CHAPTER I

Introduction

The giant tiger shrimp (*Peneaus monodon*, Fabricius 1798) is one of the most important cultured shrimp species among those reported by the Food and Aquaculture organization of the United Nation (FAO). It has presently cultured worldwide covering a vast geographic area including Australia, Bangladesh, China, India, Indonesia, Philippine, Thailand and Vietnam (Bailey-Brock and Moss, 1992).

The world shrimp production from an aquaculture sector was estimated to be 737,200 metric tons in 1998. This increased from 660,220 tons of production in 1997 for approximately 12 %. Seventy-two percent of the total production was from the eastern hemisphere. The main *P. monodon* farming is located in South – East Asia (Thailand, Indonesia, and Philippines). Of these, Thailand is a leader for cultured shrimp production for nearly a decade.

The production of *P. monodon* in Thailand has accounted for approximately 200,000 tons annually since 1992. The annual production of farmed *P. monodon* from Thailand accounted for 33.6 % of the eastern hemisphere 's production in 1998. Fresh and frozen *P. monodon* were exported to several countries including the Asian countries (Japan, China, Singapore etc.), the United State of America, Canada, Eu countries, Australia and New Zealand (Table 3).

Farming of *P. monodon* in Thailand has shown rapid expansion. Based on the fact that breeding of *P. monodon* larvae from pound-reared tiger shrimps rarely produces enough larval quality required by the industry, the farming of *P. monodon* has thus relied entirely on wild female broodstock, resulting in heavy exploitation of wild *P. monodon* females by aquaculture activity.

Table 1.1 World Prawn Farming in 1998

Area of Prawn Farming	% of World Production	Heads-on Production (metric tons)	Production Areas (Hectares)	Kilograms per Hectare	Number of Hatcheries	Number of Farms
Western Hemisphere	28	207,000	226,800	913	457	2,494
Eastern Hemisphere	72	530,200	637,550	832	3,718	168,833
Total	100	737,200	864,350	853	4,175	171,327

Source: World Prawn Farming, 1998

To overcome consequent problems from over-exploitation of wild stocks, breeding of high-quality pond-reared *P. monodon*, through domestication and subsequently selective breeding programmes are currently required. The success of these processes would resolve problems of overexploitation of *P. monodon* from natural populations.

Nevertheless, domestication and selective breeding programmes are long processes. Stock enhancement (producing and releasing artificially propagated *P. monodon* from appropriate breeding projects) of *P. monodon* is an alternative approach that can be used to supplement the natural *P. mondon* populations. To construct appropriate stock enhancemence (the so called, restocking) programs, the basic knowledge on population genetics of *P. monodon* in Thailand including the information on levels of genetic diversity and the number, if any, of reproductively isolated stocks of *P. monodon* in Thailand is required.

Table 1.2 Eastern Hemisphere Farming in 1998

Country	Percent of Production	Heads-on Production (metric tons)	Production Areas (Hectares)	Kilograms per Hectares	Number of Hatcheries	Number of Farms
Thailand	39.6	210,000	70,000	3,000	1,000	25,000
Others	28.3	150,000	200,000	750	2,000	10,000
India	13.2	70,000	140,000	500	150	100,000
Indonesia	9.4	50,000	200,000	250	300	30,000
Philippines	6.6	35,000	20,000	1,0750	90	2,000
Malaysia	1.5	8,000	4,000	2,000	100	800
Sri Lanka	0.9	5,000	3,000	1,667	66	1,000
Australia	0.4	2,200	550	4,000	12	33
Total	100	530,200	627,550	832	3,718	168,833

Source: World Prawn Farming, 1998

Table 1.3 Data indicating Thailand Export of Fresh and Frozen Marine Prawn (mainly *P. monodon*) (Q = quantity in tons, V = values in million bath)

No.	Country	Jan – Dec 1997		Jan - Dec 1998		% Change	
		Q	V	Q	V	Q	V
1	Asia	74,680.00	24,945.22	73,288.00	25,957.01	-2%	4%
	China	15,484.00	4,071.87	16,796.00	4,816.63	8%	18%
	Hong Kong	4,650.00	1,632.36	4,594.00	1,147.51	-1%	-30%
	Japan	27,804.00	12,277.14	28,021.00	12,723.67	1%	4%
	Korea	4,144.00	1,201.70	2,117.00	417.67	-49%	-65%
	Singapore	13,613.00	3,452.87	15,188.00	4,793.00	12%	39%
	Taiwan	8,985.00	2,309.28	6,572.00	2,058.53	-52%	-11%
2	U.S.A.	37,991.00	14,516.20	52,541.00	20,559.50	38%	42%
3	Canada	3,777.00	1,453.81	4,785.00	2,087.25	27%	44%
4	EU	13,161.00	3,787.82	15,519.00	5,818.82	18%	54%
	Belgium	502.00	172.98	882.00	337.45	76%	95%
	Denmark	168.00	51.39	270.00	115.92	61%	126%
	France	4,215.00	1,197.17	3,889.00	1,477.33	-8%	23%
	Germany	1,579.00	596.41	2,404.00	1,148.27	52%	93%
	Italy	1,897.00	332.64	2,244.00	554.35	18%	67%
	Netherlands	1,253.00	411.99	1,678.00	714.37	34%	73%
	Spain	959.00	201.25	842.00	218.67	-12%	9%
	United Kingdom	2,588.00	823.99	3,310.00	1,252.46	28%	52%
5	Australia	4,880.00	1,726.33	5,641.00	2,479.09	16%	44%
6	New Zealand	366.00	98.87	492.00	163.37	34%	65%
7	Others	2,225.00	655.61	3,910.00	1,278.28	76%	95%
	Total	137,080.00	47,183.86	156,176.00	58,343.32	14%	24%

Source : Department of Business Economics (1998)

1.1 Biology of the giant tiger shrimp (*P. monodon)*

1.1.1 Taxonomy

The tiger shrimp (*P. 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: Giant tiger shrimp (or black tiger prawn)

Synonyms : *Penaeus carinatus* Dana, 1852

Peneaus caeruleus Stebbings, 1905

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

Penaeus bubulus Kubo, 1949

1.1.2 Morphology

Shrimps can be morphologically divided externally into the thorax and abdomen (Fig. 1.1). The thorax (or head) is covered by a single, immobile carapace which protects internal organs and supports muscle origins. The eye stalks and eyes, the sensory antennules and the antennae (all paired) arise rostrally. The walking legs (or pereiopods) are the thoracic appendages. Gills are formed from sac-like outgrowths of the base of the walking legs and located in branchial chambers on either side of the thorax. The carapace extends laterally to cover the gills completely. The abdomen has the obvious segmentation of invertebrates. A pair of swimming legs (or pleopods) arise from each of the six abdominal segments. A tail fan comprising a telson, which bears the anus. Two uropods attach to the last (6th) abdominal segment.

The cuticle secreted by an epidermal cell layer, consists of chitin and protein in which calcium carbonate and calcium phosphate have been deposited. Pigments are deposited in the cuticle for colour and pigment cells or chromatophores are present in the hypodermis. Parts of the digestive tract are lined by chitinous cuticle.

Shrimps grow by periodically shedding their cuticle (termed moulting or ecdysis). The epidermis detaches from the inner cuticle layer and begins to secrete a new cuticle, while the old cuticle is moulted. Immediately after moulting, the new cuticle is soft and is stretched to accommodate the increased size of the prawn. Notably, actual tissue growth within the shrimp is continuous between moults.

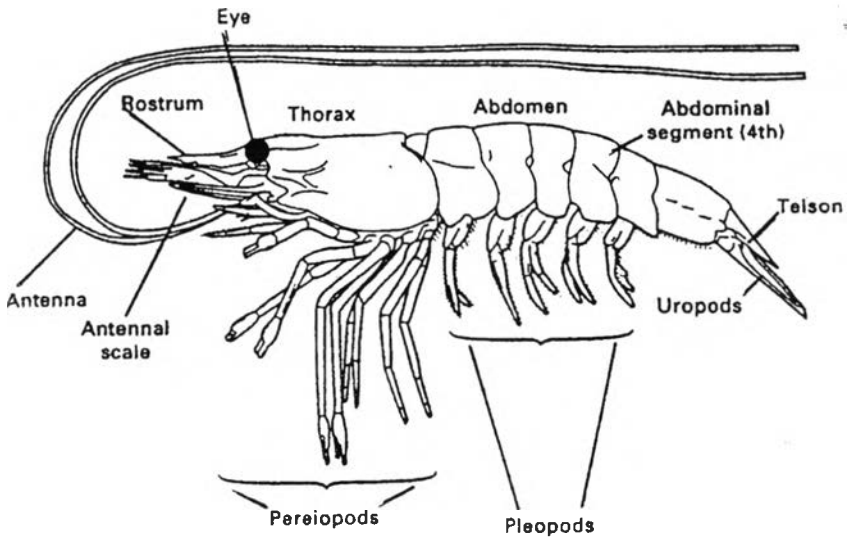

Figure 1.1 Lateral view showing important parts of *P. monodon* (Anderson, 1993)

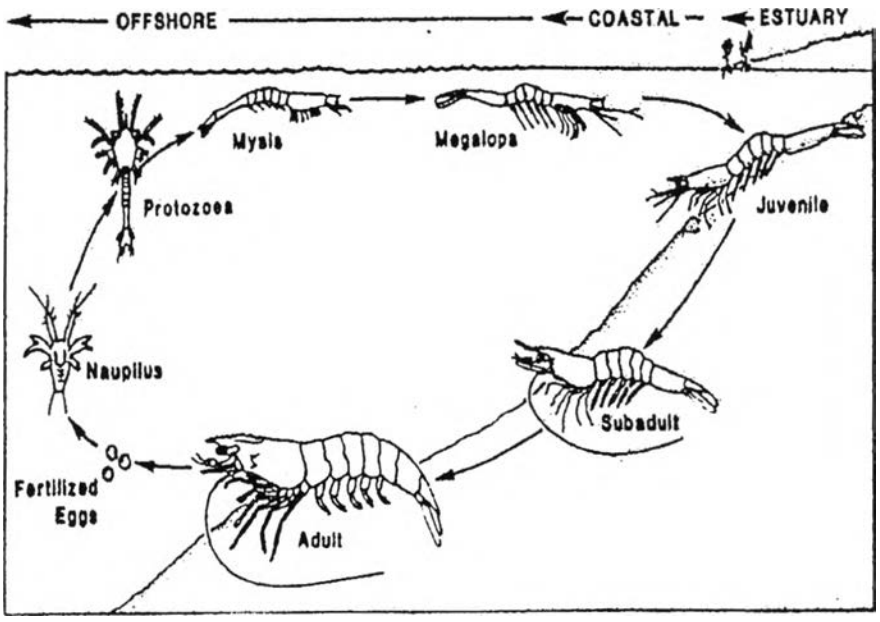

Figure 1.2 Life cycle of Penaeid shrimps (Bailey – Brock and Moss, 1992)

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 – Brock and Moss, 1992).

1.1.3 Life cycle

The life cycle of penaeid shrimps includes several distinct stages found in a variety of habitats. Juveniles prefer brackish waters of estuaries and coastal wetlands while adults are usually found off-shore at higher salinities and greater depths. Larvae inhabit plankton-rich surface waters off-shore, with a subsequent on-shore migration during the later stages of development (Fig. 1.2) (Bailey – Brock and Moss,1992).

The development of penaeid prawns is complex. Larvae hatching from the fertilised eggs pass through a series of moults and metamorphic stages before becoming adult-like (juveniles). Juveniles continue growing, moulting and finally developing into adults.

Generally, the nauplius hatches from a fertilised egg at approximately 12 hour after spawning. Larval stages consist of three to six nauplius, three protozoea and two or three mysis substages depending on the actual species (six nauplius, three protozoea and three mysis substages for *P.monodon*). The period of larval development varies with temperature and feeding levels but is usually 10-14 days. The post-larvae (PL) have all the appendages and organs similar to adult prawns.

Shrimp larvae are naturally planktonic in behaviour. Swimming is possible using antennae in nauplii, antennae and thoracic appendages in protozoa and thoracic appendages in mysis larvae. The first nauplii are about 0.3 mm long and are characterised by being totally planktonic and positively phototaxic. The larvae begin to feed at protozoea. They are filter- feeders and consume particles of the correct size at 8-200 μm (unicellular algae are typically provided as food in hatcheries). They are approximately 1 mm in length, with a narrow elongated thorax and abdomen, and a loose-fitting carapace. Paired eyes, a rostrum and feeding appendages are present for the first time. The second metamorphic change is seen when the third protozoea moults into the first mysis stage. Mysids have five pairs of functioning pereiopods (thoracic appendages). The carapace at this stage covers all the thoracic segments. The mysids swim in a more adult manner and actively seek out for phytoplankton and zooplankton. The final mysis larval stage metamorphoses to the post-larvae, where a full complement of functioning appendages is present.

Post-larval stages are given a numerical suffix to indicate the number of days since metamorphosis. Post-larvae continue to moult as they grow. They migrate shoreward and settle in nursery areas close to the shore or in estuaries, where they grow quickly to juvenile and sub-adults. Sub-adults migrate back to the sea where they finally mate and spawn. The life spawn of penaeid prawn is less than two years. (Anderson, 1993)

1.2 Distributions

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 up to 162 meters (Anderson, 1993)

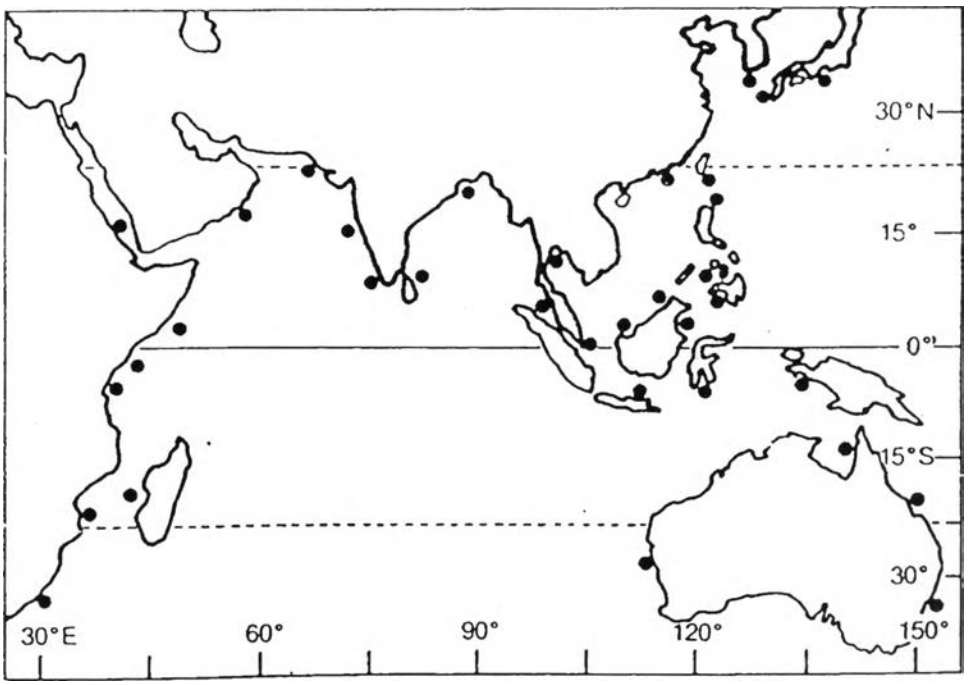

Figure 1.3 Geographic distributions of *P. monodon* (Motoh, 1981)

• = Main fisheries areas

as a result, 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 the South China Sea on the west and east coasts, respectively). The giant tiger shrimp is the most important cultured marine shrimp in Asia (Dore and Frimodt, 1987).

1.3 Molecular genetic markers and population genetics

Genetic markers and genetic distances have been used to examine relationships of organisms at different taxonomic levels. Population genetics based information can be applied to several aspects of shrimp culture; for instance, determination of stock structure in a particular area, identification of hybridization and introgression between closely related shrimp species (e.g. between *P. merguensis* and *P. indicus*), and analysis of individuality and percentage in selective breeding programmes

Genetic markers used for population genetic studies should exhibit suitable polymorphic levels for the desired application and be selectively neutral. Under this circumstance, the information from genotypes and allele frequencies is assumed to be primarily influenced by mutation, gene flow, genetic drift, rather than by selection (Smith and Brown, 1988). Molecular genetic techniques commonly used for population genetics and systematics can be classified in to those at the protein level (allozymes and polymorphism of functional proteins) and the DNA level (restriction mapping, restriction fragment length polymorphism, microsatellite analysis, DNA sequencing, amplified fragment length polymorphism etc.)

Animal mitochondrial (mt) DNA

Animal mtDNA is a circular molecule usually ranging in size from 14,000 to 20,000 base pairs (bp) (Avise, 1994). It is present in high copy numbers per cell (Brown, 1983). Generally, mtDNA is homoplasmic, (all molecules are identical in an organism) meaning that any tissue can be used as a source for purification. The nucleotide substitution rates in the mtDNA of higher vertebrates is approximately 5 - 10 times greater than that of single copy genes, enhancing the ability to detect genetic polymorphism for population genetic studies (Brown 1983; Moritz et al, 1987).

MtDNA is usually maternally transmitted and is not subject to recombination (Avise and Verijenhoek, 1987; Ovenden, 1990). Maternally inherited and the haploid nature of mtDNA result in smaller estimated effective population size compared with single copy nuclear genes unless extensive heteroplasmy is existent and a sex ratio is heavily biased towards females. This means that mtDNA is more susceptible to reduction in genetic variability due to population bottlenecks than does single copy nuclear DNA (Nei and Tajima 1981; Birky et al, 1989).

Assuming equal migration ability of both sexes, four times as much interchange among populations is required to homogenize the mitochondrial divergence, as opposed to that of the nuclear gene where a gene flow of one individual per generation may homogenize differentiation between pairs of populations successfully. (Billington and Hebert, 1991)

The animal mitochondrial genome carries genes coding for 13 proteins, 2 ribosomal RNAs (12S and 16S rRNAs), 22 transfer RNA (tRNA), and a noncoding

region which contains the origin of heavy strand replication (called D-loop region in vertebrates). The genes that code for proteins are NADH dehydrogenase (ND) subunits 1, 2, 3, 4, 4L, 5 and 6, cytochrome b, two subunits of ATP synthetase (ATPase 6 and 8) and three subunits of cytochrome oxidase (COI, COII and COIII) (Avise, 1994). The gene order in the mitochondrial genome is highly conserved within the phylum (Moritz et al, 1987).

Restriction Fragment Length Polymorphism (RFLP)

Restriction fragment length polymorphism (RFLP) is the early approach used for evaluation of genetic diversity at the nucleotide (DNA) level. Variation in restriction enzyme cleavage sites (usually detected by Southern blot hybridization) generates size differences of the resulting fragments. Therefore, the technique is called RFLP representing the polymorphism of restricted DNA fragments.

RFLP analysis can sometimes be visualized directly by staining with ethidium bromide following electrophoresis of the digested DNA in the agarose gels. For the classical RFLP approach, Southern blotting is necessary to visualise the specific interest region. Probes can be from the same species (homospecific) or from the other related species (heterospecific). The probe should represent a specific sequence that occurs in an interested region. Basically the extracted DNA is digested with the appropriated restriction endonuclease and electrophoresed through the agarose gel. The restricted DNA is transferred to a membrane before hybridised with the labelled DNA probe. Results from restriction analysis can be visualised by autoradiography.

Polymorphism of nuclear DNA using Southern blotting analysis has been carried out since the technique was developed in 1977. However, the use of this technique for analysis of mtDNA polymorphism was established in 1979 (Brown, 1979; Avise, 1994). The conventional multi-locus DNA fingerprints developed by Jreffrys et.al. (1985) is classified as a Southern blotting based technique.

PCR-RFLP

The polymerase chain reaction (PCR) is a technique for *in vitro* amplification of specific DNA sequences by simultaneous primer extension of complementary strands of DNA. PCR is a process based on a specialised polymerase enzyme, *Taq* polymerase. The PCR reaction components are composed of DNA template, a pair of primers for the target sequence, a building block like each of dNTPs and the suitable buffer.

The amplification reaction consists of three steps; template denaturation at high temperature, primer annealing at optimal temperature, and the extension of the annealed primers by DNA polymerase at the working temperature of DNA polymerase. A large number of copies of the target DNA sequence can be generated within a relatively short period of time. The amplified product can then be digested with restriction enzymes. The restricted fragments are separated on an agarose gel (or a polyacrylamide gel) and directly visualised after ethidium bromide staining. Since restriction analysis using Southern hybridisation are tedious and time consuming, PCR-RFLP analysis is rapid increasingly used for population genetic studies of various taxa where a large number of specimens needs to deal with.

1.4 Population genetic studies in penaeid shrimps

Benzie et al. (1992) examined genetic variation of *P. monodon* in Australian waters based on allozyme analysis. Samples were obtained from seven collection sites; Clarence River (N = 100), Townsville (N = 85), Cairns (N = 100) from the eastern cost, Weipa (N = 100), Melville Island (N = 64) and Joseph Bonaparte Gulf (N = 74) from the northern coast and the De Grey River (N = 73) from the western coast of Austrialia. Three loci (*GPI**, *PGM**, and *MPI**) contributed to the significant differences between the western *P. monodon* and the northern and eastern *P. monodon* ($P < 0.05$).

Sodsuk et al. (1992) reported genetic diversity of *P. monodon* origination from the South – East Asian region using allozyme analysis. Approximately 100 individuals of *P. monodon* were collected from each geographic sample including Trat and Surat located in the Gulf of Thailand and Phuket and Satun located in the Andaman sea between 1991 and 1992. 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 comparisons 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. The significant differences between specimens collected from

the same geographic location but different time should be further confirmed to indicate that the results from that study was not caused by sampling errors.

Benzie et al. (1993) used mtDNA analysis to study the population structure of *P. monodon* in Australia collected from Townsville ($N = 6$) and Cairns ($N = 6$) from the east and De Greg River ($N = 3$) and digested with *Bam* HI, *Eco* O109, *Eco* RV and *Sac* I. Frequencies of the mtDNA genotypes between the east and west coasts ($P < 0.05$) were significantly different. This studies preliminary suggested that RFLP analysis provide a better source of useful markers for penaeid prawns. It should be noted that the sample sizes used in this experiment was extremely limited for marine species. As a result, more number of specimens must be used for more reliable results.

Bouchon et al. (1994) analysed mtDNA PCR-RFLP data of three laboratory strains of *P. monodon* originating from Malaysia, Australia and Fiji and one farm population of *P. japonicus*. Using RFLP analysis, the percentage of genetic divergence of 1.68% was found between the Fiji and the Australia/Malaysia strains. This result implied the possibility on biogeography of *P. monodon* in the Indo-West Pacific region. A restriction map of the entire mtDNA of the Fiji strain was established and the small subunit of ribosomal (12S rDNA) gene region was located. The farmed population of *P. japonicus* was genetically studied. However, genetic diversity was not found in the farmed *P. japonicus*. Presumably, this *P. japonicus* population may have been established from a limited number of founder individuals having the same mtDNA haplotype.

Garcia et al. (1994) examined genetic diversity in populations of *P. vannamei* using RAPD. The population 1 was a specific - pathogen free (SPF) stock originated from at least three separated spawns of different breeding females in a hatchery from Sinaloa, Mexico. Twelve maternal families were subsequently established from this SPF 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, OPA10, OPA20, OPB11,OPB14 and OPB20). High levels of genetic diversity among *P. vannamei* populations were observed. A total of 73 scoring bands were found. 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 population 4 exhibited 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 of RAPD for examining genetic diversity of each stock used in a breeding programme.

Garcia and Benzie (1995) demonstrated the inheritance of RAPD banding patterns in six fullsib 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 family were screened. Forty-eight consistent RAPD bands were amplified from genomic DNA of parents and progeny using 14 primers. Forty-five bands (accounting for 98.8% of scorable bands) were monomorphic. Nevertheless, three polymorphic

markers (accounting 6.2%) illustrating Mendelianly inherited fashion were identified. The results indicated that RAPD was promising to generate markers assisted in selective breeding programme in *P. monodon*.

The preliminary study of nuclear rDNA polymorphism in *P. monodon* had recently shown. Inter-and intraindividual polymorphism of rDNA resulted in difficulties to analyze rDNA polymorphism statistically. Individual-specific rDNA restriction patterns were observed when digested with *Bam* HI and *Sac* I. The intergenic spacers (IGS) region of *P. monodon* rDNA may play an important role in length heteroplasmy at both between– and within–individual levels in this species. Therefore, this DNA marker was not suitable to be used for population genetic studies in *P. monodon*. (Klinbunga et al., 1998)

Restriction fragment length polymorphism (mtDNA-RFLP) of the entire mtDNA was utilised for determination of the genetic variation and population structure of *P. monodon* collected from Satun (the Andaman Sea) and Surat and Trat (the Gulf of Thailand). Twenty-eight composite haplotypes were generated from 52 restriction profiles generated from digestion of the entire *P. monodon* mtDNA with 11 restriction endonucleases. (*Ava* II, *Bam* H I, *Bgl* II, *Cla* I, *Dra* I, *Eco*RV, *Hind* III, *Pvu* II, *Sac* I, *Sca* I, and *Xba* I). Three *P. monodon* populations were allocated in to two different stocks composed of the Andaman (Satun) and Gulf of Thailand (Surat and Trat) stocks (Klinbunga et al., 1999).

Tassanakajon et al. (1997 and 1998) illustrated geographic population differentiation of *P. monodon* in Thailand using randomly amplified polymorphic

DNA (RAPD) analysis. The Andaman *P. monodon* (west) showed significant genetic difference with the Gulf of Thailand *P. monodon* (east), indicating genetic subdivision between *P.monodon* from these main fisheries regions in Thailand.

Tassanakajon et al. (1997) investigated genetic variation and differentiation of wild *P. monodon* in Thailand using RAPD analysis. Two hundred octanucleotide primers were screened, and 84 primers successfully yielded amplification products. Six positive primers (UBC101, UBC174, UBC428, UBC456, UBC457 and UBC459) that gave highly reproducible RAPD patterns were selected for population analysis of the three geographically different samples (Trat, Satun-Trang and Angsila) of *P. monodon.* A total of 70 reproducible RAPD fragments ranging from 200 to 2000 bp were scored. Forty fragments (57%) were polymorphic. The RAPD analysis of broodstocks from Satun-Trang, Trat, and Angsila, revealed different levels of genetic variability among geographic samples. The percentages of polymorphic bands were 48% and 45% in Satun-Trang and Trat, respectively. These indicated high genetic variability of these samples. Only 25% polymorphic bands were found in the Angsila sample, indicating the lowest polymorphic level among all samples examined. The primer UBC428 showed a RAPD–amplified band that appeared in *P. monodon* originating from Satun-Trang but disappeared from Trat and Angsila. This finding suggested the potential use of this marker as a region-specific marker in this species.

Tassanakajon et al. (1998a) further analyzed genetic differentiation in wild populations of black tiger shrimp *(P. monodon)* based on RAPD analysis. Specimens were collected from five geographically separated locations: Satun-Trang (N = 17), Phangnaga (N = 20), and Medan (N = 15) in the Andaman Sea and Chumphon (N =

20) and Trad (N = 28) located in the Gulf of Thailand) and were investigated using seven arbitrarily selected primers (UBC101, UBC174, UBC268, UBC428, UBC456, UBC457 and UBC459). Fifty-eight (72.5%) of eighty reproducible RAPD fragments ranging in size from 200 to 2200 bp were polymorphic. The percentages of polymorphic bands of the five geographic populations investigated varied from 51.5 to 57.7%. The genetic distance between populations and a UPGMA dendrogram indicated that the Medan population was genetically different from Thai *P. monodon* (D_{ij} = 14.975%). Within Thailand, the Satun-Trang *P. monodon* was separated from the remaining geographic populations with a genetic distance of 2.632%. RAPD analysis using those seven primers generated 252 genotypes. A Monte Carlo simulation illustrated geographic heterogeneity in genotype frequencies within *P. monodon*, suggesting that genetic population subdivision does exist in this taxon ($P < 0.001$ for all primers). Significant differences in genotype frequencies between Thai and Indonesian (Medan) *P. monodon* were observed ($P < 0.0001$). Within Thailand, the gene pool of *P. monodon* from the Andaman Sea was significantly different from that of the Gulf of Thailand (P values between 0.0000 and 0.0387), indicating fragmentation of gene pools between *P. monodon* from these two main fishery regions of Thailand.

Tassanakajon et al, (1998b) isolated and characterized microsatellites in *P. monodon*. Partial genomic library of *P. monodon* was constructed and screened using synthetic $(GT)_{15}$ and $(CT)_{15}$ oligonucleotides. The results indicated that $(GT)_n$ microsatellites were more abundant than $(CT)_n$. Among complete, incomplete and compound microsatellites isolated, two loci, CUPmo 18 and CUPmo 386, were

successfully amplified. Observed heterozygosity for these loci was 0.66 and 0.19, respectively. The ability to isolate microsatellites in this species allows the possibility to include microsatellites in breeding programmes of *P. monodon*.

Moore et. al. (1999) characterised microsatellite and amplified fragment length polymorphism (AFLP) DNA markers and established a linkage map in *P. japonicus*. Limited number of microsatellites were isolated from a partial library of *P. japonicus* genomic DNA. This caused by the existence of extended length of simple sequence repeats of isolated microsatellites. The number of microsatellites was not enough to establish a useful linkage map in this species. Isolated microsatellites displayed between 4-24 alleles and exhibited heterozygosities between 47-91% in unrelated *P. japonicus* but they did not successfully amplified *P. monodon*, *P. esculentus* and *P. stylirostris* DNA.

AFLPs were developed as an alternative approach. More than 570 polymorphic loci were identified using different primer combinations. AFLPs are quite robust and some bands were conserved across families. Sequence Tagged Sites (STS) markers could be developed in *P. japonicus*. A primary linkage map based on a three generation pedigree samples genotyped at 246 AFLPs loci has been constructed. It incorporated 129 markers in 44 linkage groups with an estimated genome coverage of approximately 57% (Moore et al., 1999).

Microsatellites were also used to examine genetic variation and differentiation of Thai *Penaeus monodon* from five geographic locations (Chumphon, Trat, Phangnga, Satun, and Trang). Using five microsatellite loci (*CUPmo*18, *Di*25, *Di*27,

*CSCUPmo*1, and *CSCUPmo*2), the number of alleles across overall loci ranged from 19 to 30, and heterozygosities ranged from 0.49 to 0.95. The mean number of alleles and effective number of alleles per locus were 21.0 to 26.6 and 13.1 to 20.4, respectively. The average heterozygosity across all investigated samples was 0.78, indicating high genetic diversity in this species.

Geographic heterogeneity analysis based on loci *CUPmo*18 and *Di*25 showed significant differences between *P. monodon* from the Gulf of Thailand (Trat and Chumphon) and the Andaman Sea. Geographic heterogeneity was also observed within the Gulf (between Trat and Chumphon) but not within the Andaman Sea. The Trat *P. monodon* were genetically different from the Andaman ($P < 0.001$) and Chumphon ($P < 0.001$) *P. monodon*. The genetic difference was not observed when compared Chumphon with each of the Andaman samples ($P < 0.05$).

Analysis of population differentiation using F_{ST} statistics also indicated consistent results (F_{ST} between Andaman-Trat was 0.0101, $P < 0.002$ and between Chumphon-Trat was 0.0118, $P < 0.002$). The gene pools of *P. monodon* from five geographic locations were divided into three distinct populations composed of the Andaman Sea (A), Chumphon (B), and Trat (C) (Supungul et al., 2000).

1.5 Objectives

The objectives of this thesis are to examine the levels of genetic variation and intraspecific population differentiation of wild *P. monodon* collected from five geographically different locales in Thailand (Chumphon and Trat from the Gulf of Thailand and Phangnga, Satun, Trang from the Andaman Sea) using PCR-RFLP of

mitochondrial genes (16S rDNA and an intergenic COI-COII) and to simplify the method for typing of *P. monodon* mtDNA. The basic information can be further applied for construction of restocking programmes of *P. monodon* in Thailand.