control of Antibiotic- Resistant Bacteria, Academic Press, London, pp. 211-226.

Mohan, C.V. and K.M. Shankar 1995. Mortality of shrimps along the east coast of India - Viral pathogen suspected. Fishing Chimes, September 1995, P. 31.

Mohanty, A.N., P. Ravichandran, T. Rajyalakshmi and S.M. Pillai 1989. Rate of ammonia excretion in *Penaeus monodon* (Fabricius). J. Aquacult. Trop., **4**: 93-95.

Moore, W.D. and C.W. Brand 1993. The culture of marine shrimp in controlled environment super intensive systems. CRC Handbook of mariculture, CRD Press. pp. 315-346.

Moriarty, D.J.W. 1985. Role of bacteria and meiofauna in the productivity of prawn aquaculture ponds. *In*: Proceedings of the first international conference on the culture of penaeid prawns/shrimps. Illoilocity, SEAFDEC, Aquaculture Department, Philippines, 1984, p. 170.

Moriarty, D.J.W. 1987. Microbial ecology in aquaculture, p.1-3. *In* : D.J.W Moriarty and R.S.V. Pullin (Eds.) Detritus and microbial ecolgy in aquaculture. ICLARM conference Proceedings 14, 420p. International Centre for Living Aquatic Resources Management, Manila, Philippiens.

Moriarty, D.J.W. 1997. Microbial biotechnology: A key ingredient for sustainable aquaculture. Aqua International, **5** (7-8): 18-20.

Motoh, H. 1984. Biology and ecology of *Penaeus monodon.* Proc. First International Conference on the culture of penaeid prawns/shrimps, Iloilo city, Philippines, pp. 27-36.

Murty, P.S.N. and M. Veerayya 1972. Studies on the sediments of Vembanad lake, Kerala state. Part 1. Distribution of organic matter. Indian J. mar. Sci., 1(1): 45-51.

Muthu, M. S. 1980. Site selection and type of farms for coastal aquaculture of prawns. Nat. Symp. Shrimp Farming Bombay, 16-18 August 1978 : 97-106.

Nair, K.K. and D.J. Tranter 1971. Zooplankton distribution along the salinity gradient of Cochin backwater before and after monsoon. J. mar. biol. Ass. India, 13: 203-210.

Nair, K.K.C., V.N. Sankaranarayanan, T.C. Gopalakrishnan, T. Balasubramanian, C.B. Lalithambika Devi, P.N. Aravindakshan and M. Krishnankutty 1988. Environmental conditions of some paddy cum prawn culture fields of Cochin Backwater, Southwest coast of India. Indian J. mar., **17**(1): 24-30.

Nandeesha, M.C. 1993. Aquafeeds and feeding strategies in India. *In*: Proc. of the FAO/AADCP Regional Expert consultation on Farm-Made Aquafeeds, 14-18 December 1992 Bangkok, Thailand. FAO-RAPA/AADCP, Bangkok, Thailand.

Nandeesha, M.C. 1995. Aquafeeds and feeding strategies in India. *In*: M.B. New, A.G.J. Tacon, and I.Csavas (Eds.) Farm-made aquafeeds FAO Fisheries Technical paper, No.343, Rome, FAO, 434 p.

Nasser 1986. A comparative study of sediment nutrients in seasonal and perennial prawn culture pond, during the southwest cand immediate post-monsoon months. M.Sc Dissertation, Cochin Univ. Sci. & Technol., 83 p. (Un pub.)

Nayyarahamed, I. Karunasagar and I. Karunasagar 1995. Microbiology of cultured shrimps in India. FAO Fisheries Report., No.514, Suppl., pp. 13-22.

Neu, H.C. 1992. The crisis in antibiotic resistance. Science, 257: 1064-1073.

New, M.B. 1976. A review of dietary studies with shrimp and prawns. Aquaculture, **9**: 101-144

New, M.B. 1987. Feed and feeding of fish and shrimp. A manual on the preparation and the presentation of compound feeds for shrimp and fish in Aquaculture, UNDP/FAO, ADCP/REP 87/26. 275 p.

New, M.B. 1990. Compound feed stuffs for shrimp culture. *In*: M.B. New, H. de Saram and T. Singh. (Eds.) Technical and economic aspects of shrimp farming. Proceedings of Aquatech'90 Conference, Kualalumpur, Malaysia, 11-14 June 1990.

New, M.B. and I. Csavas 1993. Aquafeeds in Asia; A regional overview. *In*: Proc. of the FAO/AADCP Regional Expert Consultation on Farm-Made Aquafeeds, 14-18 December 1992, Bangkok, Thailand. 434 p.

Ninawe, A.S and R. P. Raj 1993. Studies on certain nitrogen cycle bacteria in the prawn culture fields of Kerala. CMFRI Spl. Publ., **56** : 57-65

Nouv, S. and M.C. Nandeesha 1995. Aquafeeds and feeding strategies in Cambodia. *In*: M.B. New, A.G. J. Tacon, and I. Csavas (Eds) Farm-made aquafeeds FAO Fisheries Technical paper, No. 343, Rome, FAO 434 p.

Oswin, D. S. and A. A. Rahman 1997. Impact of aquaculture effluents on mangroves. *In*: R. Santhanam, V. Ramadhas and P. Gopalakrishnan (Eds.), Proceedings of the National Seminar on Water Quality Issues in Aquaculture Systems, pp. 15-22.

***Otta, S.K.** 1997. Microbial diseases of shrimp. Ph.D. thesis, University of Agricultural Scienences, Bangalore, India, 122 p. (Un pub.)

Otta, S.K. Indrani Karunasagar, P. Tauro and I. Karunasagar 1998. Review Microbial diseases of shrimp. Indian Journal of Microbiology, **38**: 113-125.

Padmavathi, R., J. D Jameson, G. Sanjeeviraj and V. Ramadhas 1997. Impact of nitrogen and phosphorus on brackishwater pond fertility and effluent quality. *In*:

R. Santhanam, V. Ramadhas and P. Gopalakrishnan (Eds.) *Proceedings of the National Seminar on Water Quality Issues in Aquaculture Systems*, Dec. 18 & 19, 1996, Fisheries College and Research Institute, TNVASU, Tuticorin, pp. 83-89.

Paez-Osuma, F., S.R. Guerrero-Galvan, A.C. Ruiz-Fernandez and R. Espinoza-Angulo 1997. Fluxes and Mass balances of nutrients in a semi-intensive shrimp farm in North-Western Mexico. Marine Pollution Bulletin, **34** (5): pp. 290-297.

Palaniappan, R. 1982. Studies on the microflora on prawns *P.indicus* (Milne Edwards)(Crustacean decapoda, Penaedae) with reference to its digestive system. Ph.D. Thesis. Annamalai University, 120p. (Un. Pub.).

Panigrahi, A and R. N. Misra 1995. Influence of different types of soil on the water quality in Culture ponds. CMFRI Spl. Publ., **61**: 69-75.

Pantha, M.B. 1995. Aquafeeds and feeding strategies in Nepal. *In*: M.B. New, A.G.J. Tacon and I. Csavas (Eds.) Farm-made aquafeeds FAO Fisheries Technical paper, No. 343, Rome, FAO 434 p.

Parker, N. C. 1987. Feed conversion indices : controversy or convention. Prog. Fish-Cult., **49**(3): 161-166.

Pascual, F.P. 1983. Nutrition and feeding of *Penaeus monodon*. Extension Manual No.2, Third edition. Aquaculture Department SEAFDEC, Iloilo City, Philippines, 10 pp.

Pascual, F.P. 1995. Aquafeeds and feeding strategies in Philippines. *In*: M.B. New, A.G.J. Tacon and I. Csavas (Eds.) Farm-made aquafeeds FAO Fisheries Technical paper, No. 343, Rome, FAO, 434 p.

Peeler, J.T. and L.J. Maturin 1992. Aerobic plate count. Chap. 3. *In*: FDA Bacteriological Analytical Manual. 7th edition. p. 17-26. AOAC International , USA

Peranginangin, R., Suparno and I. Mulyanah 1992. Quality of cultured tiger prawn (*Penaeus monodon*) and deterioration during storage: A review. FAO Fisheries Repiort No. 470, Suppl. Rome, FAO, 17-23.

Phillips, M.J. 1995. Aquaculture and the environment - striking a balance. *In*: K.P.P. Nambiar and Tarlochan Singh (Eds.) Aquaculture towards the 21st century. Proceedings of the INFOFISH-AQUATECT'94 Conference, Colombo, Sri Lanka, 29-31 August 1994.

Phillips, M.J., C. Kwei Lin, and M.C.M. Beveridge 1993. Shrimp culture and the environment. Lessons from the World's most rapidly expanding warm water aquaculture sector, pp. 171-197. *In*: R.S.V. Pullin, H. Rosenthal and J. L. Maclean ((Eds.).). Environment and aquaculture in developing countries. ICLARM Conf. Proc. 31, 359 pp.

Phillips, M.J., D.J. Macintosh and P. Bueno 1997. Environmental issues Relating to aquaculture. Aqua International - May/June, 1997 p. B - 22.

Piedrahita, H. Raol 1994. Managing Environmental Impacts in Aquaculture. Bull. Natl. Res. Inst. Aquaculture, Suppl., **1**: 13-20.

Pillai, V.K. K.J. Joseph and A.K.K. Nair 1975. The plankton production in the Vembanad lake and adjacent water in relation to environmental parameters. Bull. Dept. mar. Sci. Univ. Cochin, **7**(1): 137-150.

Pillai, V.N., T. Cherian, M.D. George and F. Anto 1973. The effect of tidal currents on the hydrography of the backwaters around Cochin barmouth during post and premonsoon season. Proc. Indian Geophysics Union, 10: 231-239.

Pillay, T.V.R. 1954. The ecology of a brackishwater bheri with special reference to the fish-cultural practices and the biotic interaction. Proc. Nat. Inst. Sci. India, **20**(4): 343-353.

Pillay, T.V.R. 1990. Aquaculture: Principles and practices. Fishing News Books, University Press, Cambridge, pp. 425-459.

Pillay, T.V.R. 1992. Aquaculture and the environment. Fishing News Books. Oxford, England. 189 pp.

Pradeep, R. and P. Lakshmanaperumalsamy. 1986. Distribution of faecal indicator bacteria in Cochin backwater. Ind. J. Mar. Sci. Vol. 15: 99-101.

Pranee Srisomboon and Achara Poomchatra 1995. Antibiotic residues in farmed shrimp and consumer health. Infofish International 4/95: 48-52.

Prescott, L.M., J.P. Harley and D.A. Klein 1990. *In*: Microbiology. Wim. C. Brown publishers, U.S.A., p. 265, 357-360.

Primavera, J.H. 1992. Prawn/shrimp culture industry in the Philippines. *In*: A.W. Fast, and L.J. Lester (Eds.) Marine shrimp culture: Principles and practices. pp. 701-728 Amsterdam. Elsevier.

Primavera, J.H. 1993. A critical review of shrimp pond culture in the Philippines. Review in Fisheries Science: **1**(2): 151-201.

Primavera, J. H. 1994. Environmental and socioeconomic effects of shrimp farming, the Philippine experience. Infofish International 1/94. p. 44-49.

Pullin, R.S.V. 1989. Third-world aquaculture and the environment. NAGA, The ICLARM Quarterly, 12: 10-13.

Pullin, R.S.V., H. Rosenthal and J.L. Maclean (Eds.) 1993. Environment and aquaculture in developing countries. ICLARM Conf. Proc. 31, 359 p.

Putro, S., A.M. Anggawati, Y.N. Fawzya and F. Ariyani 1990. Studies on microbiology of farmed shrimp. FAO Fish. Rep., 401: 6-17.

Qasim, S.Z., and C.K. Gopinathan 1969. Tide cycle and the environmental features of Cochin backwater (a tropical estuary). Proc. Indian Acad. Sci., 69(B): 336-348.

Qasim, S.Z., S. Wellershaws, P.M.A. Bhattathiri and S. A. H. Abidi 1969. Organic production in a tropical estury. Proc. Indian Acad. Sci., 69B : 51-74.

Quadri, R.B. and R. Zuberi 1977. Survey on the occurrence of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* in fish and shellfish from the Karachi coastal waters. Pakistan J. Sci Ind. Res., **20**(3): 183-187.

Quynh, D.V. 1992. Shrimp culture industry in Vietnam. *In*: A.W. Fast and L.J. Lester, (Eds.) Marine shrimp culture. Principles and practices: pp. 729-756 (Amsterdam. Elsevier.

Raj, R.P. 1992. Aquaculture Feed. *In*: Handbook on Aquafarming. MPEDA, Kochi, 75 p.

Raj, R.P., M. Rajagopalan, M. Vijayakumaran, E. Vivekanandan and R. Sathiadhas 1998. Environmental and social issues in coastal aquaculture - A case study in Tamilnadu. Proceedings of the Workshop, National aquaculture week, Jan-Feb, 1997, Aquaculture Foundation of India, Chennai, pp. 210-218.

Rajendran, A., V.K.Venugopalan and V. Ramadhas 1972. Studies on marine nitrification . J. Annamalai Univ. Sci., 30; 171-178.

Ramamirtham, C.P. and R. Jayaraman 1963. Some aspects of hydrographical conditions of the backwaters around Wellington island (Cochin). J.mar. biol. Ass. India, **5**: 170-177.

Ramamirtham, C.P., S.Muthusamy, L.R.Kambadkar, A.Nandakumar, N.P.Kunhikrishnan and and A.V.S.Murthy 1987. Estuarine Oceanography of the Vembanad lake. Part III. The region between Cochin and the 30 m depth off portmouth. Indian J. Fish., **34**(4): 414-422.

Raman, K. and M.K. Menon 1963. A preliminary note on an experiment in paddy field prawn fishing. Indian J. Fish., **10**(A) 1: 33-39.

Ramteke, P.W. 1997. Plasmid mediated co-transfer of antibiotic resistance and heavy metal tolerance in coliforms. Indian Journal of Microbiology, **37**: 177-181.

Rattagool, P., N. Wongchinda and N. Sanghtong 1990. *Salmonella* contamination in Thai shrimp. FAO Fish. Rep., 470 (Suppl.), 9 p.

Raven, P. and G. Walker 1980. Ingredients for fish feed manufacture in the United States. Aquaculture Development and Co-ordination Programme. *In*: Fish feed Technology, Rome FAO, ADCP/REP/80/11.

Raveendran, O., P.S. Gore and R. V. Unnithan 1978. Observations of faecal contamination of Cherai beach in Kerala. Ind. J. Mar. Sci., **7** (2): 128-129.

Ravi, A.S and V. Chandrika 1993. Studies on sulphur bacteria in the prawn culture ecosystem. CMFRI Spl. Publ., **53** : 13-15

Regnault, M. 1987. Nitrogen excretion in marine and freshwater crustacea . Biol. Rev., **62**: 1-24.

Reilly, A. 1998. Cholera and export of fishery. Infofish International 2/98, pp. 50-54.

Reilly, P.J.A. and D.R. Twiddy, 1992. *Salmonella* and *Vibrio cholerae* in cultured tropical prawns. FAO Fish. Rep., 470 (Suppl), pp. 9-15.

Reilly, A. and F.Kaferstein 1997. Food safety hazards and the application of the principles of the hazard analysis and critical control point (HACCP) system for their control in aquaculture production. Aquaculture Research, **28**: 735-752.

Reilly, A., C. Lima Dos Santos and M. Phillips 1998. Food safety and products from aquaculture. FAO Aquaculture Newsletter FAN, No.19: 3-7.

Rheinheimer, G. 1985. Aquatic microbiology. Weley, New York.

Rosamma Philip and P. Lakshmanaperumalsamy. 1995. On the occurrence of total heterotrophic and proteolytic bacteria in prawns from the coastal waters off Cochin. Indian Journal of Microbiology **35** (3): 235-242.

Rosenberry, B. (Ed.) 1996. World Shrimp Farming. Shrimp News International - Annual Report pp. 4-112.

Rosenthal, H., D.P. Weston, R.J.Gowen and E.A. Black 1988. Report of the ad hoc study group on environmental impact of mariculture. ICES (International Council for the Exploration of the Sea) Coop. Res. Rep. 154, 81 p.

Sabath, L.D. 1982. Emergence of bacterial resistance to antibiotics occurring during therapy. *In* C.H Stuart-Harris and D.M Harris (Eds.) The Control of Antibiotic-Resistant Bacteria, Academic Press, London, pp. 17-34.

Saclauso, C.A. 1989. Brackishwater aquaculture: Threat to the environment. Naga, The ICLARM Quarterly, July 1989, pp. 6-8.

Saitanu, K., A. Amornsin, F. Kondo and C.E. Tsai 1994. Antibiotic residues in Tiger shrimp (*Penaeus monodon*). Asian Fisheries Science, **7**: 47-52.

Sanjeev, S. 1999. Incidence, enteropathogenicity and antibiotic sensitivity of *Vibrio parahaemolyticus* from a brackishwater culture pond. Fish Tech., **36**(1): 13-18.

Sankaranarayanan, V.N. and S.Z.Qasim 1969. Nutrients of Cochin backwater in relation to environmental characteristics. Mar. Biol., **2**(3): 236-247.

Sankaranarayanan, V.N. S. Kumaran, T. Balasubramanian, Rosamma Stephan and S.U. Panampunnayil 1982. Studies on the environmental conditions of tidal ponds in Ramanthuruthu island. Proc. Symp. Coastal Aquaculture, 1: 362-368.

Sano, T. and H. Fukuda 1987. Principal microbial diseases of mariculture in Japan. Aquaculture, **67**: 59-69.

Santhi Thirumani, S. and V. Chandrika 1995. Heterotrophic bacterial activity in selected aquaculture systems near Cochin. CMFRI Spl. Publ., **61** : 22-24

***Sarnianto, P., Y.N. Fawzya, U. Rahayu and S. Putro** 1985. Preliminary studies on the environmental aspects of prawn (*Penaeus monodon*) culture in Camera Village, Karawang, West Java. Laporan Penelitian Teknogi Perikanan No.42. Balal Penelitian Perikanan Laut Jakarta. Hal: 33-37.

***Satanpornvanit, K.** 1993. Environmental Impact of shrimp farm effluent. M.Sc. Thesis. Asian Institute of Technology, Bangkok, Thailand. (Un pub.)

Shariff, M. and R.P. Subasinghe 1992. Major diseases of cultured shrimp in Asia. An overview. *In*: W. Fulks and K.L. Main (Eds.) Diseases of cultured shrimp in Asia and the United States. The Oceanic Institute, Honolulu, Hawaii. pp. 37-46.

Sharmila, R. T. Jawahar Abraham and V. Sundaraj 1996. Bacterial flora of semi intensive pond reared *Penaeus indicus* (H. Milne Edwards) and the environment. J. Aqua. Trop., **11**: 193-203.

Sheeba S. M and N. Gopinatha Menon 1993. Ecological characteristics of prawn culture fields in the Cochin area. CMFRI Spl. Publ., 56 : 149-152.

Shewan, T.M. 1961. The microbiology of sea-water fish. *In*: G. Borgstrom (ed.). Fish as food, Vol. 1. New York, Academic Press, pp. 487-560.

Shigueno, K. 1975. Shrimp culture in Japan. Association for International Technical Promotion. Tokyo, Japan, 153 p.

Shigueno, K. 1984. Intensive culture and feed development in *Penaeus japonicus*. Proc. First International Conference on the Culture of Penaeid Prawns/Shrimps, Iloilo City, Philippines, SEAFDEC Aquaculture Department, pp. 123-130.

Shigueno, K. 1992. Shrimp culture industry in Japan. *In*: A.W. Fast, and L.J. Lester (Eds.) Marine Shrimp Culture: Principles and practices. pp. 641-652. Amsterdam: Elsevier.

Shuval, H.I. 1986. Thalassogenic diseases. UNEP Reg. Seas Rep. Stud., (79): 40 p.

Sieburth, J.M. 1979. Sea microbes. Oxford University Press.

Sindermann, C.J. 1990. Principal diseases of marine fish and shellfish. Vol.2. Second edition. Academic Press, San Diego. 516 p.

Sindermann, C.J. and D.V. Lightner (Eds.). 1988. Disease Diagnosis and Control in North American Marine Aquaculture. Elsevier, Amsterdam 431 p.

Singh, I.S.B. 1986. Studies on the bacteria associated with *Penaeus indicus* in a culture system. Ph.D. Thesis, Cochin University of Science and Technology 230 p. (Un pub.)

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

Sini, J.M. 1996. Studies on *Vibrio* spp. in juveniles of *Penaeus indicus* in culture systems. Ph.D. Thesis, Cochin Unv. Sci. and Tech., Cochin, 165 p. (Un pub.).

Skinner, F.A. and J.M. Shewan 1977. Aquatic microbiology. Academic Press, London.

Solorzano, L. 1969. Determination of ammonia in natural waters by phenol-hypochlorite method. Limnol. Oceanogr., **14**: 799-801

Solyom, P and S. Carlberg 1975. Determination of nitrate. FAO Fish. Tech. Paper, 137: 156-160.

Somsueb, P. 1995. Aquafeeds and feeding strategies in Thailand. *In*: M.B. New, A.G.J. Tacon and I. Csavas (Eds.) Farm-made aquafeeds, FAO Fisheries Technical Paper, No. 343, Rome, FAO. 434 p.

Sreeja, S. and O.Raveendran 1999. Occurrence of Vibrio chlolerae non-01 and their dispersion phenomena in the coastal waters at Mangalore. Fishery Technology, **36** (2): 96-99.

Straub, D.V. and B. A. Dixon 1993. Bacteriological flora of the Brine shrimp (Atremia franciscana) from a hypersaline pond in San Francisco Bay, California. Aquaculture, **118**: 309-313.

Strickland, J.D.H. and T.R. Parsons 1968. A practical handbook of sea water analysis. 2nd edition, Bulletin No. 167, Fish. Res. Board Can., p. 310.

Subrahmanyam, M. 1974. A note on food and feeding habits of some penaeid prawns. Indian Journal of Animal Science, **44**: 914-915.

Sunarya, Budisusilowati and Ennatha Sri Haryani 1995. Influence of environmental on the microbiological quality (*Vibrio parahaemoliticus* and *Salmonella*) of cultured shrimp. FAO Fisheries Report., 514, Suppl. pp. 57-60.

Sunaryanto, A. and A. Mariam 1986. Occurrence of a pathogenic bacteria causing luminescene in penaeid larvae in Indonesian hatcheries. Bull. Brackishwater Aqua. Devl. Centre. **8**: 64-70.

Surendran, V. and V. Chandrika 1993. Studies on hetritrophic bacteria in the mangrove ecosystem near Cochin. CMFRI Spl. Publ., **53**: 66-71

Surendran, P.K. and K. Gopakumar 1981. Selection of bacterial flora in the chlortetracycline treated oil sardine (*Sardinella longiceps*), Indian mackrel (*Rastrelliger kanagurta*) and Prawn (*Metapenaeus dobsoni*) during ice storage. Fish. Technol. **18**: 133-141.

Surendran, P.K. and Nirmala Thampuran 1999. Antibiotic residues and antibiotic resistant bacteria in farmed fish and shrimp. Paper presented at National Symposium on 'Sustainable development of fisheries towards 2020 AD - Opportunities & Challenges', 21-23 April, 1999, Kochi, India. Abstracts, pp. 77-78.

Surendran, P.K., Nirmala Thampuran and K. Gopakumar 1995. Microbial profile of cultured fishes and prawns viz. a viz. their spoilage and contamination. FAO Fisheries Report, No.514, Suppl., pp. 1-12.

Surendran, P.K., Nirmala Thampuran and V.N. Nambiar 2000. Comparative microbial ecology of freshwater and brackishwater prawn farms. Fishery Technology, **37** (1) 25 – 30 .

Suseelan, C. 1975. The prawn culture practices in salt-pan reservoirs at Manakkudy near Cape Comorin. Bull. Dept. Mar. Sci. Univ. Cochin, **7**(3): 477-486.

Tacon, A.G.J. 1993. Feed formulation and on-farm feed management. *In*: Proc. of the FAO/AADCP Regional Expert Consultation on Farm-Made Aquafeeds, 14-18 December 1992, Bangkok, Thailand, FAO-RAPA/AADCP, Bangkok, Thailand, pp. 61-74.

Tacon, A.G.J. 1998. Global trends in aquaculture and aquafeed production 1984-1995. International Aquafeed Directory and Buyer's Guide 1997/98. Turret RAI PLC., Armstrong House, U.K., pp. 5-37.

Takahashi, Y., Y. Shimoyama and K. Momoyama 1985. Pathogenically and characteristics of *Vibrio* sp. isolated from cultured Kuruma prawn *Penaeus japonicus* Bate. Bull. Japan Soc. Sci. Fish., **51**: 721-730.

Takahashi, Y., T. Itami, M. Maeda and M. Kondo 1998. Bacterial and viral diseases of Kuruma shrimp (*Penaeus japonicus*) in Japan. Fish Pathology, **33**(4), 357-364.

Tansutapanit, D.S.O.A. and L. Ruangpan 1987. *Vibrio harveyi* a causative agent of white shrimp nauplii, *Penaeus merguiensis*. *In*: Abstract. Tech. Paper No.6/30. Third Natl. Seminar on Marine Science, 6-8 August 1986, NSRC, Bangkok, Thailand.

***Thatcher, F. S. and D. S. Clark** 1968. Microorganisms in food : their significance and methods of enumeration. Univ. of Toronto Press, Toronto, Ont. Pp. 3-69.

Trusell, R.P. 1972. The percent un-ionized ammonia in aqueous ammonia solutions at different pH levels and temperature. J. Fish. Res. Board Can., **29**: 1505-1507.

Trust,T.J. 1971. Bacterial counts of commercial fish diets. J. Fish. Res. Bd. Canada, **28**: 1185-1189.

Tushara, V.M. 1998. Plasmid DNA profile of bacterial pathogens of finfish and shellfish. M.F.Sc. dissertation, Central Insitute of Fisheries Education (Deemed University), Mumbai, India, pp. 32-51 (Un pub.).

Twiddy, D.R. and P.J.A. Reilly 1995. Occurrence of antibiotic-resistant human pathogens in integrated fish farms. FAO Fish. Rep., No.514, Suppl. Rome,FAO, pp. 23-37.

Uno, Y. 1984. An ecological approach to mariculture of shrimp: Shrimp ranching fisheries. *Proc. First International Conference on the culture of penaeid prawns/shrimps*, Iloilo City, Philippines, pp. 37-45.

Utama, C.M. 1995. Aquafeeds and feeding strategies in Malaysia. *In*: M.B. New, A.G.J. Tacon, and I. Csavas (Eds.) Farm-made aquafeeds, FAO Fisheries Technical paper, No. 343, Rome, FAO, 434 p.

Vanderzant, C., R. Nickelson and P.W. Judkins 1971. Microbial flora of pond - reared brown shrimp (*Penaeus aztecus)*. Applied Microbiology: **21**: 916-921.

Venkateswaran, K. and R.Natrajan 1987. Significance of bacterial indicators of pollution in the isolation of human pathogens in aquatic environment. Indian J. Mar. Sci., **16**: 51-53.

Vasudevappa, C. 1992. Growth and reproduction of the penaeid prawn *Metapenaeus dobsoni* (Miers) in brackish water environment. Ph.D. Thesis, Cochin University of Science and Technology, Cochin, 316 p. (Un pub.).

Vijayan, K.K., S.V. Alavandi, K.V. Rajendran and K. Alagarswami 1995. Prevalence and histopathology of monodon baculovirus (MBV) infection in *Penaeus monodon* and *P. indicus* in shrimp farms in the southeast coast of India. Asian Fish. Sci., **8**: 267-272.

Villalon, J.R. 1993. Commercial semi-intensive penaeid grow out techniques in Ecuador. CRC Handbook of Mariculture, CRC Press. pp. 237-272.

Wajsbrot, N., M.D. Krom, A. Gasith and T. Samocha 1989. Ammonia excretion of green tiger prawn *Penaeus semisulcatus* as a possible limit on biomass density in shrimp ponds. Isr. J. Aquacult. Bamidgeb, **41**: 159-164.

Wang, J.K. 1994. A systematic approach to physical aquaculture water management. Bull. Natl. Res. Inst. Aquaculture, Suppl. **1**, 89-96.

Wellershaus, S. 1971. On the Hydrography of Cochin backwater, a south Indian estuary. J. mar. biol. Ass. India, **14**(2): 487-495.

West, P.A. 1989. Human pathogens and public health indicator organisms in shellfish. *In*: B. Austin and D.A. Austin (Eds.) Methods for the Microbiological Examination of Fish and Shellfish, pp. 273-308. Ellis Horwood, Chichester.

Weston, D. P. 1996. Environmental considerations in the use of antibacterial drugs in aquaculture. *In*: D. J. Baird, M.C.M. Beveridge, L. A. Kelly and J.F. Muir (Eds.) Aquaculture and water resource management, Blackwell Sciences Ltd. London, pp. 140-165.

Whitefield, M. 1978. The hydrolysis of ammonium ions in sea water - experimental confirmation of predicted constants at one atmosphere pressure. J. Mar. Biol. Assoc. U.K., **58**: 781-787.

Wickins, J. F. 1976. The tolerance of warm water prawns to recirculated water. Aquaculture, **9**: 19-37. Toronto.

Williams, J. D. 1982. Epidemiological trends in antibiotic resistance and concerns for the future. *In*: C.H. Stuart-Harris, and D.M. Harris, (Eds.) The Control of Antibiotic- Resistant Bacteria, Academic Press, London, pp. 34-75.

Wood, J., J.H. Brown, M.H. Maclean and I. Rajendran 1992. Feeds for artisanal shrimp culture in India. Their development and evaluation. Bay of Bental News No. 52, Bay of Bengal Programme, Report No. BOBP/REP/52, Madras, India, 59 p.

Wood, S.R. M.C. Cashion and W. Lynch 1986. Multiple low-level resistance in *Aeromonas salmonicida*. Antimicrobial Agents and Chemotherappy, **23**, 475-83.

Wu, S.S.R. 1995. The environmental impact of marine fish culture. Towards a sustainable future. Marine pollution bull. **31**(4-12): 159-166.

Wyban, J. and J. Sweeny 1991. Intensive shrimp production technology: The Oceanic Institute Shrimp Manual. The Oceanic Institute, Honolulu, Hawaii. 158 p.

Wyban, J. and J. Sweeny 1993. Intensive shrimp production in round ponds. CRC Handbook of Mariculture, CRC Press pp. 225-286.

Xin J.C. and Y. N. Sheng 1992. Shrimp culture industry in China. *In*: A. W Fast and L.J. Lester, (Eds.) Marine shrimp culture: Principles and practices: pp. 677-689 , Amsterdam, Elsevier.

Yano, I. 1993. Ultra intensive culture and maturation in captivity of penaeid shrimp. CRC Handbook of mariculture, CRC press. pp. 289-311.

Yasuda, K. and T. Kitao 1980. Bacterial flora in the digestive tract of prawns, *Penaeus japonicus* Bate. Aquaculture, **19**: 229-234.

Yeoman, G.H. 1982. Review of veterinary practice in relation to the use of antibiotics. . *In*: C.H. Stuart-Harris, and D.M. Harris, (Eds.) The Control of Antibiotic- Resistant Bacteria, Academic Press, London, pp. 57-76.

Zaher, M. and M.A. Mazid 1995. Aquafeeds and feeding strategies in Bangladesh. *In*: M.B. New, A.G.J. Tacon, and I. Csavas (Eds) Farm-made aquafeeds, FAO Fisheries Technical paper No. 343, Rome FAO, 434 p.

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* Not referred to in original