CHAPTER I

INTRODUCTION

Among various crustaceans, marine shrimps and prawns of the family penaeidae constitute the dominant-aquaculture group. The production is expected to increase given full or close to full exploitation of wild stocks (Primavera, 1985; Uno, 1985). The long-sought goal of achieving maturation and reproduction of marine shrimp in captivity has met numerous successes over the past decade. The degree of success varies among species and commercial application is only successful in some cases. A number of species have been tested, at least on a preliminary basis but the knowledge about a quality of spawners from captivity varies greatly (Bray and Lawrence, 1984; Caubere *et al.*, 1979; Primavera, 1985).

Shrimp farm is becoming a new agricultural industry for both developed and developing countries (Bray and Lawrence, 1984). However, the present problems about shrimp culture are presently plagued by erratic supply of gravid shrimps, spawners and larvae (AQUACOP, 1985; Khoo, 1988). The constraints of spawner supply are lying also in seasonal catching, insufficient quantities and large variation in prices. (AQUACOP, 1985; Kungvankij, 1985). To overcome this problem, researchers have developed techniques in controlling maturation and subsequent spawning from adult size animals caught in the wild and maintained in tank. Under some manipulations in captivity conditions, some species mature and spawn but for others the techniques still need to be developed. At optimal condition the maturation process is very rapid (Khoo, 1988; Primavera, 1985).

The use of captive broodstock eliminates dependence on the wild stock larvae in the coastal area particularly in mangrove and estuary which are the main nursery ground (AQUACOP, 1985; Dall, 1985; Kungvankij, 1985; Primavera, 1985).

Some academic researchers studied the method of complete cycle culture. For example, the culture of shrimps from one generation to another generation using the cultured prawns for subsequent breedings. The achievement of this method may solve a problem of shortage supply of spawner from wild stock (Bray and Lawrence, 1984; Caubere, *et al.*, 1979; Khoo, 1988).

The selection of broodstock can be undertaken when harvesting a pond by sorting the fast growing animals which are then cultured at low density to ensure maximum growth. Another method is to use particular ponds to fully control the animals from postlarvae to reproductive size. These can even be produced in intensive systems giving surprisingly high quality spawners such as for *Penaeus vannamei* (AQUACOP, 1985). If the techniques in controlling reproduction of different penaeid species in captivity are sufficiently known, transfer of the techniques to commercial scale still requires a lot of improvements.

Long-term research must also be dedicated to reproductive physiology as toi give us a better understanding of individual internal mechanisms and to gain better control of culture. Even so, the time has come for lessening dependence on natural stocks. In closing penaeid cycles, genetic selection for desirable traits such as strong growth and survival, disease resistance, etc., can be accomplished. Additionally, by obtaining very little new stock a facility will decrease risk of introducing some disease organisms (Bray and Lawrence, 1984; Choy, 1987; Primavera, 1978).

Using gravid-mated female broodstocks captured from the wild is fraught with uncertainty such as limitation in quantity of broodstock supply and the seasonally of their capture in the wild (Khoo, 1988). In order to overcome this problem, the year round maintenance of broodstock under optimal conditions is necessary. And in a tropical countries where year-long outdoor culture is possible, some species have been found to reach sexual maturity satisfactorily in ponds (Rodriquez, 1981; Santiago, 1977). The induction of gonadal development, mating and spawning in adults from broodstock reared in ponds or other culture vessels completely eliminate dependence of the wild broodstock. The use of gravid female in captivity remains the basis for most researches which are aiming towards a complete understanding of captive breeding of penaeid shrimp. Many environmental and nutritional parameters for captive breeding are only broadly defined, while the consistent commercial application of these principles demands close definition for each species such as the condition for induced maturation of *Penaeus monodon* (Khoo, 1988).

Ferraris *et al.* (1987) stated that osmoregulation ability of adult prawn *Penaeus monodon* decreases when comparing to juvenile stage which dominates in estuary. This may result in offshore migrating behavior of adult shrimp to the spawning ground (New and Raband, 1985). Salinity stress may also become limiting factor when acting together with one or several other stressful condition in estuary. Osmotic stress in high salinity is possible to be a trigger of maturation process in migratory species as *P. monodon* (Sastry, 1983).

Energy expenditure process as osmoregulation can result in energy allocation in reproduction, gamete production or ovarian development (Brafield and Llewellyn, 1982; Vernberg, 1987). Thus, animals usually migrate to stable environment where osmotic work can succeed with less physiological stress. That is, will result in a successful maturation and spawning.

The gonad development of *Penaeus* spp. also occurs mostly in high salinity (Adiyodi, 1985; AQUACOP, 1985; Beard and Wickins, 1980; Primavera, 1985). The osmoregulation and salt tolerance of broodstock may relate to reproduction processes (Lockwood, 1969, 1976; Vernberg, 1983).

Nowadays, studies on osmoregulation and maturation of *Penaeus monodon* are still scanty. In particular, the combined effects of eyestalk ablation on osmotic regulation, and broodstock maturation under hypersaline condition is not very well documented.

<u>Objectives</u>

1. To determine the type of osmoregulation in subadult *Penaeus monodon* and the relationship between osmoregulation and eyestalk ablation, and the relationship between osmoregulation and acclimation time.

2. To study effects of hypersaline acclimation and eyestalk manipulation on ovary maturation of *Penaeus monodon* using ovarian index and maturity index.

3. To study hypersalinity effects on morphology of oocyte and size of mature ova in subadult and adult *Penaeus monodon* by paraffin histological method.

4. To describe and determine oocyte development by paraffin histological method and microscopic analysis.

5. To study hypersalinity and eyestalk ablation effects on condition index (body weight/carapace length) and molting interval of broodstock *Penaeus monodon.*

6. To find manipulation techniques that accelerate the ovarian development of *Penaeus monodon* broodstock in closed-recirculating water system.

Review of Literatures

1. Induced Maturation in Penaeid Prawns

Most of previous researches in prawns now were focused on defining the optimum environmental parameters and suitable methods for inducing maturation of commercial species and determining the best rearing condition to obtain healthy broodstock and sufficient seed supply for shrimp farms (Chakraborti *et al.*, 1986; Sastry, 1983; Khoo, 1988; Uno, 1985).

Kungvankij.(1985) recorded that female *Penaeus monodon* is more difficult to mature in captivity than in other penaeid species, although excellent progress on this aspect is being achieved. Thus, reliable techniques for maturation are also being developed.

The numerous techniques for inducing ovarian maturation in *Penaeus monodon* can be classified as following :

1. Environmental manipulation utilizing temperature, photoperiod, salinity, substrate and population density, 2. Nutritional manipulation of ovarian maturation, 3. Eyestalk ablation, and 4. Treatment with exogenous hormones by injection, or through thec feed. However, in this study only environmental manipulation and eyestalk ablation will be taken into consideration.

1.1 Eyestalk ablation

Hormone inhibiting vitellogenesis is produced by the X-organ-sinus gland system in decapod Crustacea. The X- organs show histological cycle associated with oocyte development (Highnam and Hill, 1977). Vitellogenesis may be controlled by alternately fluctuating levels of ovarian-inhibiting and stimulating hormones produced by neurosecretory centers in different parts of the central nervous system. However, the way in which the ovarian-inhibiting and stimulating hormones act upon the ovary is unknown (Highnam and Hill, 1977; Khoo, 1988).

Eyestalk ablation is receiving great attention as a possible and useful method of inducing precocious maturation of ovary under captivity. It has been shown that eyestalk ablation of crustaceans seems to stimulate either molting or ovarian development (Adiyodi and Adiyodi, 1970; Arnstein and Beard, 1975; Emmerson, 1983; Webster, 1985). Maturation of subadult *Penaeus monodon* is achieved by unilateral eyestalk ablation of female prawn (Primavera, 1978). Crustaceans eyestalks are known to contain the neuroendocrine centers responsible for the production and release of both the molt-inhibiting hormone (MIH) and gonad inhibiting hormone (GIH) (Beltz, 1988; Muramoto, 1988; Tombes, 1977). The manner in which these hormones control molting and ovarian development has been investigated (Cooke and Sullivan, 1982; Klenholz and Bourquin, 1984) but the mode of action is still under contention.

The stimulation is known to be dependent upon the relative interaction of the environmental factors as well as the age, molting stage and physiological condition of the animal operated on (Emmerson, 1983: Highnam and Hill, 1977; Webster, 1985). Furthermore, the ablation is a good short-term method for obtaining viable larvae in the laboratory. Moreover, attainment of sexual maturity and viable spawning in pond without eyestalk ablation has been shown for *Peneaus monodon* (Halder, 1978), *P. merguiensis* and *P. japonicus* (Bray and Lawrence, 1984).

Eyestalk removal is also known to adversely affect on the normal feeding behavior of prawns (Choy, 1987), metabolism and overstimulation of spawning may cause long-term problems, such as declined spawning, and result in death of ablated prawn (Emmerson, 1983; Kelmec and Smith, 1980; Lumare, 1979)

1.2 Environmental manipulation

At present, ecology and life history of *Penaeus monodon* with regard to seasonal abundance of fry and adults, spawning season and area, larval development, and migration and transportation are not well documented (AQUACOP, 1985).

Most of subadults and adults *Penaeus* spp. inhabit offshore waters down to about 160 m - the waters are high and unfluctuated in salinity (Motoh, 1981,1985).

The optimization of conditions to increase reproductive potential would reduce the necessity of other less effectual methods ,such as eyestalk ablation, where the viability of the eggs after treatment tends to be low (Bray and Lawrence, 1984; Primavera, 1978)

High salinity together with other parameters such as temperature and photoperiod also affect maturation and spawning (AQUACOP, 1985; Chamberlain and Lawrence, 1981; Khoo, 1988; Pudadere and Primavera, 1981; Pudadere, Primavera and Young, 1980).

Racek (1959) studied on the importance of salinity and breeding migration of *Penaeus plebeius.* Prawns prevented from leaving the estuary for two years were still sexually immature to the end of this period, although they had attained normal adult body length. Breeding migration to offshore is essential if *Penaeus* prawn are to reproduce, and shrimp that are prevented from migrating never mature. As they approach maturity, they show a pronounced urge to leave the estuarine environment as reflected by mass migration to oceanic water before spawning (Penn, Hall and Cuputi, 1985; Recek, 1959; Vernberg and Vernberg, 1972). Salinity will indeed affect reproduction and development in areas where it undergoes pronounced changes. Unfortunately, data about these effects are scanty, and it seems difficult at the moment to make general statements. Moreover, it is impossible to bring an analysis of the ecological effect of salinity on growth and development down to the molecular level (Gilles and P'equeux, 1983). It appears that salinity markedly affects both egg production and embryonic development in a variety of crustaceans. Experiments on gameto-genesis or gamete formation (egg and sperm formation) are very rare in the literature.

Not much is known about the mechanism of action of the various environmental factors in the control of the neurosecretory center but a number of papers have shown that some of these factors do induce ovarian maturation in penaeid prawns. Salinity probably controls the release of the gonad inhibiting hormone (GIH) from the X-organ-sinus gland complex and hypothetical gonad stimulating hormone (GSH) from the brains and thoracic ganglions (Gomez, 1965). It is assumed that salinity acts through these neurosecretory centers (Khoo, 1988).

2. Osmoregulation of Penaeid Prawn

Organisms living in estuaries and migrating to marine habitat as penaeid prawn must be able to osmoregulate efficiently (Gilles, 1970, 1979). Classification of organisms according to their osmoregulation capabilities divides organisms into two groups: hyper-osmoregulators and hypo-osmoregulators. The hyper-osmoregulators have higher ionic concentration in haemolymph than in surrounding medium while hypo-osmoregulators show lower ionic concentration in haemolymph than in external medium (Campbell and Jones, 1989; Castille and Lawrence, 1981; Gilles, 1970; Mantel and Farmer, 1983; Potts and Parry, 1964).

There are some crustaceans that show ability as hypo-hyperosmoregulation. They are *Palemonetes varians, Artemia salina* and *Penaeus monodon* (Castille and Lawrence, 1981; Cheng and Liao, 1986; Ferraris *et al.*, 1986, 1987; Knut, 1983). These organisms are extremely powerful osmoregulators and can cope with any fluctuation of salinity of the external medium that normally occurs in nature (Dall, 1985; Ferraris *et al.*, 1986, 1987; Gilles, 1979; Motoh, 1981; Potts and Parry, 1964).

Osmotic receptors in *Penaeus monodon* are thought to be located near neurosecretory cells in eyestalk complex and other parts of nervous system (Tombes, 1977; Beltz, 1988; Waterman, 1960). As they sense a change in salinity of the external medium they send a signal to the central nervous system (CNS), thus, initiating osmoregulation. Moreover, hormones implicated that function in salt and water balance are produced by neurosecretory cells, in eyestalk, brain, and thoracic ganglion. The crustacean thoracic ganglion contains a substance that increases the permeability of the body surface to water, whereas the eyestalk has a substance that either inhibits the release of the thoracic ganglion factor or acts directly on the body surface decreasing its permeability to water (Fingerman, 1976; Waterman, 1960).

The combined effect of salinity and ablation of eyestalk in some prawns and crabs may lead to increase in weight and decrease in osmolality or ion concentration of haemolymph, presumably because of increased water uptake or retention (Beltz, 1988; Muramoto, 1988). Eyestalk ablation can induce maturation or molting and results in hormonal imbalance which affects osmotic stress (Highnam and Hill, 1977). However, no significant changes in osmolality or ion content occur when the ablated animals are hypoosmotically regulating (Mantel and Farmer, 1983). Thus, in hypersalinity where maturation occurs eyestalk ablation may not affect osmoregulation of broodstock. The prolonged osmotic stress may produce some shortage of energy demands for growth or maturity, which is reinforced at a certain moment by some generalized reduction of energetic metabolism i.e. reduction of

ATP production (Beltz, 1988; Gilles, 1970, 1979; Fingerman, 1976; Lassere, 1976; Towle, 1984; Yancey *et al.*, 1982).

Therefore, it is believed that hypersaline acclimation should be able to induce maturation of *Peneaus monodon* in captivity. In order to know whether hypersaline acclimated prawn has reached maturity or not, gonad index and maturity index will be employed (Giese and Pearse, 1974; Grant and Tyler, 1983a, 1983b)

3. Method of Estimating Sexual Reproductive Condition

3.1 Gonad Index

The most widely used quantitive method for estimating reproductive stage is gonad index. The gonad index is calculated in several ways, but usually it is the ratio of gonad wet weight (or volume) to the wet weight (or volume) of the whole animal, expressed as a percentage (De Vleming, Grossman and Chapman, 1982; Giese and Pearse, 1974; Vernberg and Vernberg, 1972).

Determination of the gonad index is an attempt to measure the relative reproductive condition of animals of variable sizes so that changes in their gonads at different times can be compared (Giese and Pearse, 1974; Grant and Tyler,1983a). However, A major limitation of the gonad index method is that, unless accompanied by microscopic examination of the gonads, it indicates little as to what is occurring within the gonads (Giese and Pearse, 1974; Grant and Tyler,1983b).

3.2 Maturity Index

Examining sections of gonads also yields more detail information on reproduction than it is possible to obtain from an examination of gonad indices alone. The simplest assessment of paraffin sections is to subjectively place in a number of development stages. It seems better to call this method as **maturity index** (Bell and Lightner, 1988; Grant and Tyler, 1983b).

If microscopic preparations of the gonads are being produced to estimate maturity indices, it is definitely worthwhile measuring oocyte sizes in the females as the amount of information gained. The oocyte size can be measured using eyepiece graticule calibrated against a stage micrometer. Only those oocytes sectioned through the nucleus are measured (Grant and Tyler, 1983a, 1983b).

Additionally, in order to given the need of keeping female prawn alive for spawning, most penaeid workers use the *in vivo* classification by looking at the ovaries externally through the dorsal exoskeleton (Motoh, 1981; Primavera, 1978).