CHAPTER IV

Discussions

Population genetics and fishery management of marine organisms

The levels of genetic diversity within exploited species are basically important and help deciding whether such species should be conserved or exploited. The heavy exploitation of a fishery composed of numerous unidentified interbreeding populations is likely to lead to either the serious erosion of population structure or possibly permanent extinction of a section of the resource (Ovenden, 1990).

If the exploited species is truly panmictic, treating the fishery as a single stock would have no consequences in terms of recruitment and distribution from overfishing. If, however, degrees of reproductive isolation do exist, the unit stock harvest may result in local overexploitation diminishing the sustainable of the overall fishery yield as long-term effects. (Carvalho and Hauser, 1994). Furthermore, undetected stock may become eliminated, with a corresponding reduction in interpopulation genetic divergence. (Leslie and Grant, 1990; Jennings and Beverton, 1999)

Distributions of mtDNA haplotypes in Thai P. monodon

Mitochondrial DNA analysis has been widely used for determination of population structure in various animal taxa because mtDNA polymorphism is expected to be more effective than does allozyme electrophoresis. This is mainly due

to a smaller effective population size estimated from mtDNA polymorphism resulting in its more sensitive to genetic drift (Ward and Grewe, 1994; O'Connell et al., 1995).

A total of 37 composite mtDNA haplotypes was observed from restriction analysis of 16S rDNA and an intergenic COI-COII with polymorphic restriction endonucleases used in this study. The patterns of composite haplotype distributions based on the analysis of mtDNA- RFLP in *P. monodon* were different from those often observed in other species because of the deficiency of fixed population-specific haplotypes and the presence of many private haplotypes (haplotypes observed in a single individual).

Two common composite haplotypes (I, ABBBBA and VII, BAAAAB), found in restriction analysis of *P. monodon* 16S rDNA and COI-COII, were not population-specific but they were shared across geographic samples. Nevertheless, a ratio of haplotypes I/VII in each of the Andaman samples (1.00, 0.75 and 0.45 in Satun. Trang and Phangnga, respectively) and Chumphon (0.45) was greater than that of Trat (0.17). This implied the existence of biogeography of *P. monodon* in Thailand.

Two phylogenetic clusters of composite haplotypes were found. These were overlappingly distributed among geographically different samples. A possible explanation for distribution patterns of mtDNA of *P. monodon* in Thailand is an ancient separation of the ancestral population into two allopatric populations, which have evolved into two separate mitochondrial lineages. Alternatively, *P. monodon* from the Andaman and Gulf of Thailand may have been coincidentally colonised by separate populations. Further studies on genetic differentiation of this taxon over

large geographic areas (e.g. the South East Asian regions) will provide a clearer conclusion.

A large number of privaye haplotypes (haplotype observed only once) were also observed in the Pacific oyster (*C. gigas*) but the mtDNA distribution pattern in *C. gigas* is different from that of *P. monodon*. In *C. gigas*, two common haplotypes were distributed in all of the British Columbia samples with roughly the same frequencies. Accordingly, population subdivision was not observed in that species (Boom et al., 1994).

Considering digestion patterns obtained from restriction enzymes used in the present study, only those from digestion of an intergenic COI-COII with *Alu* I and *Taq* I could represent the frequency of each phylogenetic cluster accurately. The restriction patterns B, C and D from *Alu I* digested COI-COII were consistently found in the cluster I while patterns B and C were specifically found in *P. monodon* exhibiting the cluster II genotypes. Likewise, patterns A and others (B, C, D and E) of *Taq* I digestion represented cluster I and II haplotypes precisely.

Therefore, frequencies of these two phylogenetic clusters in a particular location can be examined by digestion of an amplified COI-COII with *Alu* I and *Taq* I rather than the use of a battery of restriction enzymes. This practically opens the possibility to construct stock enhancement (restocking) programmes of *P. monodon* in Thailand without the anthropological disturbance of its local gene pools.

Genetic diversity of P. monodon in Thailand

The levels of haplotype and nucleotide diversity found in *P. monodon* indicated relatively high genetic variability in this species. The high haplotype diversity was obviously caused by the occurrence of a large number of private haplotypes and their relatively low frequencies.

The average haplotype diversity in *P. monodon* was as high as that previously reported in other marine organisms, such as 0.89 in the horseshoe crab, *Limulus polyphemus* (Saunders et al., 1986), 0.80 in the American oyster, *Crassostrea virginica*, from the Gulf of Mexico (Reeb and Avise, 1990) and 0.66 in the Japanese scallop , *Patinopecten yessoensis* (Boulding et al., 1993).

Comparisons of haplotype diversity between all pairs of samples were not statistically different ($P > 0.05$) indicating similar levels of genetic diversity of five *P. monodon* samples in Thailand. However, estimation of haplotype diversity is based on haplotype frequencies alone; as a result, it is sensitive to the number of restriction enzymes used in the experiments. If more enzymes are employed, additional haplotypes can be detected, increasing the value of haplotype diversity (Grave and McDowell, 1994).

The average nucleotide diversity within samples of Thai *P. monodon* was greater than common mtDNA sequence diversity, which is usually lower than 1.0% (Ovenden, 1990; Palumbi and Wilson, 1990, Boulding et al., 1993). The mean nucleotide diversity between pairs of populations was 3.3648% which was slightly

greater than that within samples (3.3283%) implying a low degree of population substructure in this species. The mean nucleotide divergence in *P. monodon* (0.0365%) was higher than that previously reported at -0.0017% in the Japanese scallop, *P. yessoensis* (Boulding et. al., 1993), -0.017% in the banana shrimp, *P. merguiensis* (Duad, 1995) and 0.011% in the Pacific oyster, *C. gigas* (Boom et al., 1994), indicating a slightly greater degree of population differentiation in *P. monodon* compared to those species. Nevertheless, a stronger level of population differentiation was previously reported based on restriction analysis of the entire mitochondrial genome of *P. monodon* from Trat, Satun and Surat (Klinbunga et al., 1999). The mean nucleotide divergence for all pairs of samples in their study was 0.26% which was several times greater than 0.038% reported by this study. Results from the present study suggests a lower level of population differentiation over the same geographic areas but different sampling periods reflects a consequence of disturbance of *P. monodon* gene pools through farming activity.

A large number of mtDNA haplotypes, relatively high level of nucleotide diversity and divergence in Thai *P. monodon* found indicated that the level of genetic diversity in this species was high in comparison to previously reported studies of various marine invertebrates (Ovenden, 1990).

Does intraspecific population differentiation exist in Thai P. monodon?

Population subdivisions in Penaeidae inferred from allozyme analysis were reported in *M. bennettae, M. macleayi, M. endeavouri, P. latisulcatus* (Mulley and Latter, 1981a and 1981b), *P. keraturus* (Mattoccia et al., 1987), *P. merguiensis*

(Daud, 1995), *P. monodon* (Benzie et al., 1992; Sodsuk et al., 1992; Daud, 1995; Sodsuk, 1996). In addition, Benzie et al. (1993) reported the genetic structure of *P. monodon* among four samples in Australia based on restriction analysis of the entire mtDNA.

Analysis of geographic heterogeneity and estimation of nucleotide divergence levels between pairs of samples indicated the existence of genetic population differentiation of *P. monodon* in Thailand. Five *P. monodon* samples were then allocated to two different stocks composed of the Andaman (Satun, Trang and Phangnga) and Gulf of Thailand (Chumphon and Trat) stocks. The results in this study are concordant with those of Sodsuk (1996) who examined the population structure of *P. monodon* using allozyme analysis of 46 loci. Several polymorphic loci (*AAT-1*, ALAT*, GPI*, IDHP*, MDH-1*, MPI** and *PGM**) contributed significant differences in allele and genotype frequencies between geographic locations and indicated significant population subdivisions between the Andaman Sea (Kedah, Medan, Satun, Phuket) and the eastern populations of the Thai/Malaysian peninsula (Trat, Surat, Dungun, Northern and Southern Java).

The exact test based on Ewen's sampling theory for selective neutrality of specimens used in this study did not show statistical significant in any geographic sample ($P > 0.05$). This confirmed that intraspecific differentiation of five *P. monodon* samples in Thailand did not occur from artifacts or from sampling errors.

It is now clear that *P. monodon* in Thailand can no longer be treated as a single panmictic species as revealed by the intraspecific genetic differentiation found

in this study. Two genetically different populations (The Andaman Sea and the Gulf of Thailand *P. monodon*) should be recognised as different stocks and must be managed separately by fishery managers and government organisations. At this time it is not clear whether there has been any differentiation in gross morphology or physiology of these populations. Accordingly, further studies on those topics should also be carried out.

Geological and hydrological changes may have responded for genetic differentiation of P. monodon in Thailand

Intraspecific genetic subdivisions of *P. monodon* in Thailand may have been explained by biogeographic barriers resulting from changing of sea levels during the Pleistocene for which the sea levels have dropped as much as 150 metres below and risen to 5 m above the present sea level (Dale, 1956; Pianka, 1994). The most recent rise of sea levels at the Straits of Malacca was approximately 5000 years ago at 5 m above the present level whereas at 10,000 years ago, the Straits of Malacca was shallower and narrower (about 50 m below the present level). This created geographic barriers effectively cut off gene flow between *P. monodon* from either side of the Malaysian Peninsula allowing genetic differentiation to occur (Klinbunga et al., 1998).

The Straits of Malacca would have been the most direct route for mixing between the Andaman Sea and Gulf of Thailand samples. The fact that mixing of two phylogenetic clusters of *P. monodon* composite haplotypes in these areas has not been complete is probably explained by the restriction on gene flow between *P. monodon*

from the Andaman Sea and the Gulf of Thailand. Nowadays, the currents in the Straits of Malacca are not affected by the major reversals in monsoon driven surface current systems seen in the Java and South China Sea (Dale 1956, Morgan and Valencia 1983). The main current in the Straits of Malacca is presently from south to north throughout the year. This would have restricted the gene flow particularly from the Andaman Sea to the Gulf of Thailand.

Potential female gene flow homogenises genetic differentiation between Chumphon and Trat P. monodon

Our parallel studies based on RAPD and microsatellite polymorphism of the same sample sets of *P. monodon* clearly demonstrated obvious genetic differences between *P. monodon* from different areas of Thailand (Wuthijinda et al., 1999; Supungul et al., 2000). Geographic heterogeneity analysis and F_{st} estimates showed significant genetic differences between Trat and the Andaman Sea populations. The *P. monodon* from these areas should, therefore, be regarded as separate stocks. Surprisingly, the Chumphon *P. monodon* showed significant genetic differentiation with Trat located in the same coastal area (Wuthijinda, 1999 and Supungul et al., 2000).

Recently, Klinbunga et al. (1999) determine genetic variation and population structure of *P. monodon* collected from Surat (a few hundred kilometers further south from Chumphon), Satun, and Trat using restriction analysis of the entire mitochondrial genome with *Bam* HI (G/GATCC), *Bgl* II (A/GATCT), *Cla* I (AT/CGAT), *Dra* I (TTT/AAA), *Eco* RV (GAT/ATC), *Hind* III (A/AGCTT), *Pvu* II

(CAG/CTG), *Sac* I (GAGCT/C), *Sca* I (AGT/ACT) and *Xba* I (T/CTAGA)], and *Ava* II (G/G[A or T]CC). They found high genetic diversity and significant geographic heterogeneity between the Andaman Sea and the Gulf of Thailand samples (mean nucleotide divergence between pairs of population = 0.26% and $P < 0.0001$, respectively). Nevertheless, they did not observe significant geographic heterogeneity and differentiation between Surat and Trat ($P > 0.05$).

The present study illustrated the existence of genetic population structure of *P. monodon* in Thailand ($P = 0.0005$ based on a Monte Carlo simulation). Highly significant genetic difference was observed when compared the Andaman *P. monodon* with Trat ($P = 0.0002$), and Chumphon ($P = 0.0011$) and pooled Gulf of Thailand samples ($P < 0.0001$). Nevertheless, genetic heterogeneity between Chumphon and Trat was not observed. Results from the present study were consistent with those previously reported by Klinbunga et al. (1999).

What are the possible causes for the failure to detect genetic heterogeneity between Chumphon and Trat sample using restriction analysis of the16S rDNA and an intergenic COI-COII?

An ability to differentiate the Chumphon *P. monodon* from Trat (both located in the Gulf of Thailand) using RAPD and microsatellite loci, but not mtDNA-RFLP suggests several possible reasons. Under a presumption of selective neutrality for genetic markers (RAPD, microsatellites and mtDNA), the possibilities to explain contradictory results between nuclear and mtDNA markers are: A) a recent introgression of nuclear DNA (but not mitochondrial DNA) from the Andaman Sea

into Chumphon *P. monodon* through intraspecific hybridisation, B) transferring of a *P. monodon* stock from the Andaman Sea into the Chumphon region has created a "mixed stock" in that area or C) biased female-mediated gene flow of *P. monodon* may exist between Chumphon and Trat.

Of these three possibilities, the possibility A seems to be unlikely unless it can be proved that only transferred males of *P. monodon* from the Andaman Sea are mature and significantly contribute on subsequent generations of shrimps in Chumphon whereas transferred females did not significant contribute to the gene pool in that area.

The possibility B may be the simplest explanation for this circumstance. This should have been true if the present study on mtDNA polymorphism indicated an Andaman Sea-derived distribution of mtDNA haplotypes for the Chumphon sample. The circumstance could be tested by of an unbiased χ^2 analysis (such as a Monte Carlo simulation in this study) on mtDNA haplotype distributions between the Andaman Sea and Chumphon. Results indicated non-significant differences in haplotype distribution between Chumphon and Phangnga (P = 0.2465) and Satun (P = 0.1552) but significant differences were observed when compared with Trang (P = 0.0080) and the pooled Andaman samples (P = 0.0011). Accordingly, the possibility of localised wild stock displacement by aquaculture activity cannot be eliminated until larger sample sizes of *P. monodon* in Thailand were analysed genetically.

The Chumphon samples in this study did not show any genetic differentiation from Trat. This was consistent with results described by Klinbunga et al. (1999). An

interesting question arises with this circumstance. *Why would nuclear DNA differences develop between Chumphon and Trat and not mtDNA differences between these geographic samples?* Considering the reproductive biology of penaeid shrimps, the potential female-mediated dispersal may exist between Chumphon and Trat. This has not been reported in *Penaeus* species and could apparently explain an anomalous pattern of population differentiation of *P. monodon* within the Gulf of Thailand appropriately.

Applications of the knowledge on intraspecifc genetic differentiation for conservation programmes in P. monodon.

In terms of aquaculture, the success of domestication and subsequent, selective breeding programmes is an important step to partly resolve problems of over-exploitation of wild *P. monodon* females.

Previously, the practical management of *P. monodon* has ignored that *P. monodon* from different geographic origins is possibly genetically different. Under this concept, massive numbers of postlarvae and broodstock have been transferred over broad areas. Moreover, fishery managers have not concerned about the effects of escapees released into the new environments either accidentally or intentionally.

The most important problem that can be arisen from introduction and transplantation of non-native stocks to the new environment is a spread of unfamiliar pathogenic to a local stock causing an outbreak of diseases over vast geographic areas (Avise, 1994).

The mtDNA-RFLP analysis of *P. monodon* in this study indicated the existence of population subdivision in this species. Therefore the previous assumption of a large single breeding stock in *P. monodon* must be changed (Ward and Grewe, 1994). More importantly, two genetically different populations (the Andaman Sea and Gulf of Thailand) should be recognised as different stocks and must be managed separately (Carvalho and Hauser, 1994). The controls on the movement of live broodstock and unnecessary transplantation should be implemented to prevent national shortages of good quality broodstocks.

Overexploitation of this species has also resulted in actively enhancing natural populations with hatchery-reared larvae by the government's organisations without consideration on the characteristics on gene pools of *P. monodon* in Thailand. Results from several stock enhancement programmes have been successful in a number of species if measured from economic point of view alone. Nevertheless, genetic interaction between exotic and local stocks of *P. monodon* should be aware following an assumption that local stocks are optimal adapted.

Direct ecological effects causing competitions between introduced and native stocks have been reported in a number of species and should not be overlooked in *P. monodon*. Moreover, transferred stocks may create consequence problems from intraspecific hybridisation, introgression and competitive replacement of local stocks resulting in an irretrievable loss of local gene pools and destruction of overall genetic diversity of *P. monodon* (Daud, 1995, Klinbunga, 1996).

Interbreeding between exotic and indigenous populations of *P. monodon* may open the possibility allowing introgressive non-adaptive genes from the former to the local stock producing intraspecific hybrid progeny exhibiting less well adapted for survival and reproduction These effects have been documented in several economically important aquatic species (Altukhov and Salmenkova, 1987; Thrope et al., 1995).

As a result, unnecessary introduction and transplantation of non-native stocks of *P. monodon* should be strictly regulated and not be permitted without careful analysis of ecological and genetic risks. If transplantation is unavoidable, genetic compositions of transplanted *P. monodon*, should be determined regularly using appropriate molecular markers (e.g. mtDNA, microsatellite and single copy nuclear DNA markers) to follow the effects of transplantation and its successfulness in a particular area.

All of the results from the present study indicated that mtDNA-RFLP analysis is applicable to the studies of population genetics and phylogenetics in *P. monodon.* This study has simplified the method for examination of mitochondrial DNA distribution frequencies in *P. monodon* based on only one restriction enzyme allowing the practical possibility to use this information for restocking of *P. monodon* using the genetic based data.