CHAPTER 1.
INTRODUCTION
1. Introduction

Shrimp has proved to be one of the great aquaculture cash crops since 1980 and a source of prosperity in a number of developing countries. Shrimp constitutes an important aquatic resource for human consumption and thus its commercial exploitation is on the growing trend. Ever increasing population growth has provided impetus for rapid growth in aquaculture (FAO, 1999). However, demand is elastic income and shrimp consumption has continued to grow, spurred by the generally increasing prosperity in the major industrial markets. During the 1970s and the 1980s, several countries were able to develop a shrimp culture industry, which found a seller’s market as they plugged the gap between supply and demand. World aquaculture production has soared, growing from 25000 T in 1975 to 340000 T in 1987. In 1999, the global average production was 650 kg/hectare. According to the MPEDA (Marine Product Export Development Authority) statistical report of 2004-2005 total export of frozen shrimp from India was 138,085 MTs and its export value was 938.41 million $US (MPEDA, 2005). Shrimp export from Asia was 294,658 MTs, it was 8.84% higher than the previous year 1998 (Asian shrimp market report), but it was 0.98% lower than the year 2004 (Infofish, 2006).

Today, shrimp farm makes up only 3-4% of global aquaculture production. Around 80% of cultured shrimp come from Asian countries such as Thailand, China, Indonesia and India and these countries are the top producers. The giant tiger shrimp *Penaeus monodon* accounts for more than half of the total shrimp aquaculture output and other important commercial species are *P. vannamei, P. indicus, P. merguiensis* and *P. chinesis* (Ronnback, 2001).

Shrimp farming is currently practised in over 50 countries worldwide and the sector has grown at an annual average of over 18.8% since 1970 (FAO, 2001). The explosive growth of shrimp farming in the later decades has been associated with the tropical giant tiger shrimp *Penaeus monodon*. This species comprised ~60% of the total production, the Asian white shrimps *P. indicus* and *P. merguiensis* came next.
with ~20% followed by *P. vannamei, P. chinensis, P. stylirostris, P. japonicus* which contributed to the rest. Shrimp aquacultures are mainly influenced by various physical, chemical and biological factors. Therefore, knowledge of the changing pattern of environmental factors of pond water along with the availability and size of shrimp is essential for obtaining a scientific view of its basic productivity. Traditional culture practices differ from extensive methods in that they are completely dependent on the natural tidal entry for seed, food and water (Shiva and Karir, 1997). Where as, traditional and extensive shrimp aquaculture uses natural production in the ponds or in the incoming waters, the semi-intensive and intensive production systems are heavily dependent on formulated feeds (Tacon, 1996). The growth and health of shrimp depends upon the intake of food containing essential nutrients. For the increasing demand of shrimp feed, the feed manufacturers focus exclusively on the formulation and they manufacture nutritionally complete artificial diets or aqua feed. There are many recommendations on how to make shrimp aquaculture environmentally and socio-economically sustainable.

The use of formulated feeds constitutes approximately 50 – 60% of the operational cost (Pascual, 1989). Large-scale shrimp farming depends upon cost effective pelleted feed. It can be possible by developing nutritionally balanced formulated feed using low cost and locally available raw materials. In particular, intensive farming systems for shrimp species currently depend upon the use of fish meal and other fishery resources as a sole or major source of dietary proteins and lipid in formulated aqua feeds (Bimbo and Crowther, 1992; Rumsey, 1994; Tacon, 1994; Wijkstrom *et al.*, 2003). At present, the shrimp farming industry totally depends upon marine captured fisheries for dietary animal protein and lipid. From 25 – 50% of the ingredients in most commercial shrimp aquaculture feed are derived from marine capture fisheries, including fishmeal, fish oil, shrimp/crustacean meal and others (Cruz-Suarez *et al.*, 1998; Tacon, 1999; Davis, 2000).

The nutrient composition of marine animal protein sources approximates very closely to the known dietary requirements of shrimp, so it is no surprise that these feed ingredients usually have a higher nutritional value for shrimp than individual
plant feed stuffs (D’Abramo et al., 1997). Therefore, the challenge of feed formulation is to blend complementary plant and animal feed ingredients to obtain a more balanced nutrient profile.

The protein, lipid and carbohydrate levels of shrimp tissue would indicate the basic energy giving nutritional requirements. Protein is the most essential and expensive component in feed. Thus, knowledge on the protein requirement of candidate species at different growing stages of its life is essential in the formulation of well balanced diets (Shiau et al., 1991). Carbohydrate is one of the essential nutrients in crustaceans and a number of reports are available describing its change in relation to maturation (Diwan and Nagabhusanam, 1974; Anilkumar, 1980; Ajmalkhan and Natarajan, 1980; Varadarajan and Subramaniam, 1982; Mohamed and Diwan, 1992). Lipid is also a major constituent and it contributes appreciably towards yolk formation during oocyte maturation in crustaceans. The haemolymph and ovarian lipids have been studied to some extent (Adiyodi, 1968; Varadarajan and Subramaniam, 1982; Mohamed and Diwan, 1992).

In addition to plant proteins, it is also worth mentioning here that the good long-term prospects of using single cell proteins (SCP), including bacteria, fungi/yeast, and algae as dietary fish meal replacement for shrimp. Single cell proteins produced from renewable resources such as hydrocarbons and/ or agriculture waste materials probably represent the best long-term alternative to fish meal for the animal and aqua feed industries (Anonymous, 2000).

Disease outbreaks are being increasingly recognized as a significant aquaculture problem, affecting the economic development of the developing countries (Subasinghe, 1997). The massive use of antibiotics in aquaculture induces the antibiotic resistance microorganisms and promotes the human health hazards (Witte et al., 1999). Researchers are trying to use probiotic bacteria in aquaculture to improve water quality by balancing beneficial bacterial population in water and reducing pathogenic bacterial load. "Probiotics" generally includes bacteria, cyanobacteria, micro algae, fungi, Photosynthetic bacteria, Lactobacillus,
Actinomycetes, Nitrobacteria, Denitrifying bacteria, Bifidobacterium, yeast, etc (Verschuere et al., 2000). Probiotics principally inhibit the growth and decrease the pathogenicity of the pathogenic bacteria, enhance the nutrition of the aquacultured animals, improve the quality of the aquaculture water and decrease the use of antibiotics and other chemicals and thus decreases environmental contamination by the residual antibiotics and chemicals. "Probiotics", "Probiont", "Probiotic bacteria" or "Beneficial bacteria" are the terms synonymously used for probiotic bacteria (Harris, 1993). *Lactobacillus acidophilus* is used commonly to control and prevent infections by pathogenic microorganisms in the intestinal tract of many terrestrial animals. In addition, the use of probiotics can increase the population of food organisms, improve the nutrition level of aquacultural animals and improve immunity of cultured animals to pathogenic microorganisms. The use of antibiotics and chemicals can be reduced and frequent outbreaks of diseases can be prevented (Holzapfel et al., 1998).

Cyanobacteria otherwise called a blue green algae, has a very good market value. *Spirulina* has received the attention of researchers from few decades ago. This cyanobacteria has the highest protein content (55-65%) as compared to other algal forms and is amenable to both sophisticated and simplified technology for biomass production (Venkataraman, 1983). Besides, its use as protein source for feed or food, the presence of \( \beta \)-carotene, B-complex vitamins, polyunsaturated fatty acids and minerals contributes to its overall quality. The greatest promise of this cyanobacteria, particularly in India lies in its potential as poultry feed (Venkataraman, 1983).

Considerable interest and research have been aimed at to use the unicellular organisms such as yeast, molds, bacteria, microalgae and fungi as additives to aquaculture feeds. Moreover, the composition of many microorganisms can be manipulated to produce higher levels of protein and lipids, specific essential amino acids or fatty acids (Dabrowski et al., 1985; Murray and Marchant, 1986). Phytoplanktons are the main food of larval stage of some crustaceans (Preston et al., 1992). A number of studies have reported the inclusion of photosynthetic microalgae in aquaculture feeds (Day et al., 1990; Day et al., 1991; Day and Tsavalas, 1996).
Many species of algal diets are supplemented or partially substituted with protein and lipid diets for aquatic animals (Cacrs et al., 1998). There have been several studies on algae meal as a dietary protein source in fish diet (Nose, 1960; Meske and Pfeffer, 1978; Tsai, 1979). Small amount of algae added to the fish diet significantly improves growth, lipid metabolism, body composition and disease resistance (Yones, 1989; Xu et al., 1993). High dietary protein of marine algae provided the best growth of juveniles (Knuckey et al., 2001). Some brown algae (Phaeophyceae), red algae (Rhodophyceae) and green algae (Chlorophyceae) have traditionally been consumed as marine foods in Japan.

There is a general conception, Indian shrimp farmers use local moist feeds which do not produce higher shrimp yields as they anticipate. On the other hand, the manufactured feeds in pellet form of high nutrient quality and water stability would give improved yields compared to those in other shrimp-producing area. By knowing the lacuna in the earlier studies, the present study aims at the formulation of aqua feed using different dietary constituents.

1.1 Outline of the thesis

The aim of this thesis is to perform quantitative studies on formulation of shrimp feed and to evaluate its performance. This focus of the study is on the formulation of different experimental feeds with locally available raw materials of both animal and plant origin. In addition to the cyanobacteria, the seaweeds and the probiotic organisms (Lactobacillus sp. and yeast) were added in separate preparations. The effects of novel composition of feed for P. monodon were examined towards growth, survival, feed conversion ratio, feeding efficiency, protein efficiency and shrimp proximate composition like, protein, carbohydrate, ash, and other contents. A special emphasis was given to economics of feed production and viability in terms of its technology. The thesis has subdivided in to different chapters. The chapter -1 has given the background to the study of formulation of aqua feed. Chapter- 2 is deals with a review of literature from the fields on the aquaculture, economic status, shrimp feed formulation studies, microalgal feed, live feed, microbial diseases, evaluation of probiotic feed and live feeds. Chapter- 3 is a study
Research scheme

CYANOBACTERIAL SURVEY
(Samples from shrimp ponds)

Water quality test  Identification of cyanobacteria

FEED FORMULATION STUDY
On shrimp growth performance study

CYANOBACTERIA  SEAWEED MEAL  LIVE FEED STUDY
SUPPLEMENTED FEED  SUPPLEMENTED FEED  SUPPLEMENTED FEED
Cyanobacterial meal  Seaweed meal (5%)
(5%) supplemented feed  supplemented feed
Phormidium tenue (C-1);  Chondrococcus harnemanii (SW-1);
Synechococcus elongatus (C-2);  Padina tetrastrumatica (SW-2);
Phormidium sp. (C-3);  Lobophora variagata (SW-3);
and Spirulina sp. (C-4).  and Dictyota alternara (SW-4).

Optimizing dietary Spirulina meal concentration
(5%, 10%, 15%, 20%, 25% (w/w))

Water quality
Salinity, pH, Dissolved oxygen (DO),
Total ammonia Nitrates, Phosphorous,
Sulphates analysis.

Growth analysis
Mean weight gain, FCR, FE, PER,
Survival and Proximate analysis

Algal and Cyanobacterial live feeds
(Chlorella sp., Tetraselmium sp., Isochrysis sp.,
Synechococcus elongatus and Phormidium sp.)

Probiotic supplementary feed
(Lactobacillus sp. and Yeast)

Water quality
Salinity, pH, Dissolved oxygen (DO),
Total ammonia Nitrates, Phosphorous,
Sulphates

Effect of feed on culture tank
Total bacterial/ fungal count, identify the frequent bacteria/fungi
on cyanobacterial abundance in shrimp culture pond in Chidambaram and Cuddalore regions of Tamil Nadu. Chapter -4 is a study on feed formulations and evaluation of shrimp growth. In the chapter- 5 is a study on quality and microbial status of formulated shrimp feed on storage. Chapter- 6 is consist of a study on probiotic feed experiment and chapter-7 covers the study on the efficacy of microalgae and cyanobacteria as a live feed on juveniles of shrimp *P. monodon*. In the chapter- 8, a study on feed economics was discussed by comprised by comparing with commercial feed. Finally chapter-9 consists of all-important findings discussed in the previous chapter are concluded.

1.2 Objectives of the work

- To survey cyanobacterial abundance in culture ponds of shrimp *Penaeus monodon* in Chidambaram and Cuddalore regions, Tamilnadu.
- To study the effect of cyanobacterial meal (5%) on shrimp *P. monodon* diet.
- To find the growth performance of *P. monodon* fed with *Spirulina* meal supplemented in different concentration.
- To test the quality and microbial status of formulated shrimp feed on storage at two different temperatures.
- To investigate the effect of different seaweeds as a dietary supplement on growth and survival of *P. monodon*.
- To study the effect of probiotic feed supplements (*Lactobacillus* sp. and yeast) on growth performance of *P. monodon* and microbial quality of culture tank.
- To study the effect of feed on water quality of shrimp culture tank.
- To study the efficacy of microalgae and cyanobacterial cells as live feed on shrimp growth.