2.1 Broodstock collection and transport

Information on broodstock availability in India is very limited. Presently broodstock is obtained as by catch from shrimp trawling and catamaran using specialized traps, except in seasons of peak demand and value, when exclusive fishing for gravid female broodstock is done by a small percentage of trawler operators for short duration. Near-shore trawlers and catamarans supplied about 90 percent of the broodstock requirement while the deep-sea trawlers may have fulfilled the rest. But the information is very limited. Most trawlers and catamarans fished near shore at a depth of between 20 and 50 m where *P. monodon* broodstock are available (FAO, 2007). Off the east coast of Tamil Nadu fishing for broodstock is conducted 5 to 10 km from the shore where there is soft loam or sandy clay or clay-loam substrates with seaweed. Although trawling usually lasts from three to four hours, to reduce stress, broodstock-specific trawling lasted only 1 to 1.5 hours. As shrimp broodstock is largely by catch, the fishermen need to modify present practice in order to reduce stress, improve general quality and minimize the time from capture to delivery of broodstock to the auction centres. There is a need for targeted short-duration trawling with nets having mesh size larger than the 1 cm mesh currently used. Additionally the fishermen require training in selecting the right quality broodstock and in handling, storage and transportation techniques. The main season for fishing in Tamil Nadu is June to April with the banned season from Middle of April to May (Government of India, 2005).
2.2 Screening of Broodstock

Currently the quarantine facilities in Indian hatcheries are inadequate, and disposal of infected broodstock from quarantine is to prevent contamination of other stocks. Also in most hatcheries the understanding of the concepts of bio-security and quarantine is little weak. Construction and operation of shrimp quarantine is discussed in MAF (2001), Anon. (2002) and AQIS (2003). In the east coast of India, shrimp hatcheries practice with broodstock as well as wild spawners with developed ovaries for naupli production but due to limited supply of wild spawners, naupli production depends on wild brood stock supply from catamaran and trawler operators. Broodstock or spawners at captive reproduction are the source of virus infection through vertical or horizontal transmission in the shrimp seed. To avoid the possibility of infection, quarantine is much important before proceeding for maturation process at shrimp hatchery system. The presence of pathogen in broodstock is an indication that breeding, rearing of larvae and culture in the farm are not sustainable. Methods for the detection of pathogens and the diagnosis of disease that are currently in use by shrimp pathologists and by diagnostic lab have been reviewed (Baticados, et al 1990; Limsuvan, 1993; Lightner and Redman, 1998, Uma et al ,2005). Among the most important of these are gross and clinical signs, with the most commonly applied laboratory test being direct examination and microscopy (Lightner and Redman, 1998). Molecular methods have been applied to the diagnosis of infectious diseases of penaeid shrimp (Lightner and Redman, 1998). One way to achieve sustainable production is through a combination of broodstock screening at quarantine level and larval rearing stage with novel techniques and proper environmental management. There are many virus diseases affecting *P. monodon broodstock* and larvae viz. white spot syndrome virus (WSSV) (Wongteerasupaya et al. 1995), Yellow head virus (YHV) (Boonyaratpalin et al. 1993),
Infectious hypodermal and haematopoietic necrosis virus (IHHNV) (Bonami et al. 1990), Hepatopancreatic parvo like virus (HPV) (Lightner and Redman, 1985), Monodon baculovirus (MBV) (Lightner and Redman, 1981), but among all viruses White Spot Syndrome Virus (WSSV) and Monodon baculovirus (MBV) are more virulent in broodstock, larval rearing and in the farming areas (Uma et al., 2005).

2.2.1 Monodon baculovirus (MBV)

Monodon baculovirus (MBV) causes an epizootic disease in larval and adult shrimp (Lightner and Redman, 1981; Natividad and Lightner, 1992; Ramasamy et al., 1995). This virus has been reported to be widely present in shrimp broodstock and larvae in several parts of Asia (Baticados et al., 1991; Lightner et al., 1992a), but there are few reports from India (Ramasamy et al., 1995; Manivannan et al., 2002; Karunasagar et al., 1998; Otta et al., 2003). In the case of MBV, transmission occurs horizontally through fecal oral route (Uma et al., 2005). Prevalence of MBV in Penaeus monodon broodstock has been reported from several countries in Asia, but there is little information from India. Data on prevalence of viruses in broodstock would be important to develop strategies for health management.

2.2.2 White spot syndrome virus (WSSV)

Transmission of WSSV occurs vertically from infected broodstock to larvae or horizontally through water or infected animals. Disease outbreaks have caused mass mortalities among cultured penaeid shrimps worldwide, especially in Asian countries (Kim et al. 1998). White spot viral disease has caused high mortalities and severe damage to the shrimp culture industry in China (Huang et al. 1994), Thailand (Wongteerasupaya et al. 1995), Japan (Takahashi et al. 1994), Taiwan (Wang et al. 1995), Indonesia and India (Anon, 1994). The white spot disease virus is believed to have been transmitted through seed brought to India clandestinely from Southeast Asian countries, where the virus has
been amplified before (Shankar and Mohan 1998). During 1994-95, white spot viral
disease caused severe mortality of cultured shrimp *P. monodon* and *P. indicus* along the
east coast of India (Anon 1994). Karunasagar *et al.* (1997) reported the white spot viral
disease outbreak on the west coast of India.

### 2.3 Antibiotics and Probiotics

A number of reports raised a legitimate public concern over safety of Antibiotics drug usage in Aquaculture (Alderman and Hastings, 1998). A variety of Antibiotics and chemicals used in Indian shrimp hatcheries for increased and controlled production of seed in the hatchery. Chloramphenicol, Oxytetracyclin, Erythromycin and Prefuran were used widely at shrimp hatcheries in controlling pathogen. Treflan and Malachite green were used as antifungal agents. The Antibiotics Chloramphenicol and Nitrofurans (Prefuran) are banned world wide and in India for use in food production because of potentially serious side effects. Chloramphenicol can cause fatal aplastic anemia and Nitrofurans are classified as carcinogen (GESAMP, 1997; Graslund and Bengtsson, 2001). Oxytetracyclin known to enhance the production of plasmid mediated resistance in aquatic bacteria (Shotts *et al.*, 1976) and Erythromycin develop strain resistance (Primavera *et al.*, 1993). Dierberg and Kiattisimkul (1996) reported that malachite green is a respiratory poison, persistent residue in the tissues of seafood. Treflan a possible human carcinogen induces thyroid and liver tumors in mammals (Hurley, 1998).

The abuse of antimicrobial drugs, pesticides and disinfectants in aquaculture disease prevention and growth promotion has led to the evolution of resistant strain of bacteria and question of safety (Esiobu *et al.*, 2002; Boyd and Massaaut, 1999). Thus research in the use of Probiotics for aquaculture animals is increasing with demand for environmental friendly sustainable aquaculture (Gatesoupe, 1999). Probiotics have been
defined as microbial dietary supplement of benefit to the host (Fuller, 1989). The benefits of such supplement includes improved food value, enzymatic contribution to digestion, inhibition of pathogenic microorganisms, antimutagenic and anticarcinogenic activity, growth promoting factor and increase immune response (Verschuere et al., 2000). The effect of Probiotics for shrimp and fish have been reported by many authors (Mohanty et al., 1993; Sharma and Bhukhar, 2000; Wang et al., 2005; Wang and Xu, 2006).

2.4. Broodstock Management (Maturation, spawning, hatching and nauplii production)

Highly nutritive and balanced diet is very important for captive reproduction at shrimp hatchery which will reflect in nauplii quantity, quality and eventually at good quality seed production with good survival rate (Wouters et al., 2001). Maturation diet plays a key role in shrimp hatchery operation and in culture system. The weight of the ovaries of maturing shrimp can increase four to nine fold when the animals fed with nutritive diets (Jeckel et al., 1989; Mourente & Rodriguez, 1991; Ravid et al., 1999). Sufficient nutrient is needed to accumulate in the egg yolk to sustain the normal development of the embryo and pre feeding larvae.

It has been reported that an unbalanced or incomplete diet causes poor reproductive performance or may even stop animal from reproducing (Bray & Lawrence, 1992). Food represents the highest operation cost in most broodstock facilities (Kawahigashi, 1998). Shrimp maturation and reproduction are greatly influenced by environmental factors (Bray & Lawrence, 1992; Ogle, 1992). In natural conditions these factors determine the existence of breeding seasons. Breeding seasons are characterized by the presence and availability of specific food organisms, adequate photoperiod and water temperature. In the wild adult shrimp can eat a wide variety of micro invertebrates, (gastropods, bivalves, crustaceans and polychaetes) and plant materials (Rothisberg, 1998). The changes in the abundance
and distribution of these food organisms or their nutritional quality, explain in part the observed changing pattern of reproductive performance at different times of the year (Crocos & Coman, 1997). In captivity, one tries to mimic the condition of breeding season in an attempt to trigger the hormonal machinery that controls maturation. Fresh or fresh frozen marine organisms are used for acceptable maturation and reproduction outputs. Often these marine organisms are found to give the best results when they are in reproductive stage. Squid, bivalves (mussel, clam, and oyster) and polychaete worms are generally the main food items fed at high daily rations for the consistent production of high quality shrimp naupli (Du et al., 2004). Crustaceans like Crab and Krill are also fed for shrimp Broodstock, but due to the risk of disease transmission, they are used less frequently nowadays (Anirban Chakrobarthy, 2002).

Polychaete worm (Glycera dibranchiate) is expensive maturation diet, but hatchery operators feel that it is indispensable for stimulation of ovarian maturation (Kawahigashi, 1998). In the wild, 85% of ingested food of adult P. monodon consisted of small crabs and shrimps and mollusks (Morte, 1980). The more occurrences of mollusks and other non-crustaceans during months when P. monodon showed a higher feeding index may reflect changes in dietary requirements related to gonad development during the spawning season (Marte, 1982). Marte (1980) observed that feeding activity of female P. monodon was significantly higher than that of males. Several studies have been performed to study the life cycle pattern and improve reproductive performance of different Penaeid species in captivity (Ogle, 1992; Luis & Ponte, 1993; Machinouchi & Hirata, 1995; Ramos et al., 1995). Fecundity in penaeids has been reported to increase with female size (Motoh, 1981; Makinouchi & Primavera, 1987; Cavalli et al., 1997; Hoang et al., 2002). It has been generally accepted that larger, presumably older, penaeid females present a superior
spawning performance; the influence of age has been poorly explored (Menasveta et al., 1994; Minagawa et al., 2000). Hoang et al. (2002) reported that larger F. merguiensis females produced a superior overall spawning performance than smaller females of the same age.

2.5 Eyestalk ablation

Eyestalk ablation has been used to induce ovarian maturation in various Penaeid species (Adiyodi & Adiyodi, 1970; Primavera, 1985). Male penaeid generally mature in wild so that induced maturation mainly concern with females. Santiago (1977) observed that ablation of one eye was sufficient to induce maturation in P. monodon. The method most commonly used are incision-pinching as described in detail for P. monodon by Primavera (1978) and Cutting and cautery have also been used to ablate P. duorarum (Caillouet, 1972; P. orientalis (Arnstein & Beard, 1975), and P. herathurus (Lumare, 1979). Cautery prevented bleeding and produced less mortality in ablated female in P. monodon and P. indicus (Muthu & Laxminarayana, 1977) but female ablated by pinching method gives more stress and further mortality at maturation tanks (Makinouchi & Primavera, 1987). In India hatchery operators are adopted cutting and cautery method to induce maturation in female broodstock and gives better results than any other methods. The great majority of captive maturation in P. monodon has been from ablated females and Very few workers have been reported maturation in unablated females (Santiago, 1977; Primavera, 1978; Aquacop, 1980) with successful spawn (16.7% to 82%) only from Emmerson (1983).

2.6 Spawning and hatching

The act of spawning occurs in one or several minutes and description available for several penaeid species AQUACOP (1977a). Spawned eggs go through a striking process of changes immediately after extrusion and accompanying the fertilization process (Clarck
et al 1980). Egg fertilization and classification have been described by AQUACOP(1977a) and Primavera and Posadas (1981) for *P. monodon*. Hatching of *P. monodon* eggs was well documented by Emmerson (1980) ; Primavera and Posadas (1981); Lin and Tin (1986a) Choy(1987). The induction of maturation and spawning in female penaeids through unilateral eyestalk ablation of various species has been well documented (Primavera, 1978; Lumare, 1979; Emmerson, 1983; Makinouchi and Primavera, 1987). Excellent reviews on the reproduction of penaeid shrimps in captivity have been published by Primavera (1985), Bray and Lawrence (1992) and Browdy (1992). Considerable success has been achieved in the maturation and spawning of the Gonadal Maturation and Spawning of *Penaeus semisulcatus* (Browdy and Samocha, 1985; Browdy et al., 1986). Abnormal spawn for *P. monodon* eggs laid in masses remain unfertilized and unhatched (Villaluz et al., 1969) perhaps due to a failure of the cortical reaction (Aquacop, 1977a). The wide range of numbers of eggs could be due to varying female sizes (50-200gms) and inclusion of egg counts from both partial and complete spawns. Similarly hatching rates range from 0 to 90% depending on nutrition ratio, water depth and other physiological factors that may affect egg quality and/or mating efficiency.

2.7 Larval rearing

The pioneering work of Hudinaga (1942) in Japan with spawning and rearing of *P. japonicus* larvae laid the ground work for modern penaeid shrimp larviculture. Now there are 24 penaeid species worldwide whose culture have been either partially or fully established (Liao, 1985). Propagation of *P. monodon* by Liao et al. (1969) paved way for penaeid larviculture worldwide. Successful larviculture and mass production of high quality formulated shrimp feed made possible systematic and large scale production of *P. monodon* in Asian countries using intensive culture methods. Techniques used for mass
production of penaeid larvae are generally divided into three categories; intensive, semi-intensive and extensive. The intensive method referred as clear water system or Galveston method which is predominant in western hemisphere for the culture of *P. vannamei*, *P. stylirostris*, *P. azetecus* (Smith et al., 1993). In the clear water system, water quality is carefully controlled through rigorous water treatment, control of inputs into the system and a high rate of water exchange. The semi intensive and extensive styles were also known as Taiwanese, Japanese and Asian methods which are predominant in the eastern hemisphere for the culture of *P. monodon*, *P. japonicus* and *P. chinensis* (Liao, 1985; Liao et al., 1973). These three styles differ in terms of larval stocking density, feeding regime, live feed method, culture method, tank designs, water exchange, cost and labour. The fecundity and relatively fragile larvae of *P. monodon* are well suited to less intensive culture system.

Several larval rearing schemes are being practiced. Basically, the difference lies in variations of feeding and management practice which is influenced by site, seasons (Hudinaga & Kittaka, 1975) and skills of the technicians (Liao 1986; Kungwankij et al., 1986). Penaeid larval rearing techniques were developed primarily using the community culture method practiced by Hudinaga and Kittaka (1966). Consequently methods and procedures tended to be more specialized needing more control of culture condition, thus requiring smaller tank system, separate algal production tanks and more intensified stocking and monitoring procedures. Familiarity with larval stages are important in determining the right kind of feed to give at right larval stages either natural or formulated and in carrying out other hatchery procedures. The egg and larval developmental stages are well described by Motoh (1979, 1981). Larval feeds are classified as either natural or artificial feeds (Liao, 1988b). Natural, live food organisms include phytoplankton and
zooplankton. Phytoplankton species which are popularly cultured and used as feed includes *Chaetoceros* sp., and *Skeletonema* sp. (Kungwankij, 1972; 1976; Simon, 1978). Phytoplankton is main food during larval stages and adequate timing and proper planning are necessary to meet day-to-day food requirements. *Chaetoceros calcitrion* is used almost exclusively for Zoea and early Mysis stages. Since zoea is the most difficult larviculture stage in shrimp larvae, provision of high quality feed during this stage improves survival at latter stages (Liao, 1988b). Late Mysis and Post Larvae stages require animal protein in their diet. Common animal protein sources are Artemia nauplii and formulated supplementary feeds which are more commonly used. Formulated feeds come in different forms, including dried powder and micro particulate feeds. Microparticulate feeds may be either micro encapsulated diet or micro bound diet, or micro coated diet. Most hatcheries regardless of the size, require the culture of at least two types of living food organisms—unicellular algae for Zoea stage and Artemia for Mysis to post larvae stages (Wickins, 1986). Proper identification of the most adequate live food organism propelled the development of hatcheries in to successful industrial practice.

2.8. Use of Antibiotics and Probiotics.

2.8.1 Oxytetracyclin

Oxytetracycline is a broad spectrum antibiotic that is active against a wide variety of bacteria. However, some strains of bacteria have developed resistance to this antibiotic, which has reduced its effectiveness for treating some types of infection.
2.8.2 Chloramphenicol

Chloramphenicol is a bacteriostatic antimicrobial agent originally derived from the bacterium Streptomyces venezuelae, isolated by David Gottlieb, and introduced into clinical practice in 1949. It was the first antibiotic to be manufactured synthetically on a large scale and is considered the prototypical broad-spectrum antibiotic. Chloramphenicol is an effective against a wide variety of Gram-positive and Gram-negative bacteria, including most anaerobic organisms.

![Structure of Chloramphenicol](image)

2.8.3 Probiotics

Probiotics are defined as microbial dietary adjuvants that beneficially affect the host physiology by modulation the mucosal and systemic immunity, as well as improving microbial balance by preventing the colonization of undesirable bacteria in the intestinal tract. In the present investigation Probiotics (Brand name: BACIMOR Hi-Spore Hatchery manufactured by Hi-line Aqua PVT. LTD Adayar, Chennai, India) was used to study the effect on maturation, spawning, hatching and larval rearing. The following strains of Bacillus are present in the Probiotics: Bacillus subtillis, Bacillus licheniformis-I, Bacillus licheniformis-II, Bacillus polymyxa, Bacillus megaterium, Bacillus tumulus.
2.9 Larval Health Analysis

All type of commercial larviculture systems have been plagued by problem with pathogenic microorganisms. Shrimp disease is considered as the single most limiting factor to successful commercial production. Diseases of Penaeid shrimp may be caused by living agents like virus, bacteria, fungi and parasites. Moreover stress from nutritional deficiency in the shrimp, exposure to toxic substances and advance rearing water parameters may also leads to diseases (Lightner et al 1992a, Flegel et al 1995). Early detection and diagnosis are crucial factors to well timed and prompt control. Some of the diseases observed in *P. monodon* larval rearing systems in India are: Viral diseases (White spot syndrome virus, Monodon baculovirus, Yellow head virus, Infectious hypodermal and hematopoietic necrosis virus, Hepato pancreatic parvovirus), Bacterial diseases (Filamentous bacteria, *Vibrio* spp, Mycobacteria, Necrotizing hepatopancreatitis), Fungal disease (larval mycosis) and Parasites (protozoan). Shrimp larval quality is considered as another key factor in influencing the successful shrimp culture. Proper management in hatchery and nursery system would ensure good survival and growth rate of shrimp post larvae before stocking. In recent years, shrimp health management has become the main focus of improving production and minimizing infectious diseases in shrimp ponds. However, the ultimate goal of shrimp health management is to (1) prevent the disease from occurring; (2) reduce the incidence of infectious disease when it occurs and (3) reduce the severity of the disease when it occurs.