CHAPTER I

Introduction

Thailand has produced high quantity of the black tiger shrimp (*Penaeus monodon*) from aquaculture since the last decade. Previously, the main shrimp production was from ocean capture fisheries. During the early 1980s, shrimp production from the aquaculture sector contributed 10 % of the total production. Nevertheless, farming of *P. monodon* had been consistently increased. Contribution of production from fisheries and aquaculture was approximately equal in 1989. Subsequently, shrimp production from the latter has (and still is) predominate (Table 1.1). Thailand has been the world' s largest shrimp producer since 1992. The quantity of exported shrimp production had rapidly increased from 33,903 MT in 1987 to 187,072 MT in 1994 (Table 1.2) where the main exporting market are the United States of America and Japan (Table 1.3 and1.4). This corresponds to an income of 4-5 billion bahts annually.While only one – tenth of *P. monodon* from Thailand was imported by Japan, one – quarter of *P. monodon* was exported to the United States in 1997 (Table 1.5). The remaining markets are Europe, Asian and other countries. Although, Thailand was still the most important shrimp exporter to the United States and Japan in 1997, India, Indonesia and Ecuador also exported the large quantity of culture shrimp to those countries (approximately 70,000 MT). The total exported production of *P. monodon* of Vietnam, China and Bangladesh to the United States and Japan in 1997 accounted for between 15 – 30 % of that from Thailand.

Rapid expansion of shrimp farming leads to overexploitation of wild *P. monodon*. Currently, this species has been managed as a large single panmictic population, therefore, harvest of boodstock has been carried out without consideration of different population sizes in different geographic sources of wild *P. monodon*.

Therefore, information on genetic structure of *P. monodon* in Thailand is currently needed. The knowledge gained from such information would lead to construction of suitable conservation programmes to ensure protection of genetic diversity levels and isolated gene pools, if available, in this species.

1.1 Biology of *Penaeus monodon*

1.1.1 Taxonomy

Penaeus monodon can be morphologically classified as follows (Bailey-Brook and Moss, 1992) ;

Phylum Arthopoda

Subphylum Crustacea

Class Malacostraca

Subclass Eumalacostraca

Order Decapoda

Suborder Natantia

Infraorder Penaeidea

Superfamily Penaeoidea

Family Penaeidae Rafinesque, 1985

Genus Penaeus Fabricius, 1798

Subgenus Penaeus

Species monodon

Scientific name : *Penaeus monodon* (Fabricius,1798)

English Common name : Black Tiger shrimp (or prawn)

Synonym names : *Penaeus carinatus* Dana, 1852

Peneaus caeruleus Stebbings, 1905

Penaeus monodon var. *manilensis* Villaluz and Arriola, 1938

Penaeus bubulus Kubo, 1949

1.1.2 Morphology

Externally, the shrimp can be basically divided into thorax and abdomen. The thorax is covered by single immobile carapace which protects internal organs and supports muscle origins. The internal organs in this part consist of eyes and eye stalls, sensory antennules, antennae and walking legs (pereopods). The abdomen has segmentation commonly observed in invertebrates. It consists of swimming legs (pleopods), which arise from each of six abdominal sagment and a tail (Figure 1.1).

Rostrum has 7-8 dorsal and 3-4 ventral teeth and curves down very slightly. Rostral ridge lack a distinct groove behind it and the hepatic ridge is long and curved. Telson has a groove but is without lateral spines Carapace and abdomen have black bands giving a tiger - striped appearance to this species. Pereiopods may be red (Bailey - Brook and Moss, 1992).

Table 1.1 Shrimp production from ocean capture, pond culture and culture areas in Thailand

Year	Production by normal fisheries (MT)	Production by culture (MT)	Total production (MT)	Shrimp culture area(Rai)*
1981	122,706 (91.9%)	10,729 (8.1%)	133,435	171,619
1982	156,523 (93.9%)	10,091 (6.1%)	166,614	192,453
1983	127,584 (91.7%)	11,550 (7.3%)	139,134	222,107
1984	104,394 (88.9%)	13,007 (11.1%)	117,401	229,949
1985	91,632 (85.3%)	15,840 (14.7%)	107,472	254,805
1986	102,227 (85.1%)	17,886 (14.9%)	120,113	283,548
1987	128,100 (84.5%)	23,566 (15.5%)	151,666	279,812
1988	110,200 (66.4%)	55,633 (33.6%)	165,833	342,364
1989	110,800 (54.2%)	93,495 (45.8%)	204,295	444,785
1990	107,400 (47.6%)	118,227 (52.4%)	225,627	403,787
1991	129,100 (44.3%)	162,070 (55.7%)	291,170	470,826
1992	116,800 (38.7%)	184,884 (61.3%)	301,684	454,975
1993	100,000 (30.7%)	225,514 (69.3%)	325,514	449,292
1994*	97,773.0 (27.1%)	263,445 (72.9%)	361,218	457,793
1995*	100,700.0 (28.0%)	259,540 (72.0%)	360,240	468,386
1996*	103,700.0 (33.1%)	210,000 (66.9%)	313,700	455,182

Souce : Asian Shrimp News, 3rd Quarter 1996.

*Fisheries Statistics and Information Technology Sub-Divitions, Fisheries Economics Division, Department of Fisheries, 1998.

6.25 Rai = 1 ha

Table 1.2 Exported quantity of fresh and frozen shrimp (*P. monodon*) from Thailand since 1987-1997

Year	Quantity(MT)	% Change	Value(Mbath)	% Change
1987	33,909		5,748.885	
1988	49,810	46.89	9,697.988	68.69
1989	74,294	49.15	16,057.080	65.57
1990	84,724	14.04	20,453.749	27.38
1991	121,240	43.10	26,680.926	30.45
1992	130,516	7.65	31,695.554	18.79
1993	148,886	14.07	37,841.650	19.39
1994	187,072	25.65	49,155.576	29.90
1995	175,091	-6.4	50,302.047	2.33
1996	161,486	-7.77	43,404.490	0.14
1997	137,082	-15.11	47,184.950	8.71

Source : Fisheries Statistics and Information Technology Sub-Divisions, Fisheries Economics Division, Department of Fisheries, 1998.

Table 1.3 Imported quantity of fresh and frozen shrimp from Thailand separated by country since 1992-1995 (in metric tons)

Country / Year	1992	1993	1994	1995
JAPAN	51,166	53,873	66,082	50,738
U.S.A	37,150	46,034	53,332	44,385
EU	16,790	15,027	17,377	20,712
ASEAN	10,176	10,731	11,854	13,996
TAIWAN	1,690	8,338	11,649	11,743
EFTA	1,793	1,788	1,697	1,938
OTHER	11,751	13,095	25,081	31,534
TOTAL	130,516	148,886	187,072	175,091

Source : Fisheries Statistics and Information Technology Sub-Divitions, Fisheries Economics Division, Department of Fisheries, 1998.

Table 1.4 Imported quantity of fresh and frozen shrimp from Thailand separated by country in 1996 and 1997 (in metric tons)

Country	1996 Quantity(MT)	1996 Value(Mbath)	1997 Quantity(MT)	1997 Value(Mbath)
ASEAN	90,631.00	24,126.61	74,680.00	24,945.22
U S A	41,812.00	12,092.60	37,991.00	14,516.20
CANADA	3,912.00	1,080.15	3,777.00	1,453.81
EU	17,617.00	4,063.88	12,993.00	3,736.43
AUSTRALIA	4,118.00	1,193.94	4,882.00	1,727.42
OTHER	3,396.00	847.31	2,759.00	805.87
TOTAL	161,486.00	43,404.4	137,082.00	47,184.95

Source : Shrimp culture newsletter, April 1998.

Table 1.5 Quantity of shrimp exported from varrious countries to USA and JAPAN in 1997

Country	U.S.A.	JAPAN	TOTAL
Thailand	73,545	24,075	97,620
India	20,045	59,112	79,157
Indonesia	12,864	57,342	70,206
Ecuador	63,864	4,922	68,786
Vietnam	3,545	31,119	34,664
China	12,909	15,142	28,051
Bangladesh	9,773	5,495	15,268
Other	98,137	70,040	168,177
TOTAL	294,682	267,247	561,929

Source : Shrimp culture newsletter, April 1998.

1.1.3 Life cycle

In the natural environment, adult shrimps migrate offshore to the oceanic water (Fig 1.2). After the pre-mating moult stage, each female is copulated. The spermatophores are inserted and kept in the sperm receptacle (thelycum) located between the 4^{th} and 5^{th} pereopods. When spawning, eggs are released from a pair of genetic pore and spermatozoa are extruded from the thelycum. Generally the embryonic stage until hatching takes approximately 14 hours. The larval stages of *P. monodon* are composed of 6 nauplii, 3 protozoea (also called zoea) and 3 mysis substages before metamorphoresed to postlarvae. During these periods, the larvae were carried passively by the surface currents. Some of them are swept to the nursery ground (e.g. estuaries, mangrove swamp) and further develop to adolescent, subadult and adult stages, respectively. It is estimated that only 1% of those spawned in nature reaches the adult stage (Treece and Yates, 1990).

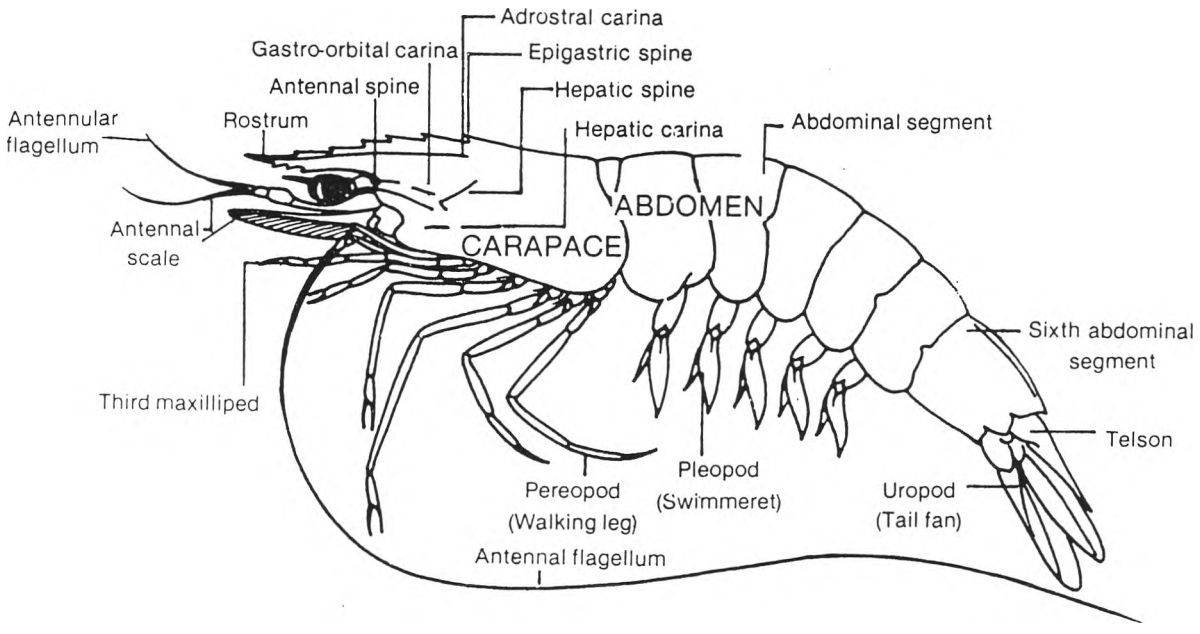

Figure 1.1 Lateral view showing important parts of *P. monodon* (Motoh, 1981)

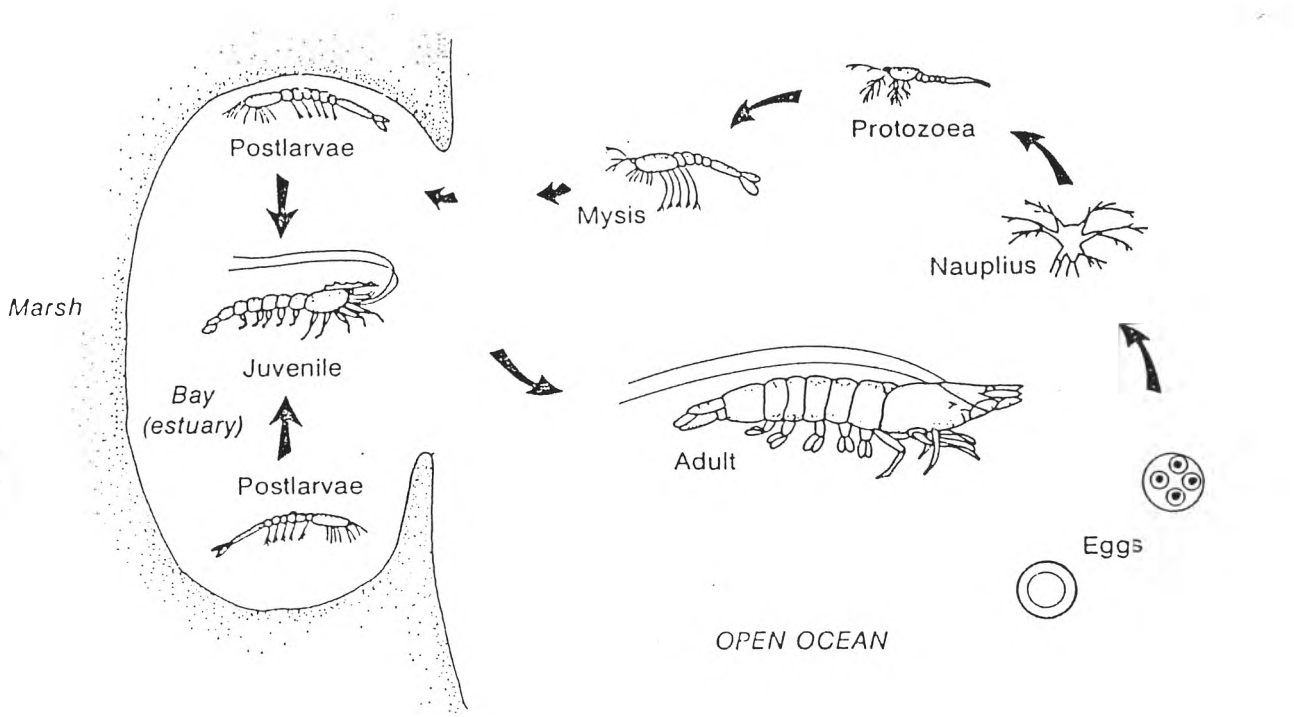

Figure 1.2 Life cycle of panaeid shrimps (Treece and Yates, 1990).

1.2 Distribution

The black tiger shrimp (*P. monodon)* distributes over the major part of the Indo – West Pacific region. It is principally found in the East and Southeast of Africa, through the Red Sea and Arabian Gulf, around the Indian subcontinent, throughout the Malasian Archipelago, Northern Australia and Japan (Fig 1.3). It is a marine species inhabits mud or sand bottoms at all depths from shallow to 162 meters (Anderson, 1993), so it can be caught offshore or inshore as well as from tidal zones . It is a local species in Thailand found in both sides of the Thai – Malaysian Peninsula (the Andaman and South - China Sea on the West and East coasts, respectively). This species is probably the most important culture marine shrimp species in Asia (Dore and Frimodt, 1987).

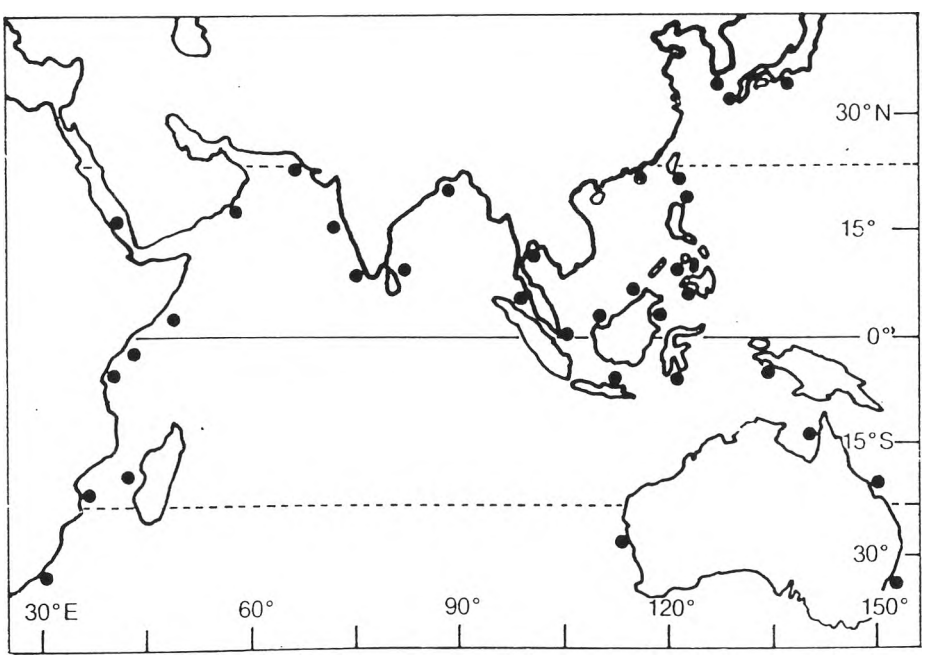

Figure 1.3 Geographic distribution of *P. monodon* (Motoh,1981)

● = Main fisheries areas

1.3 Genetic diverity of *P. monodon* : a basic descipline for fisheries management

Previously, the main shrimp production was from ocean capture fisheries. The important shrimp fishing areas in Thailand are the Gulf of Thailand and the Andaman Sea. After the 200 miles exclusive economic zones were ratified in 1981, Thailand lost about 50 % of the previous fishing grounds. This is one of the reason to explain the dramatic reduction of production from normal ocean fisheries since 1981 (Table 1.5). Nevertheless, the limited of fishing grounds was compromised by delvelopment of advanced technologies for shrimp industry in Thailand (Menasveta, 1992)

Farming of *P. monodon* in Thailand has been rapidly developed since the last decade. Accordingly, Thailand has become the world 's largest shrimp producer for six consecutive years since 1992. Practically, Thai farmers claim that the quality of postlarvae bred from female broodstock originating from the Andaman Sea show higher success and better in performance (e.g. higher growth and survival rates) than do the postlarvae bred from the Gulf of Thailand origin. Using molecular marker such as region-specific DNA markers can be further clarified whether the economically important phenotypes between Andaman and Gulf of Thailand *P. monodon* were different.

Based on the fact that culture of *P. monodon* is still in the open pattern, postlarvae used in the industry are entirely from wild breeding females leading to overexploitation of natural populations. Moreover, poor management practice has created several subsequent problems including e transferring of female broodstock from one to other places without consideration of any genetic structure of this species. To be fair, *P. monodon* has been practically managed without any assumption on sensible fisheries management even though differences of

performance between broodstock from different geographic regions have been claimed by farmers.

Level of genetic diversity in any wild species is important to indicate whether such a species need to be conserved. Moreover, this parameter also reflects the bottleneck effects for the wild species and indicates founder effects for domesticated species and / or strains. Inter- and intraspecific hybridization of organisms can happened naturally or artificially. Unintentional transplantation through mismanagement can lead to the loss of local gene pools. Theoretically, the status of wild and cultured stocks need to be established before they can be used for farming (McAndrew, 1996). Genetic variation level is a basic indicator for long-term genetic management of conserved species (Cataudilla and Crosetti, 1993).

Knowledge of genetic diversity level of domesticated stocks is the major requirement to monitor progress of selective breeding programmes (Porsuksiri, 1994). Clarification for response to desired selection among different stocks of *P. monodon* seems to be crucial for significant reduction of selection period. This cannot operated unless the stock structure of *P. monodon* is investigated.

Monitoring of genetic diversity at different generations is essential for improving the efficiency of selective breeding programmes. Data on rare alleles, decreased heterozygosities and increased level of inbreeding are important during domestication of *P. monodon.* The most interesting circumstance at present is to examine whether levels of genetic diversity of each domesticated stocks are positively correlated to ecomomically important traits (e.g growth rate and/or disease resistance)(Garcia et al., 1994).

Characteristics of different *P. monodon* populations should be investigated because each stock (either wild or domesticate) need a particular conservation

scheme depending on several factors for example, potential of the stock , population size and budget to carry out the protecting programme.

1.4 Molecular genetic markers as tools for genetic diversity studies

Understanding distribution patterns of genetic variability within species would assist the efficient use of natural resources. On the one hand, genetic variation is the basic resource for any successful selective breeding programmes, on the other hand, genetic diversity between populations is also critical when one considers hatcheries conserving genetic resources (Kamonrat, 1995).

Previously, traditional methods such as morphology and comparative anatomy have been widely used for taxonomic identification and systematic evaluation of various taxa. These, however, have been replaced by the molecular approaches (based on polymorphisms found in protein or DNA molecules). Morphological characters have been used for classification and identification of organisms at different taxonomic levels (genera, families and species) but variants can be resulted from environmental influences. Several important molecular techniques have become available for various applications in genetic and systematic studies. These approaches can also be used to measure rates of genetic divergence between taxa (genera, species or populations). Useful genetic markers are generally developed from allozyme electrophoresis, restriction mapping and / or restriction fragment length polymorphism (RFLP) of investigated DNA and randomly amplified polymorphic DNA (RAPD) analysis.

The effective markers used for population structure studies should be selective neutrally. Under this circumstance, the information from genotypes and allele frequencies is assumed to be primarily generated by mutation, gene flow, genetic drift, rather than by selection (Smith and Brown, 1988).

1.4.1 Allozyme

Allozyme electrophoresis has been successfully applied to various organisms from bacteria to animals and plants since the 1960s (May, 1992). A tissue extract is prepared and electrophoresed on the supporting media (usually starch or polyacrylamide gels). Proteins are separated according to their net charges and sizes. The protein bands can be visualized by a specific histochemical stain of investigated enzymes. Once the electrophoresed gel are stained, the status of homo- or heterozygosity at such a locus can be examined. The number of band is reflected from configuration of enzyme molecules. The position of polymorphic band are genetically informative (Weising et al., 1995).

Allozyme electrophoresis still remains one of the most important tool used for genetic variation studies at intraspecific level even though it is being increasingly replaced by direct DNA analysis. The advantages of allozyme electrophoresis due primarily to its speed and cost-effective. Data from hundreds of individuals at several loci can be assessed within a few days or weeks. Equipment needed for allozyme analysis is modest. Although interpreting gel patterns usually requires considerable experience, operator can be trained quickly. Disadvantages of this method are the strict requirement of fresh or good quality frozen tissue, the need of more material than most DNA methods, and a small proportion of protein coding sequences that can be investigated. Nevertheless, there has remained a debate about whether allozyme variation is selectively adaptive or neutral. This latter point results to an assumption that genetic drift is responsible for population differentiation (Carvalho and Pitcher, 1995).

Benzie et al (1992) reported population differentiation of *P. monodon* in Australia based on allozymes. Specimens were obtained from seven different geographic sites; Clarence River, Townsville and Cairn from eastern

coast, Weipa, Melville island and Joseph Bonaparte Gulf from northern coast and De Grey River from western coast of Australia. Of the 75 enzymes originally screened, eight polymorphic loci were consistantly scorable (*GPI**, *LGG**, *LT-1**, *MDH-I**, *MDH-2**, *MPI**, *PGDH** and *PGM**). Three loci (*GPI**, *PGM** and *MPI**) showed highly significant genetic differences between the western *P. monodon* and the northern and eastern *P. monodon*. Geographic heterogeneity test using typical χ^2 (chi-square) and analysis of population differentiation using *F*-statistics indicated highly significant differences between *P. monodon* from the west to the east and the north of Australia.

Analysis of genetic variation of *P. monodon* originating from the South-East Asian region using allozymes has additional reported. (Sodsuk et al., 1992). A total of 100 *P. monodon* individuals was collected from each of Trat and Surat in the Gulf of Thailand and Phuket and Satun in the Andaman Sea between 1991 and 1992. Five polymorphic loci (*ALAT**, *MPI**, *IDHP**, *PGM** and *GPI**) of 35 investigated loci were consistently resolved. In the 1991 samples, Phuket and Surat was significantly different at *IDHP** ($P< 0.05$) and *GPI** ($P <0.01$). Significant distribution between allele frequencies between Trat and Surat was surprisingly observed at the *GPI** locus ($P<0.05$). For the 1992 samples, all pairwise compairisons with the exception between Surat and Satun were significant different with at least one enzyme (*ALAT**, *GPI**, *MPI**). Heterogeneity of allele distribution of samples collected from the same locations but different years was observed in Trat (*MPI**, $P<0.05$) and Surat (*PGM**, $P<0.05$) but this circumstance was not observed in Phuket. Low level of population subdivision was found between the Andaman and the Gulf of Thailand.

1.4.2. Restriction fragment length polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is one of several techniques used to determine DNA variation based on the assumption that digested DNA fragments having identical migration distance on the gel are the same restriction fragment resulted from the homologous fragment. For conventional RFLP approach, the target DNA digested with restriction endonucleases are size-fractionated by agarose gel electrophoresis and transferred to a supporting membrane (usually nylon or nitrocellulose membranes). The investigated fragment(s) is identified by hybridization with the specific radiolabeled probe (Davis et al,1996).

The extensive use of restriction analysis for molecular population genetics of animal taxa has emphasized surveys of genotype frequencies, diversity and population differentiation based on polymorphism of mitochondrial genome. Genotype (or haplotype) frequencies can be quantified by the presence or absence of restriction sites (or fragments) among individuals. This can be directly carried out if pure mtDNA was digested with appropriate restriction enzymes followed by gel electrophoresis. On the other hand, the large and complex molecule like nuclear DNA cannot be directly analyzed after restriction enzyme digestion. The labeled DNA probe is required for hybridization and can be labelled using either isotopic or non-isotopic methods. The RFLP markers are regarded as a Type I marker in linkage maps. Accordingly, RFLP markers have been extensively used to develop genetic maps and phylogenetic trees (Yu et al.,1993).

Studies on mtDNA variation are primarily carried out by restriction analysis of the entire mitochondrial genome. Nevertheless, analysis of the entire mtDNA by restriction enzymes is increasingly replaced by PCR-RFLP where the specific regions of mtDNA are amplified in vitro through the polymerase chain

reaction (PCR), and the products are digested with restriction endonucleases. Alternatively, the PCR amplified product can be further analyzed by direct sequencing (Chapman and Brown, 1990).

1.4.3. Randomly amplified polymorphic DNA (RAPD)

Randomly amplified polymorphic DNA was developed by Williams et al., (1990) who introduced this method to assess DNA polymorphism. It is a simple method to generate genetic markers and DNA fingerprinting patterns without prior knowledge of the genome subjected to be analysed. This technique is based on the polymerase chain reaction(PCR) requiring a single short arbitrary oligonucleotides, (usually 10 bp long with GC content at least 50 % and do not contain palindromic sequences) (Ellsworth et al., 1993).

RAPD is utilized to amplify target DNA on the basic that the nuclear genome may contain several priming sited closed to one another that is located in an inverted orientation. Accordingly, the primer is utilized to scan genome for small inverted sequences resulting in amplification of DNA segments of variable length (Bowditch et al, 1993). The RAPD products are detected as DNA fragment length polymorphism for multiple loci by the presence or absence of band at various positions (Mullis, Ferre and Gibbs, 1994).

The allele distribution of RAPD amplified fragments is generally inherited in a dominant fashion. Accordingly, the presence of amplified fragment may reflect either a homozygous (AA) or heterozygous (Aa) of the amplified product where each A indicates the successful amplification product from a haploid genome whereas "aa" indicates an inability to amplified such a particular fragment.

The reasons for increasing uses of RAPD – PCR for population genetic studies are due to its several advantages. First, RAPD analysis is a

relatively simple and rapid technique which requiring less technical expertise. Second, it is a PCR-based techniques requires tiny amount of DNA used in the reaction. Third, the same single short primer generated informative data in one species can produce such information in other investigated taxa, Fourth, the sequence data of the target DNA is not necessary, Fifth, the availability of the unlimited number of random primers makes RAPD-PCR become a powerful tool for population and systematic studies of unicellular (bacteria, alga) to muticellular (animals and plants) organisms. Finally, RAPD-PCR does not require probes, DNA libraries, and radioactive chemicals obviating complicated processes and the use of harzardous chemicals (Williams et al.,1993 ; Narang et al., 1994).

There are some disadvantages to use RAPD-PCR approach for population genetic, genetic mapping, and taxonomic studies. First, many fragments (especially those arising from mispairing of a primer with the genomic DNA) may not be reproducible among different laboratories because amplification is sensitive to slight changes in temperature cycles. Second, in contrast to RFLP, which is usually inherited codominantly, most RAPD-PCR amplified fragments are inherited in the dominant fashion. Therefore, homozygotes and heterozygotes can not be differentiated. Third, RAPD bands of the same size may not actually identical, therefore, comigrating RAPD bands may not be allelic (Narang et al., 1994).

RAPD markers can be used to measure similarity among individuals within (natural or artificial) populations or a species. Diversity within population and species is influenced by a multiplicity of factors including life history , generation time, outcrossing or inbreeding, pollen dispersal, geographic ranges and ecological niche. Population of a species showing differences in some of these biological traits can be disassociated by appropriate APD markers. (Williams et al., 1993)

Welsh and Mclelland (1990) developed arbitrary primer - polymerase chain reaction (AP-PCR). This technique requires the mismatch amplification resulted by low - stringency amplification for two cycles. The primary mismatched products are further amplified at higher stringent conditions before labeled dNTPs are added to the reaction for signal detection. Using this approach, they were able to genetically distinguish 24 different strains belonging to 5 *Staphylococcus* species and a strain isolated from *S. pyogenes*.

Identification of genetic diversity in shrimp by RAPD suggested the potential usefulness for various application. D' Amato and Corach (1996) used RAPD analysis to study genetic diversity of population of freshwater shrimp (*Macrobrachium borelliib)*. The Arroyo Pescado population (a stream that flows into Rio de la Plata) and the Canteras de Berisso population (an artificial pond located approximately 20 km from Arroyo Pescado) were chosen. A total of 20 primers (A1 - A20) were tested and four chosen primers (A4, A5, A7 and A8) were used for genetic diversity analysis. Apparently, the lower level of genetic diversity was observed in an artificial pond population. Within each population the Arroyo Pescado population show a greater diversity than did the Berisso population. The estimated genetic distance (d = - 0.001) indicated a high genetic similarity between populations. These results demonstrated that RAPD analysis can be useful measures of genetic diversiry in this taxa.

Garcia and Benzie (1995) had investigated RAPD patterns in six families of *P. monodon.* (each of six wild-caught females was singly mated to each of six different wild-caught males). Fifty offspring from each of the six families were screened. A total of 48 bands were amplified from genomic DNA of parents and progeny using 14 primer. Forty-five bands (98.8%)were monomorphic. Three polymorphic markers (6.2%) illustrating Mendelian transmission were

identified. Therefore, RAPD was promising to generate markers assisted in selective breeding programme.

Garcia et al.(1994) determined genetic diversity in three populations of *P. vannamei* using three molecular tecniques, allozyme, RFLP and RAPD analyses. The population 1 was a specific – pathogen free (SPF) stock originated from at least three separated sprawns from different breeding females in a hatchery in Sinaloa, Mexico. Twelve maternal families were subsequently established from this population. The population 2 was also a SPF stock originated from a hatchery stock in Ecuador. The last population (population 4) was originated from wild captured postlarvae at Oaxaca, Mexico. A total of 141 individuals were investigated using 5 RAPD primers (OPA9, OPA 10, OPA20, OPB11, OPB14 and OPB20). The results across all investigated individuals showed 73 scoring bands. The percentage of polymorphic bands were 55% for both families 1.5 and 1.6 of the population 1. A slightly lower percentage of polymorphic band was observed in the population 2 (48%). The wild originating population 4 possessed the highest polymorphic bands (77%). A population – specific marker was found in all individuals of the population 2 when the OPA20 was used. Large genetic differences among groups of *P. vannamei* was illustrated by this technique implying the potential usefulness to use RAPD for examining genetic diversity of each stock in a breeding program.

The first publication for analysis of genetic variation in wild *P. monodon* using RAPD was reported by Tassanakajon et al., (1997). Three hundred octanucleotide primers were screened. Six of which (UBC101, UBC174, UBC428, UBC456, UBC 457 and UBC459) yielded reliable and consistent results.

Analysis of population structure using these primers were carried out in three population (Satun- Trang from the Andaman sea and Trat and

Angsila from the Gulf of Thailand). Seventy amplified bands (200-2000 bp in length) were consistently scored. Forty of these (57%) were polymorphic. The percentages of polymorphic bands observed were 48% in Satun–Trang and 45% in Trat, suggesting high genetic variability of these two geographic samples.

Only one - half of the polymorphic bands was found in the Angsila *P. monodon* indicating a possibility of inbreeding in this sample. Primer 428 detected a RAPD marker that was found only in *P. monodon* originating from Satun-Trang, implying the potential use of this marker as a population specific marker in this species.

1.5 Objectives

The objectives of this thesis is to examine genetic variation and population structure of 5 geographically separated samples (from the Andaman Sea and the Gulf of Thailand) of black tiger shrimp, *P. monodon* in Thailand using RAPD analysis.