CHAPTER I

INTRODUCTION

General Introduction

Since 1985, the giant tiger prawn, *Penaeus monodon* Fabricius, has been the most commonly cultured marine shrimp in Thailand (Fast, 1992). Because of its rapid growth to large size and high value per kg. which are important export quality, this species has become the most commercially important species of Thailand fishery industry. Japan and the United States of America are the two major consumer markets that have caused the explosive growth of this industry. From the world cultured shrimp production from 1986 to 1994 (Table 1.1), Thailand has become the world leader in shrimp exporter since 1991. Furthermore, Shrimp Market Report reported that in 1995 Thailand remained the world leader in frozen shrimp exporter for the fifth consecutive year with the exported frozen prawn of 219,901 MT, value more than $1,280 millions followed by Ecuador and India (Asian Shrimp News, 1996).

The general characteristics of giant tiger prawn can be briefly summarized as below:

Table 1.1 World Cultured Shrimp Production from aquaculture sector (1,000 MT, live weight) (After Fedouse, 1994: cited in Suraswadi, 1995)

Country/year	1986	1987	1988	1989	1990	1991	1992	1993	1994
Thailand	18	30	70	100	110	150	160	209	225
Indonesia	41	52	85	90	120	140	130	80	100
India	-	-	25	25	45	35	45	60	70
China	83	153	199	185	150	145	140	50	35
Vietnam	-	-	-	-	-	30	35	40	50
Bangladesh	-	14	25	20	25	25	25	30	35
Taiwan	70	90	45	20	30	30	30	25	25
Philippines	30	35	45	45	30	30	25	25	30
Ecuador	36	66	70	45	73	100	95	90	100
Mexico	1	2	3	4	4	5	6	9	12
Honduras	-	-	-	-	5	5	8	9	10
Columbia	-	-	-	-	-	9	8	9	10
Others	62	64	53	31	33	25	24	27	31
Total production	341	506	620	565	625	730	731	663	733

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1. Taxonomy of *P. monodon*

The taxonomic definition of the giant tiger prawn, *P. monodon* is as follows (Bailey-Brock and Moss, 1992):

Phylum Arthropoda
- **Subphylum** Crustacea
  - **Class** Malacostraca
    - **Subclass** Eumalacostraca
      - **Superorder** Eucarida
        - **Order** Decapoda
          - **Suborder** Natantia
            - **Infraorder** Penaeidea
              - **Superfamily** Penaeoidea
                - **Family** Penaeidae Rafinesque, 1815
                  - **Genus** *Penaeus* Fabricius, 1798
                    - **Subgenus** *Penaeus*
                      - **Species** *monodon*

Scientific name : *Penaeus monodon* Fabricius, 1798

Common name : Giant tiger prawn

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2. Distribution

The giant tiger prawn, *P. monodon* Fabricius, is one of the largest penaeid prawns. It is widely distributed throughout the greater part of the Indo-West Pacific region (Figure 1.1). However, the main fishing grounds are mostly located in tropical countries, particularly Malaysia, Indonesia, Phillippines,

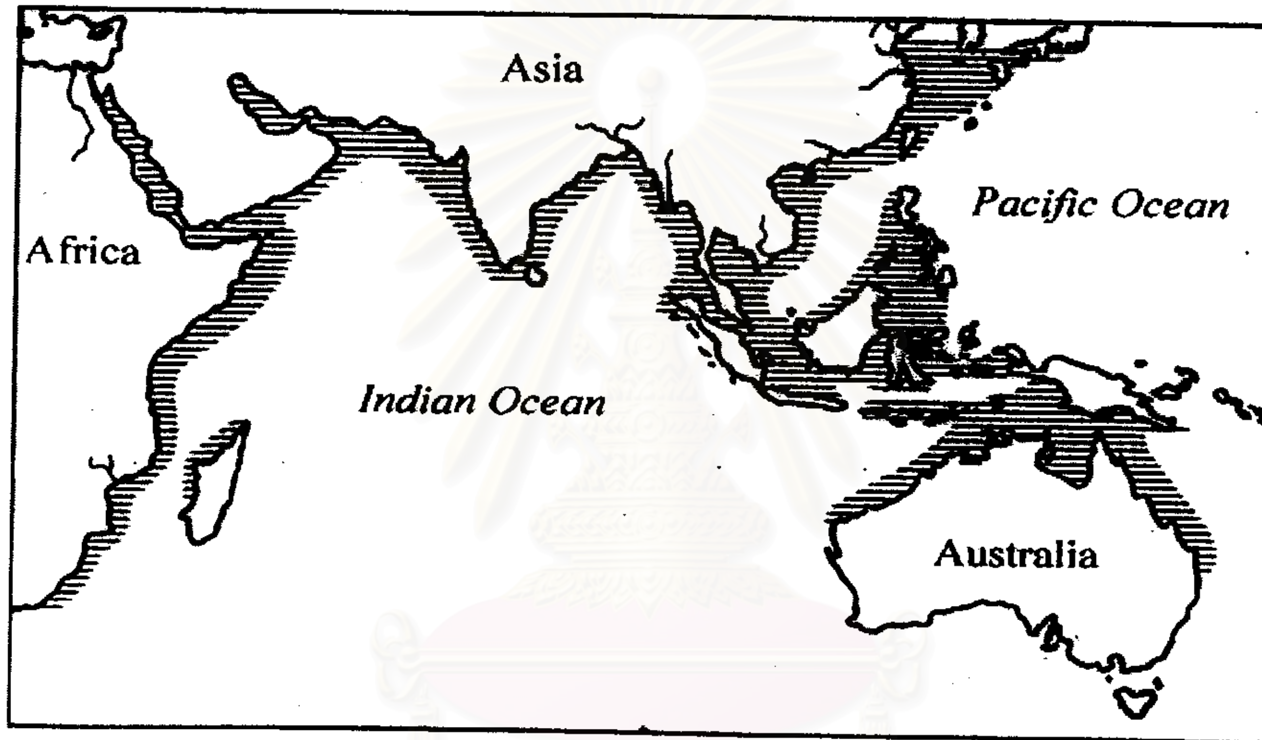

Figure 1.1 Geographic distribution of *P. monodon* in Indo-West Pacific region (Grey et al., 1983)

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India, Bangladesh and Thailand (Sodsuk, 1995).

In Thailand, survey of the distribution and abundance of *P. monodon* in the Andaman Sea conducted by Kungvankij et al. (1973) (cited in Sodsuk, 1995) showed that it was the dominant species in sandy bottom conditions at a depth of 35-40 m. Promsakha (1980) (cited in Sodsuk, 1995) reported that *P. monodon* in the Gulf of Thailand had breeding grounds at a depth of 26-50 m. 11-15 miles off the shorelines, and where water salinity was higher than 31 ppt.

In general, the life cycle of *P. monodon* can be divided into three stages; planktonic larval (nauplius, protozoa, mysis and post larval stages), juvenile and adult stages. The fry, juvenile and adolescent stages of *P. monodon* inhabit nearshore littoral areas to the depths of about 160 m., and are non-burrowers. The adults spawn at sea and their planktonic larvae migrate to inshore estuaries and mangrove areas. They migrate into deeper water as sub-adults (Figure 1.2). The life-span of giant tiger prawn is about 3 years (Liao, 1992).

The reasons for the widespread culture of this species are due to several advantages over other penaeid prawns. First of all, it exhibits a higher growth rate among the cultured penaeids (Liao and Chao 1983, cited in Liao 1992). The second advantage of this species is that it is an euryhaline animal. Its growth is not retarded even when reared in salinities near to freshwater. It tolerates salinities as low as 1 ppt. and as high as 57 ppt. (Motoh 1981, cited in

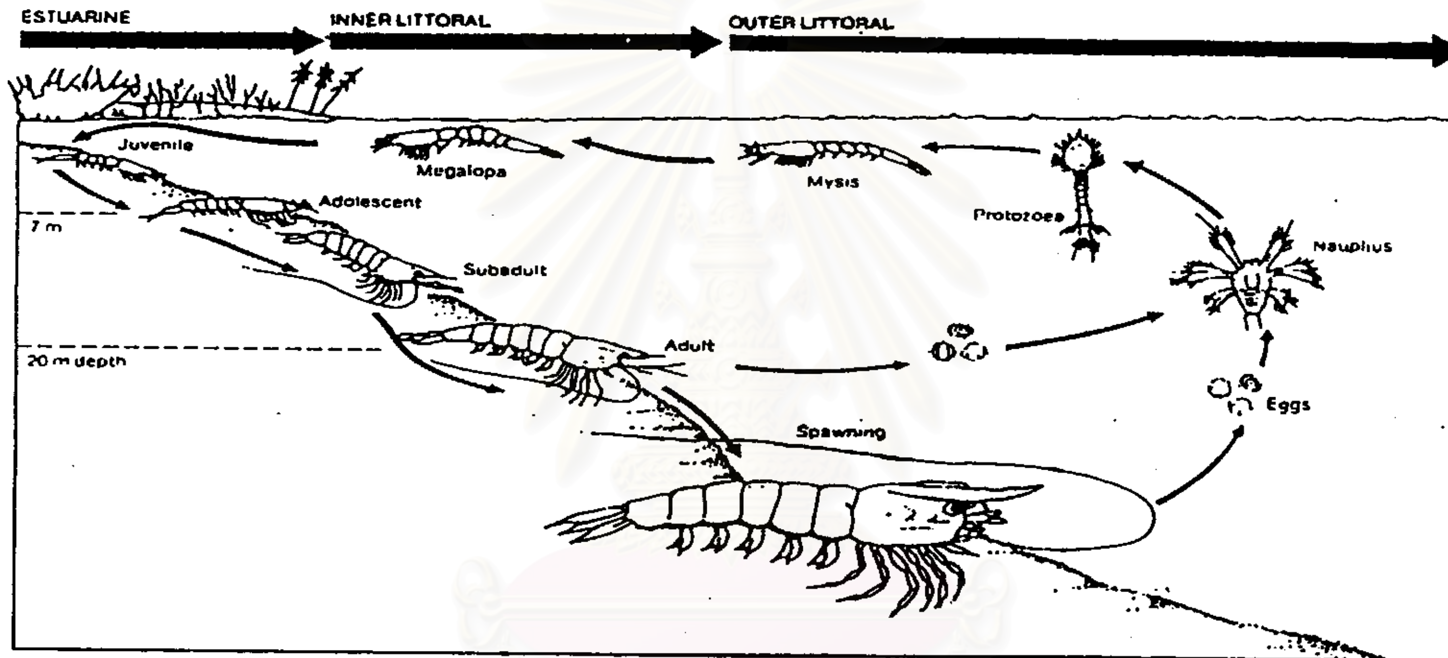

Figure 1.2 The life cycle of the giant tiger prawn, *P.monodon*, with stages in different habitats (Motoh, 1981)

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Liao, 1992). Furthermore, *P. monodon* has a wide temperature tolerance. Favorable culture temperature range from 21 to 32°C, but it can tolerate temperatures as low as 12°C or as high as 36°C for a short period of time. The general tolerance of this species is reflected in the large geographic range over which it is cultured. The third feature in favor of this species is that it is omnivorous rather than carnivorous. Its natural food includes crustaceans, organic detritus, radiolarians and mollusks (Su and Liao 1984, cited in Liao, 1992). These food habits and its low protein requirements help reduce production costs. Forth, simple culture facilities such as clay bottom ponds are more than adequate for grow-out and generally give good results for intensive culture. The last important feature is that *P. monodon* has a high survival rate in culture. These characteristics make *P. monodon* an ideal culture species.

Exploitation

Due to a strong market demand and highest price in international markets, prawn culture area in Thailand has increased rapidly from 40,768 hectares in 1985 to 80,000 hectares in 1993 (Royal Thai Department of Fisheries, 1993). Because the farming of this species relies entirely on wild caught broodstocks for the supply of juveniles, this has resulted in declining of broodstock in the wild population. Many problems has followed the rapid expansion of giant tiger prawn culture including ecological problems such as

pond waste and mangrove forest conversion (Menasvesta, 1986; Sodsuk, 1995; Browdy, 1996).

Some prawn culturists has been developing a higher technology for the management of pond culture for more than a decade. At present, approximately 85% of total culture area in Thailand has been converted to an intensive culture system. The system has advantageously resulted in not only constantly increasing of prawn production but also significantly decreasing the rate of mangrove forest conversion and the rate of expansion of culture area (Menasveta, 1996).

As the industry grows, some of the most heavily fished stocks are showing signs of over exploitation that can be a serious bottleneck to continued maturation of the industry (Browdy, 1996). The supply of broodstocks in Thailand is available from the Gulf of Thailand and the Andaman sea but farmers prefer prawn larvae from the Andaman sea brooder to those from the Gulf of Thailand (Sodsuk, 1995). High demand, availability of the preferred broodstocks and larvae from certain areas stimulate the movement of broodstock leading to overfishing in some areas. The decrease in wild prawn production has resulted in a government policy to increase prawn production by releasing hatchery reared prawns into areas where natural populations are depleting. Thailand has released about 30 million larvae of giant tiger prawn, mainly from the Andaman sea sources, into the Gulf of Thailand each year

(Royal Thai Department of Fisheries, 1993). There are also the escapes from prawn hatcheries and growout ponds. These will lead to stock mixing and break down of any existing natural population structures as mentioned by Ryman (1981) (cited in Sodsuk, 1995).

With the sign of over exploitation, the government policy and some private sectors have started focusing on genetic management. The ultimate goals include maintainance of genetic diversity, disease control and selection to improve the commercially important traits; i.e. to obtain a greater yield or higher quality traits. Genetic approaches can provide valuable information upon which management decisions are based.

The use of molecular genetic techniques in fishery research has increased dramatically over the past several years. The basic principle of all molecular genetic methods is to employ inherited, discrete and stable markers to identify genotypes that characterize individuals, populations or species. Such genetic data can provide information on the levels and distribution of genetic variability in relation to mating patterns, life history, population size, migration and environment. In addition, they continue to provide an impetus for the development of increasingly sensitive population discriminators, yielding information that can be valuable for both sustainable exploitation and the conservation of populations (Carvalho and Pitcher, 1995).

Effective genetic management often involves the analyses of distinct

alleles at loci. The first step of this requires the genetic markers to evaluate the degree of genetic diversity in natural populations across the geographic ranges for management of wild stock and initiation of trait selection and long term management of potential stocks aquaculture (Wolfus et al., 1997).

Morphometric variation studies

Several studies of morphometric variation in several *Penaeus* species have been performed with the objective of determining the traits that could then be used in a selection program as in the works of Lester (1983) and Goswami et al. (1986). In most analyses, it was indicated that morphometric variation was due to size differences, both within and among species. There was usually very little variation among members of a species in shape. Size differences were sometimes difficult to measure accurately because of measurement error. The characteristics of this variation, known as natural markers, are affected not only by genetic but also by environmental and physiological factors (Lester et al., 1992).

Genetic markers

Genetic markers are inherited in a simple Mendelian fashion and are assumed to be selectively neutral, i.e. the marker has no genetic effect on the fitness of an individual. The polymorphism of genetic markers observed in

organisms is maintained by forces such as mutation, migration and genetic drift (Park and Moran, 1994). Several genetic markers have previously been proven to be useful. These include allozyme loci, mitochondrial DNA and Variable Number of Tandem Repeat (VNTR).

1. Allozyme

Allozymes are the variant protein products of alleic genes in the same chromosomal DNA locus. Allozymes from tissues of organisms are revealed after separation in an electrical field. The different allozymes migrate with different distances depending on their net charge. The protein bands can be visualized by histochemical stains specific for the enzymes. Allozyme electrophoresis has been used as a predominant molecular technique to characterize population level genetic variation in many fishery researches because of its following advantages. It is a relatively easy and inexpensive technique, fairly rapid to perform on a large scale, and a number of loci can be screened simultaneously. Protein electrophoresis has been used for species identification as evidence by the study of Lavery and Staples (1990). However, the technique has certain limitations. Because of redundancy in the DNA code that dictates protein sequences, changes in a gene may not result in a change in the overall charge of the protein expressed. Thus many genetic variants are not detected by protein electrophoresis. Furthermore, protein electrophoresis is

limited to detecting actively expressed gene product that are detectable with histochemical staining (Hunter and Markert, 1957; Morizot and Schmidt, 1990, cited in Park and Moran, 1995). Unfortunately, these genes constitute only a small percentage of the whole genome of an animal, only a small proportion of DNA sequence variability can be detected. Protein loci also evolve more slowly than non-coding DNA sequences (Park and Moran, 1995). The studies of Benzie et al. (1992) and Sodsuk (1995) in *P. monodon* population had shown that allozymes exhibited low level variability and were not sufficient to discriminate between diverged populations.

2. Mitochondria DNA

In recent years, mitochondria DNA (mtDNA) analysis has been found more favourable and is generally assumed to be more powerful than allozyme analysis for revealing population structure. This is largely because mtDNA is haploid and maternally inherited; it therefore has an effective population size only one quarter to that of nuclear, thereby accentuating genetic drift effects (Nie and Li, 1979; Birky et al., 1989 cited in Ward and Grewe, 1995). The significant disadvantages are that although the mitochondrial genome contains over thirty genes, it is usually treated as a single locus because of the absence of recombination in the mtDNA molecule, whereas allozyme electrophoresis permits the examination of many independent characters (loci) (Ward and

Grewe, 1995). Because of its maternal mode of inheritance, the mtDNA analyses provided no information about the genetic contribution of males. Moreover, in some species, especially marine fishes, populations often appear to be dominated by only one or two mtDNA haplotypes together with many rare variants. In the study of Klinbunga (1996), the mtDNA RFLP analysis was carried out to investigate the genetic variation of hatchery stocks. He observed the large number of composite haplotypes and the relatively high value of haplotype diversity in his survey and suggested high level of genetic polymorphism in *P. monodon* mtDNA. He also suggested that the more appropriate approaches to provide additional information on the genetic variation were by using single VNTR locus, microsatellite loci and RAPD markers because of their ability to detect high polymorphism.

3. Variable Number of Tandem Repeat (VNTR)

VNTR markers are characterized by a core sequence which consists of a number of identical repeated sequences. They can be divided into two categories based on the repeat length. These are minisatellites, 9-65 base pairs (bp) (Wright, 1993) and microsatellites, 1-10 bp (Tautz, 1989).

3.1 Minisatellites

Minisatellites are tandemly-arrayed sequences of 9-65 bp in length,

frequently GC rich, sequence units which form arrays typically 0.1 to 20 kilobases long (Wright, 1993). An increase or decrease in the size of a repeat array results from changes in the repeat copy number thought to arise from high rates of unequal crossing-over during meiosis (Avise, 1994). The original use of minisatellites as genetic markers was by Jeffreys et al. (1985).

The original Jeffreys' probes were isolated from a myoglobin intron in human DNA and applied to forensic studies in humans (Avise, 1994). The 33-bp sequence of the first intron of human myoglobin gene was used as a probe to identify several minisatellite clones, for instance 33.5, 33.6 and 33.15 in the human genomic library (Jeffreys et al., 1985; Zhang and Tang, 1993). In the procedure, digested genomic DNA is cross-hybridized with the probe under low stringency conditions using the Southern blotting procedure to produce multilocus bands of 4-25 kbp size range (Bentzen et al., 1991; Zhang and Tang, 1993). The band pattern produced is specific for each individual and is inherited in a Mendelian fashion. These characteristics of minisatellites have allowed their application in pedigree construction (Wright, 1993).

The probes developed from human myoglobin genes by Jeffreys, others developed from gene III of bacteriophage M13 and synthetic probes have been used to DNA fingerprint several other animal species (Bentzen et al., 1991; Harris et al., 1991; Bruford and Burke, 1991). The disadvantage of DNA fingerprinting using minisatellite probes is that a complex multilocus pattern

(about 20 or more bands per individual) is produced. It is difficult to determine a specific locus to which the band belongs and therefore alleles cannot be identified and allelic genotypes cannot be determined (Avise, 1994). Heterozygosity and homozygosity cannot be determined and genotype frequencies cannot be calculated, hence the application of population genetic analysis to determine gene flow and other population variables cannot be done (Avise, 1994).

3.2 Microsatellites

Microsatellites consist of short, tandem repeats, typically 1 to 10 nucleotides of simple DNA sequence which is inherited in a Mendelian fashion. They are abundant, widely spread throughout the chromosome and are highly polymorphic in eukaryotic genomes (Tautz, 1989). Arrays of the repeat have been found to vary dramatically in length, from several to hundreds of basepairs, providing a new and plentiful source of allelic polymorphisms (Tautz, 1989; Weber and May, 1989; Litt and Luty, 1989). Microsatellite arrays are embedded in unique DNA sequence, sometimes within the coding region of genes, but more commonly in the untranslated regions of the genome (Valdes et al., 1993).

Microsatellites are five times more abundant in mammals than in plants (Lagercrantz et al., 1993). The most common repeats are the

dinucleotides $(GT/AC)_n$, $(CT/AG)_n$ and $(TA/AT)_n$ (Tautz and Renz, 1984; Rafalski and Tingey, 1993), although trimeric and tetrameric tandem repeats have also been isolated in humans (Edwards et al., 1992) and fish (Wright, 1993). The $(GT/AC)_n$ repeats are the most abundant and informative in vertebrates, whereas in plants it is the $(TA/AT)_n$ repeats (Rafalski and Tingey, 1993).

Recently microsatellites have been increasingly used as the markers of choice. In a species like giant tiger prawn with a well documented low level of polymorphism, microsatellite markers would be a useful supplement to other marker systems. There are some advantages of utilizing microsatellites over other markers, which make them desirable. First, microsatellite loci have been found in large numbers and relatively evenly spaced throughout the genome. The frequency of occurance of $(GT/AC)_n$ sequences is estimated to be from once every 7 kbp in Atlantic cod, *Gadus morhua* (Brooker et al., 1994), to once every 139 kbp in European flat oyster, *Ostrea edulis* (Naciri et al., 1995). Second, a small quantity of DNA is used in an analysis of microsatellites. Because each microsatellite locus (i.e. tandemly arrayed repeat) is embedded in a unique sequence, if the sequences flanking the microsatellite are known, complementary primers can be designed and used to amplify the repeated segment by the polymarase chain reaction (PCR). With this approach, the length of microsatellite alleles at a specific locus can then be detected by size

fractionation with acrylamide sequencing gels. This was first demonstrated by Tautz (1989). A PCR analysis of small fragments also allows the analysis of degraded samples in which the mean fragment size of the genomic DNA has been severely reduced through environmental insult (Paabo et al., 1989). Third, these microsatellite arrays are highly susceptible to length mutation. The estimation of the mutation rates of microsatelittes was as high as 10^{-3}-10^{-4} (Weissenbach et al., 1992). The mutational process thought to account for this length change is slipped-strand mispairing or slippage during DNA replication (Wright, 1993; 1994). The high rate of mutation at these microsatellite loci leads to extensive allelic variation and high levels of heterozygosity. Hence, microsatellites have been found to be variable even in populations which have low levels of allozyme and mitochondrial variation (Paetkau and Strobeck, 1994). Finally, microsatellite alleles are codominant markers inherited in a Mendelian fashion, such that heterozygotes can be distinguished from homozygotes, as is not the case with dominant RAPD (Wright, 1993) or haploid mtDNA markers. Codominant Mendelian inheritance makes microsatellites more informative in pedigree studies, as well as in population studies (Wright and Bentzen, 1995). These characteristics make microsatellites to be a useful supplement for a species like giant tiger prawn with a well documented low level of polymorphism as detected by other types of markers.

Microsatellites are useful for a number of analyses. One of the earliest

uses of microsatellites, before the inception of PCR, was as multi locus probes for DNA fingerprinting (Tautz and Renz, 1984). The single locus PCR approach proved to be vastly superior to multilocus fingerprinting as a tool for forensic uses, clone or strain identification and in genetic mapping efforts (Queller et al., 1993; Weissenbach et al., 1992). Extensive genomic maps have been extensively using linkage analysis for human (Dausset et al., 1990; Hazen et al., 1992), rodents (Love et al., 1990; Beckman and Weber, 1992) and some economical livestock species (Rohrer et al., 1993; Georges et al., 1993; Fredholm et al., 1993), with the intent to identify genes responsible for disease, growth or other economically important traits. As a result of human mapping efforts, some human genetic diseases were found to be caused by expanded forms of microsatellites (Cakey et al., 1992). Conservation of microsatellite loci among different taxonomic groups has been achieved by amplification of homologous loci in closely related domestic mammalian species (Moore et al., 1991). A similar approach was taken with salmonid species (Morris, 1996). Microsatellites may also be useful to behavioral ecologists for studies involving in the determination of parentage and kinship (Quiller et al., 1993; Colbourne et al., 1996) and pedigree analysis (Herbinger et al., 1995). Finally, microsatelllites have found applications in the area of population genetics and stock discrimination of important fisheries (McConnell et al., 1995; Kamonrat, 1996)

Therefore, the aim of this thesis is to isolate and characterize microsatellites in *P. monodon* genome and develop genetic markers to be a valuable tool for the future genetic studies of population genetics, evolution and breeding management in this commercial prawns.