CHAPTER 5

CONCLUSION

Channa striata is a freshwater fish, native to the South-Eastern region of Asia. It is a favorable source of protein and is better known for its wound healing properties that is not restricted to fish itself but also to the consumers of the fish too. Pharmaceutical interest in the fish as well as nutritional has added an extra stress on the natural stock, as it has made it a target for over exploitation over the past years.

This study has been conducted to investigate the reasons behind the decline in the numbers of species in the wild, over the past few decades. It aims at studying the genetic content of which is crucial to examine whether or not the fish is on the verge of extinction.

The project started off with a hypothesis of matching the genetic distances of the data analysis to the actual geographical location of each population and looking at the genetic diversity across the Malaysian states. However our findings revealed rather interesting aspects of the individuals around the Peninsular. In fact some results arose that had us wondering whether there other factors, that are greater than just the mere mating habits and resulting genetic content, that might be involved in the changes affecting the existence of the species.

During the course of this project a total of seven polymorphic microsatellite loci were isolated and developed for *Channa striata*, using 5’ RAM primers. This was a result of 110 microsatellite primers that were designed and 60 of those that have been tested for polymorphism.

A common realization when working with this species in our laboratory was that microsatellites were tougher to isolate using this method, compared to other organisms. Higher repeat motifs were more abundant than di- repeats and their allelic variation was also not up to our expectations. However, the genetic content was variable enough to confirm that the species still has enough variation to maintain heterozygosity in the future. True enough this reflected on the observed heterozygosity data obtained, which for the most part showed that the species was highly heterozygote, at least for the loci we have studied.

The values of HWE showed that less than 50% of all loci in our seven populations are significantly rejecting the null hypothesis. This could mean that the actual species is not going through drastic genetic stress, or even reduction in variation. This is also highlighted by the inbreeding coefficient results, which show that the fish is not by any chance inbreeding, in fact a few loci show that the species is outbreeding. However more studies will have to confirm whether or not it has reached the state of outbreeding depression. According to Frankham and others, there are two ways to determine if a population is undergoing outbreeding depression (Frankham, Ballou, & Briscoe, 2002). One is to study the phenomenon using molecular markers (Coulson, et al., 1998) and the other is to cross the suspected populations (Lynch M. , 1991).

When investigating the variation among the populations, this study has shown significant differentiations among the locations studied. Impaired gene flow would be the logical explanation. This is caused, or eventually will lead to genetic drift (fixation and loss of alleles).

AMOVA results did indicate that the main source of variation is within species differentiation, rather than among populations, which is not what we had anticipated since F_{st} values were quite high. This goes to show that the high heterozygosity values as well as the extremely negative F_{is} values (some close to -1), could very well be a logical argument as to why the variation exist within the individuals as opposed to within populations. This probably means that in this particular case and using the loci that we have isolated, it is the high genetic variation that are caused by outcrossing individuals that are most likely genetically dissimilar, that is causing this genetic variability among individuals of our studied species. As a highly active fish this is rather acceptable if not predicted.

When summing up these results and looking at the genetic distance trees and structure plot. One cannot help but wonder whether the unusual uniform pattern of hybridizations that are found in three of the seven locations (which are spatially isolated from one another), are in fact a result of outbreeding. If so then the only logical explanation would be that human tempering could be involved. The movement of this fish for fishery and industrial purposes could be a logical cause to the genetic results.

Ancestral origins could also be a plausible explanation to the hybridizations observed in the structure of the seven populations. Seeing that the loci chosen were of long repeat motifs and short repeat numbers, makes them rather conserved and less prone to mutation.

It would be worth mentioning that the studied loci in this project were combined with five more loci that were isolated by a fellow colleague using the same method (4 of which were di- repeats and the fifth was a tetra- microsatellite marker). However the study was only conducted on six locations out of the seven (excluding Pahang), since sampling of that location was done much after the analysis.

When the combined data sets were analyzed, tree clusters were almost identical to that of the seven loci in this study and F_{is} values were still negative, however to a lesser extent (lower values). F_{st} values were also reduced, with the highest being between the Negeri Sembilan and Selangor populations at 0.23752. The lowest was recorded at 0.04078 between the Pahang and Selangor populations. STRUCTURE bar plot showed the same pattern of hybridization, except the Penang population, which not only showed matching hybridizations to the Selangor population, but also to the Terengganu population hybridization became more obvious. This in other words confirms the results of the current project. Furthermore pooled results will be included in a later publication.

After looking at all the genetic aspects that could be causing the decline in stocks, a disregard to the ecological and environmental habitats, would be of complete ignorance. There have been drastic deforestation and climate changes over the past decades that should be enough to alter mating habits and genetic disposition of the species around the peninsular.

The changes in the water ways, river basins and canals, to suite human needs such as: flood prevention, hydropower supply, agricultural irrigation systems, *etc.*; have altered tremendously in the cycles and paths of these freshwater sources, creating somewhat new ecosystems and surroundings with modified nutrients that might lead to mating of species that were geographically secluded (World Resources Institute, 2000). Dams are increasing in number; in a bid to collect water for human consumption, however this has altered the natural course and flow of the rivers, forcing the fish to mate in an unorthodox manner.

Also the climate change and global warming affects have a lot to do with stock populations of *Channa striata*. Studies show that there is an increase in rainfall (3%) and temperatures (0.3^{o}C) over past years (Salleh, 2009). This is bound to have an effect on the inhabitants of these areas.

Water pollution and use of pesticides might hinder the behavior of change the natural habitat and therefore survival of the species. An example of this is the Pahang river basin, whereby erosion is taking over the river basins in the area. Add to that high turbidity and acidity with very low pH (Khan, Lee, Cramphorn, & Zakaria-Ismail, 1996). Sewage and factory waste is also a cause of concern, since the later especially contains a high percentage of harmful chemicals (Kechik, 1992).

Over fishing and exploitation of the species also could lead to drastic changes in their population sizes. A statistical study showed that 7.7 billion metric tons of fish were caught from freshwater sources in 1997 around the world (World Resources Institute, 2000). A common practice in Malaysia is the use of cyanide, insecticides and dynamite to catch fish (Zakaria-Ismail M. , 1991). Although these actions have been halted by authorities, other illegal means of fishing are still on the rise, with blasting and electric shocks still being practiced despite all actions taken against them (Bernama , 2009; Simamora, 2009).

Therefore there are many players when it regards the decline in stocks. All aspects play an equal role in this devastation. Genetics merely is an indication of the physical impact that our current environmental situation has on the fish. However this study shows that this impact will not only leave its mark on the current generation of Haruan, but on the future progeny as well. Hence this is a situation that calls for all environmentalists, ecologists and conservation geneticists alike to handle the matter, before it escalades further. Predictions currently estimate by the year 2020, 50% of existing freshwater fish that have existed in the 80s and 90s would be declared extinct (Zakaria-Ismail M. , 1992).