CHAPTER I

INTRODUCTION

Among various crustaceans, marine prawns and shrimps in Family Penaeidae constitute the dominant aquaculture group at present. Although shrimp culture contributes only a few of current total world shrimp production, shrimp culture has shown tremendous development in the last decade. Due to a constant increase in shrimp demand and limited supply in the world market, it has appeared clearly to many investors that the gap could not be filled by capture fishery which is declined by overfishing, coastal pollution and increasing operating costs. Shrimp farming becomes a new agricultural industry for both developed and developing countries. Marine shrimp farming is a century-old practice in many Asian countries. However, sluggish development of this industry is mainly due to an inadequacy of hatchery technology.

The commercial species cultured in Thailand belongs to giant tiger prawn or jumbo prawn, *P. monodon*. This species is the most common and well-known in Southeast Asian countries. *P. monodon* is the fastest growing prawns for pond culture (Pinij Kungvankij, 1985). Although several hatcheries have been established, fry production is still not steady due to dependence on spawners which are difficult to obtain from the wild and more difficult to mature in captivity than those of other Penaeid species.

Reliable techniques for maturation are also being developed. Many hatcheries have been developing techniques for maturing *P. monodon* in captivity but so far the results are not consistent. Thus techniques for gonadal maturation of captured and pond-reared adult *P. monodon* need to be improved. In fact, endocrine manipulation has been synonymous with unilateral eyestalk ablation in order to induced maturation. However, the lower hatch rates may be caused inherently poor egg quality or unmated females. Besides, broodstock copulated prior to the capture or exhaustion of stored spermatozoa shows decrease in sperm quality in seminal receptacle during the sequential spawning (AQUACOP, 1977, quoting Beard and Wickins, 1980).

Generally, newly-caught wild or pond-reared broodstocks mated before stocking in maturation tank. Hatching and viability of nauplii from captive spawnings are dependent on sperm from copulation in the wild or pond environment. However, unsuccessful mating in captivity will eventually produce nonhatched eggs due to loss of spermatophores after molting. In comparison to closed thelycum species, the failure of mating and consequent absence of spermatophores is more frequent in open thelycum ones which spermatophores are more easily dislodged. Low frequency of mating is also observed in closed-thelycum species, i.e. *P. monodon* (Lin and Ting, 1984). Artificial transfer of spermatophore has been developed to solve the problem. Introduction of spermatophore transplantation method together with a development of reliable criteria for selection of fully mature spermatophore to assure a reproductive success is important. The technique would be a significant progress as it allows reuse of valuable broodstocks and improves hatching efficiency in the hatcheries.

Most researches concerned with developing technology for maturation and reproduction of penaeid species have focused primarily on female reproduction. However, some evidences show that male reproduction also has problems which are responsible for the limitation of reproduction in captive penaeid species. Since artificial insemination of penaeid shrimps have been accomplished (Bray, Chamberlain, and Lawrence, 1982; Sandifer *et al.*, 1984; Lin and Ting, 1986). Sperm quality of the male, especially, in the closed thelycum species is still not very well documented. With this respect, the present investigation was undertaken to study sperm quality of *P. monodon* by several methods and criterias.

Objectives

The objectives of the present study are:

1. To determine the size of the initial maturation of the pond-reared male *P. monodon*
2. To quantify and to study the sperm quality of pond-reared *P. monodon* at different sizes.
3. To determine optimal size of pond-reared male *P. monodon* recommended for broodstock.
4. To compare quality and quantity of sperm of pond-reared stock and wild stock at different sizes.
5. To quantify and study the sperm quality of normal male and unilateral eyestalk ablated male from pond-reared stock in captivity at different period of captivity.

Review of Literatures

P. monodon is dioecious. The sexes can be distinguished by external characters (genital organs), petasma and appendix masculina in male and thelycum in female (Solis, 1988).

Structural Male Genital System of Giant Tiger Prawn.

Motoh (1981) reported that the male genital system consists of internal organs, viz. paired testes, paired vas deferens and paired terminal ampoules, and five lateral lobes located in the cardiac region dorsally to hepatopancreas under the carapace. The lobes are connected to each other at their inner ends and lead to the next organ, the vas deferens. The vas deferens arise from the posterior margins of the main axis of the testes and open to the exterior through genital pores located on the middle of coxopod of the 5th pereiopods. Each vas deferens consists of four distinct portions; a short narrow proximal portion (proximal vas deferens), a thickened large medial portion having a double fixture (medial vas deferens), a relatively long narrow tube (distal vas deferens) and a muscular portion (terminal ampoule). The terminal ampoule, a bulbous structure, possesses a thick muscular wall lined with extremely tall columnar epithelial cells. The terminal ampoules have two chambers internally, one containing the spermatophores and the other, calcareous material of a slightly gray color. The paired terminal ampoules open at the base of the coxopod of the 5th pereiopods.

The Petasmal Development of Giant Tiger Prawn.

Motoh (1981) reported that specimens at average size of 11 mm in carapace length (CL) possess a small rudimentary petasma in the form of a knife-shaped projection situates on the subapical portion of the protopod. Until at a size of 31 mm CL, the modified endopod closely resembles the petasma of the adult. When the prawn reaches about 34 mm CL, the petasma has almost assumed the adult form. The two halves are now large enough so that their inner margins meet at the median line and are thus united or fused together with the aid of numerous minute hooks. However, the two components could be easily separated by physical force. The relationship between carapace length and petasmal length in wild male, *P. monodon* derived from the least square method are: PL = -3.5075 + 0.3025*CL, where CL = the carapace length and PL = petasmal length in mm.

Size at First Maturation of Male *P. monodon*.

Motoh (1981) reported that subadult stage began at the onset of maturation, i.e. males possessing spermatozoa in terminal ampoules, and females possessing first spermatozoa inside the thelycum through copulation. A sex size disparity occurred at almost 30 mm CL, and hereafter, the growth of females became greater than males. The size at onset of maturation in both male and female were independent of the fishing area and the season. Motoh also pointed that males were sexually mature at a smaller size than females in *P. monodon* from the wild. It seems to have a size gap between *P. monodon* from the wild and those from brackish water fishponds in terms of possessing spermatozoa on both sexes. The minimum sizes of males and females

possessing sperm, the brackish water reared ponds showing smaller than the wild. Primavera (1978) reported both pond-reared and wild male *P. monodon* as possessing morphologically normal sperm at the average 10 months.

The Formation of Spermatozoa and Spermatophore in Penaeid Shrimp.

Shigekawa and Clark (1986) studied spermiogenesis in the marine shrimp *Sicyonia ingentis* and found that spermiogenesis of the penaeid shrimp can be divided into 7 discrete stages; consisted of (1) vesicular cytoplasmic stage, (2) proliferation of the rough endoplasmic recticulum, (3) anterior granule formation, (4) acrosomal vesicle formation, (5) flocculent chromatin stage, (6) membrane pouch formation, and (7) subacrosomal development. Each of these is distinguished by morphological characteristics and the occurrence of particular developmental processes.

Decapod crustacean sperms have been recognized as unique cells vastly different from the sperm of other animals. Generally, they are nonmotile sperm cells consisting of a spherical main body housing an uncondensed nucleus, a central cap with acrosomal vesicles, and an anterior spike (Kleve, Yudin, and Clark, 1980; Shigekawa and Clark, 1986)

The sperm of the decapods could be divided into two major groups based on their morphological characteristics. The first group has been further categorized as the reptantian (lobsters, crayfishs, crabs). The second group is natantian type (shrimps or prawns). The sperm of these two groups differ in the number and structure of its appendages. Reptantian sperm have several stellate appendages (radial

arms) which are continuous with the nucleus and usually contain chromatin and/or microtubules (Shigekawa and Clark, 1986 quoted in Moses, 1961a, 1961b, Pochon-Masson, 1965, Brown, 1966, Hinsch, 1969, Talbot and Summer, 1978, Talbot and Chanmanon, 1980, Lopez-Camps *et al.*, 1981). On the other hand, natantian sperm possesses a single appendage (spike) which is not continuous with the nucleus and may contain microfilament (Shigekawa and Clark, 1986 quoted in Pochon-Masson, 1968, Brown *et al.*, 1976, Lu, 1976, Brown, 1978, Kleve *et al.*, 1980). Many papers dedicated to the morphology of mature sperm of caridea natantians have illustrated the great diversity of sperm structures between the palaemonid and penaeid shrimps (Lynn and Clark, 1983 quoted in Pochon-Masson, 1969, Lynn, 1981, Sandifer and Lynn, 1981, Sellos and Legal, 1981). However, natantian sperm is still poorly understood compared to the reptantian (Shigekawa and Clark, 1986 quoted in Brown, 1966, Hinsch, 1971, Talbot and Chanmanon, 1980, Goudean, 1982).

Malex and Bawab (1974b) reported that three successive stages could readily be recognized during the formation of the spermatophore in *P. kerathurus*. All of them involved participation of certain secretions yielded by appropriate glands which lined throughout the vas deferens. At the first stage, the sperm cells are individually released from the testes and converted into a compact mass. The process starts in the proximal region of the vas deferens but is not completed until the sperm cells reached the blind pouch. In the second stage, the main layers constituting the spermatophores are deposited partly around the sperm mass and partly as an independent, solid, accessory wing. All of these layers are produced in the ascending

limb, and their subsequent fusion together in the adjoining descending limb resulted into the formation of an incomplete spermatophore. The third stage occurs in the terminal ampoule, when the spermatophore is eventually molded to its final and subsequently hardened. Perez Farfante (1975) described the characteristics of spermatophores in five species of the American penaeids and concluded that sperm of penaeids are similar structure, but are quite different in appearance.

Weerachai Singhaniyom and Verapong Vuthiphandchai (1989) investigated a male reproductive organ of *P. monodon* by light microscope and suggested that spermatogenesis takes place in convoluted tube. The immature sperms gradually develop while move along convoluted tube through the duct and to the sac. The process is evidenced by increasing spike length. Sperm needs to be stored in the sac for a certain period of time prior to completion of the process. However, Lin and Hanyu (1990) studied on vas deferens transplantation in *P. penicillatus* and reported an average hatching rate of 76.2 percent, a value exceeding hatching rate for female implanted with spermatophore. Lin and Hanyu also confirmed that the development of larval shrimp using their technique is normal. This indicates that spermatozoa in vas deferens of some penaeid shrimps are functionally mature. However, understanding of mature sperm and spermatophore in *P. monodon* is still not clear.

Following transfer to the female, the spermatophore protects sperm until spawning and fertilization occurred (Kooda-Cisco and Talbot, 1983 quoted in Allen, 1916, Fasten, 1917, and Mattews, 1954a, 1954b). Lin and Ting (1986) reported that sperm rooted on the seminal receptacle for many days. Anderson, Clark, and Chang (1985) found that

sperm of *S. ingentis* can live in seminal receptacle for weeks to about one month.

Fertilization.

Clark *et al.* (1984) studied the process of fertilization in the marine Penaeidae *S. ingentis* which could be considered a good representative description of fertilization process in the natantian group. They concluded that a process of penaeid shrimp fertilization can be divided into 7 stages as follows: (1) Primary binding, (2) Primary acrosomal reaction, (3) Secondary binding, (4) Jelly extrusion; this series of events is very similar to jelly formation as described in the ova of *P. aztecus* and *P. setiferus* (Clark *et al.*, 1980). Thereafter, Lynn and Clark (1987) studied biochemical structure of jelly, and showed that the penaeid material also differs in its biochemical properties from the jellies of other animal ova. It contained a substantial amount of protein compared to carbohydrate (70-75 percent protein and 25-30 percent carbohydrate), (5) Secondary acrosomal reaction (6) Fertilization; the most notable feature of fertilizing sperm is the loss of the anterior granule, and (7) Hatching envelope formation.

Recently, Griffin, Shigekawa and Clark (1988) found that formation and structure of the acrosomal filament is temporally separated from acrosomal exocytosis by 10-20 minutes *in vivo* and 30-45 minutes *in vitro*. They also reported that isolated sperm incubated in egg water undergo a complete acrosomal reaction and formation of the acrosomal filament (Griffin *et al.*, 1987).

Optimal Age at Maturation of *P. monodon*.

Santiago (1977) used 15 month-old pond-reared broodstock and found that average survival rate of larvae was about 41.6 percent from nauplii stage 3-4 to post-larvae 5-6. While, Primavera, Borlongan and Posasdas (1978) reported that only 22.90 percent nauplii 3-4 survived to post-larvae 15 in 1-2 year-old broodstocks from cultured pond. Many Thai researchers also reported that *P. monodon* over one year old pond-reared can be efficiently used for seed production with a good quality as a wild broodstock (Sakchai Chotikul *et al.*, 1985, 1986; Anand Tansutapanich *et al.*, 1989)

Factors Affecting on Fertilization of The Prawn .

Beard and Wickins (1980) quated in AQUACOP (1977) reported that eyestalk ablation techniques, sometimes affected egg quality because of some non-fully developed mature ova, and low fertilization efficiency caused by low quality in sperm. Paitoon Akayanon, Tanan Tattanon, and Paiboon Boonliptanon (1986) did an experiment on captive reproduction of *P. monodon* and suggested that percentage of fertilized eggs was affected by the number of sperms.

AQUACOP (1983) reported a low percentage of fertilization in captive *P. vannamei* spawn, even with artificial insemination, and suggested that poor sperm quality was responsible. They noted that males often had very small spermatophores or brown spermatophores with necrosis and that sperm masses removed from spermatophores were very small and lacked adhesive qualities. Chamberlain and Gervais (1984) working with adult *P. stylirostris*, reported that low incidence of successful mating during a 4 months study period was due to a swollen

vas deferens.

The Decline in Sperm Quality in The Prawn.

Brown *et al.* (1979) noted that extensive damage to spermatophores, including complete destruction of the anterior "wings" portion, geminate body, and flanges occurred in both estuarine and oceanic water culture. They also noted that a bacterial infection (*Vibrio* sp.) was associated with the deterioration. Chamberlain and Lawrence (1981b) worked with *P. stylirostris* and *P. vannamei* and stated that the reproductive capabilities of the males may be a limiting factor in the laboratory. Chamberlain, Johnson, and Lewis (1983) reported that over 70 percent of *P. stylirostris* and *P. setiferus* held in captivity for several months exhibited swollen proximal and medial vas deferens and 58 percent of both species had slightly to extremely melanized spermatophores. The incidence of melanization was particularly high in groups of males which had been manually ejaculated for using in artificial insemination. The report by Chamberlain *et al.* (1983), male *P. setiferus* became infertile within 35 days in captivity and could not be used for breeding concurred with that reported by Talbot *et al.* (1988, 1989).

Bray *et al.* (1985) preliminary investigation on sperm quality of captive *P. setiferus*, found that at 30 day-period, mean sperm counts were significantly lower in the ambient temperature and EDTA treatments. Whereas the chilled and inoculated treatments showed no decline in sperm counts during the first 30 day-period, but the percentage of abnormalities had increased. At 60 day-period, only the chilled treatment contained any males bearing sperm. Bray *et al.*

summarized evidences of decreasing sperm quality in captivity as an effect of low temperature, bacterial infection, and chemicals such as EDTA. Many reports also suggest that symptoms of male gonad disease, including spermatophore structural deterioration, melanization or necrosis of spermatophores and swelling of the vas deferens could take place in a variety of culture situations. These range from oceanic and estuarine flow-through seawater in raceways and tanks (Brown *et al.*, 1979; Chamberlain, Hutchins, and Lawrence; 1981, AQUACOP, 1983; Chamberlain *et al.*, 1983) to estuarine semi-closed system (Leung-Trujillo and Lawrence, 1985).

Leung-Trujillo and Lawrence (1987) studied on the decline in sperm quality of *P. setiferus* under laboratory conditions. They found that there was no significant decline in sperm quantity during the first 2 weeks. However, number of sperms declined in the third week, and no sperm could be found on spermatophore in the sixth and seventh weeks. It is clear that sperm quality of penaeid shrimp is affected by the period in captivity.

<u>The Effect of Eyestalk Ablation on Sperm Quality of The Prawn</u>.

Maturation of shrimps in the captivity can be achieved by unilateral eyestalk ablation of females (Khoo, 1988 quoted in Panouse, 1943, Aoto and Nishida, 1956, Kamiguchi, 1971, Bomirshi and Klek, 1974, Rcykaert *et al.*, 1974, Laubier, 1975, Primavera, 1978, Liang *et al.*, 1983, and Yano, 1984). Leung-Trujillo and Lawrence, 1985 Alikunhi *et al.* (1975) found that ablation of both males and females were necessary to induce maturation of *P. monodon* in brackish water. They also reported that small immature *P. merguiensis* males matured

within 7-8 days following the operation while ablation in mature males made the spermatophore to appear even more whitish and conspicuous. Using *P. vannamei*, Chamberlain and Lawrence (1981b) reported that unilateral eyestalk ablated males increased gonad size and mating frequency.

Leung-Trujillo and Lawrence (1985) studied the effects of eyestalk ablation on spermatophore and sperm quality in *P. vannamei* by investigating in bilateral, unilateral, and unablated males. They found that unilateral ablation enhanced gonad weight, gonad index and spermatophore weight. Unilateral ablated males demonstrated a significantly higher mean sperm count. Additionally, neither unilateral nor bilateral ablation caused a decreased in sperm quality. Talbot *et al.* (1989) suggested that unilateral eyestalk ablation in *P. setiferus* enhanced the reproductive capabilities of males by increasing sperm count, without diminishing sperm quality. This result concurred with previous studies in crabs (Adiyodi and Adiyodi, 1970) and other penaeid shrimps (Lawrence *et al.*, 1979; Chamberlain and Lawrence, 1981b).

Sreekumar and Adiyodi (1983) found that spermatogenesis of the freshwater prawn, *Macrobrachium idella* was precise correlation with specific stages of the molting cycle. Lin and Ting (1986) reported that the operated males, *P. monodon* were capable of regenerating spermatophore in 7 to 11 days after the extraction. Salvador *et al.* (1988) found that unilaterally eyestalk ablated wild *P. vannamei* can be reused after approximately 10 days spermatophore ejaculation. The experiment showed that eyestalk ablation was a viable way of improving male reused.

An outcome of this study will eventually enable to a background information for understanding the reproductive biology of *P. monodon*. It might be worth to try other methods for the consistent production of *P. monodon* instead of using the wild prawns broodstock. The results will enhance on high quality seed production and reuse valuable broodstock. Throughout a basic knowledge for apply and develop to sperm bank, artificial breeding, hybridization, and genetic studies.