

---

# Identifying stock structure of two *Henicorhynchus* species in the Mekong River using mitochondrial DNA

David A. HURWOOD\*, Eleanor A.S. ADAMSON and Peter B. MATHER

School of Natural Resource Sciences Queensland University of Technology

---

## ABSTRACT

A primary concern in wild fisheries is the identification of stocks for management. Many freshwater fish species exist as discrete populations where a lack of gene flow among local populations results in ecological and/or genetic differentiation due to the effects of random genetic drift and for adaptation to local environments. Successful sustainable harvesting of a wild fishery requires management plans specifically designed that consider the stock structure of the target species. Ecological approaches (e.g. tagging studies) to identifying discrete stocks where they exist are, at best, inferential because they cannot confirm whether dispersing individuals contribute genes to the receiving population. The application of population genetic techniques can be an effective approach for assessing population structure because estimates of gene flow can give a measure of the movement of individuals among locations. The current ACIAR/MRC project used mitochondrial DNA (mtDNA) analysis to determine the stock structure of two commercially important cyprinid species (*Henicorhynchus siamensis* and *H. lobatus*) in the MRB. Analysis of *H. siamensis* from the lower MRB showed high levels of genetic diversity and indicated that the samples represent a single panmictic population for management purposes. Although mtDNA haplotypes were shared among sampling sites both above and below the Khone Falls, the data suggest that gene flow (dispersal) occurs predominantly downstream (i.e. over the Khone Falls) resulting in significant population structure above and below the falls. In contrast, the *H. lobatus* analysis revealed extremely low levels of genetic diversity, with no differentiation from Chau Doc in Viet Nam to Nong Khai in northern Thailand. However, the sample from Ubon Ratchathani (just upstream of the Khone Falls) represents a highly divergent *H. lobatus* population from other Mekong samples. These results suggest that *H. siamensis* and *H. lobatus* have experienced vastly different demographic histories and consequently may display very different ecologies. While previous morphological studies have suggested that these two species are closely related, genetic data reveals levels of divergence among the species more consistent with that seen among different genera of cyprinids. These data highlight the need for development of different strategies to achieve sustainable management of both species.

KEY WORDS: Mekong; Stock structure; DNA; *Henicorhynchus* sp.

## INTRODUCTION

Many species exist as a collection of independently breeding units (populations) across the range of their natural habitat. This occurs where dispersal among locations is insufficient to allow random mating across the species entire range. A knowledge of spatial distribution of individuals that interbreed (population structure) is of particular importance for taxa that require management for conservation and/or commercial considerations. Successful management of a species must reflect its underlying biological processes and, as such, the level of population (or stock) is the scale at which management should be focused. Understanding population structure allows predictions to be made regarding the likelihood of natural recolonisation of a local stock following stock depletion due to environmental disturbance, either natural or anthropogenically generated (e.g. overharvesting of wild stocks).

---

\* School of Natural Resource Sciences Queensland University of Technology 2 George Street, Brisbane Queensland, 4001 Australia  
Email: d.hurwood@qut.edu.au

Species found in freshwater systems are often structured spatially, because unlike in marine systems, there are many barriers to effective dispersal among locations (Gyllensten, 1985; Ward *et al.*, 1994). Firstly, river drainages are isolated from each other by terrestrial and marine environments, therefore fully aquatic species find dispersal among drainages difficult. Secondly, there are several factors, either environmental (extrinsic) or biological (intrinsic), that can limit dispersal within a drainage. Environmental factors include instream barriers (e.g. waterfalls, rapids or impoundments) and the unidirectional flow of the river. Furthermore in large systems, even physical distance among sites may present a significant barrier to dispersal across the whole system. Intrinsic biological attributes means that some species may be inherently poor dispersers, are territorial, or are extremely habitat specific, all of which may limit dispersal over a wide area.

Identifying stock structure in freshwater fishes where it is present using traditional field assessment methods is often difficult if not impossible. It is not easy to recapture tagged individuals and direct observational studies of individual movements are generally not practical. Moreover, even if direct methods can measure levels of dispersal, they may not identify 'real' stocks as they do not provide any insight into whether dispersing individuals are entering the breeding population. Application of population genetic techniques can address some of these difficulties. The basic concept is that with high levels of dispersal and subsequent interbreeding (gene flow), populations become similar genetically (genetically homogeneous). Conversely, where there is minimal gene flow, populations will tend to become genetically differentiated due to the effects of chance (random genetic drift) and at some loci natural selection favouring different genes in different local environments. Measuring the genetic variation that is partitioned among locations provides an insight into the level of connectivity (effective dispersal) and assists with the delineation of 'real' stocks.

The primary aim of the present study was to demonstrate the utility of a molecular (DNA-based) approach for defining management units for fisheries in the Mekong River Basin (MRB). The species chosen for the study were two cyprinids (*Henicorhynchus siamensis* and *H. lobatus*) that are generally considered to be some of the most abundant species in the MRB (Roberts and Baird, 1995) and form a major component of the wild fisheries in the Mekong River. As with many important species in the Mekong River, relatively little is known about their biologies and what is known has largely been inferred from anecdotal evidence. It is well documented that both species undertake mass migrations during the wet season each year, but little if any knowledge exists as to whether populations across the system are homogeneous or structured in some way. This knowledge is fundamental to developing effective stock management practices for both species in the future.

Furthermore, due to their similarities in both morphology (they are often misidentified (Roberts, 1997)) and fluctuations in relative abundances over the annual cycle, it suggests that a single management strategy will provide a sustainable fishery for both species. While the population structure for both species is expected to be complex, current taxonomic uncertainty has resulted in the grouping of *H. siamensis* and *H. lobatus* (and other *Henicorhynchus* species) together for

the purposes of ecological description (MRC, 2003). It is a secondary aim therefore to identify and highlight differences in the patterns of variation among the species that are inferred from the genetic data.



Figure 1. *Henicorhynchus siamensis* and *H. lobatus*

## METHODS

### *Sampling and DNA analysis*

Samples of both *H. siamensis* and *H. lobatus* were collected from the Mekong River, from Viet Nam to northern Thailand, and from Tonle Sap Lake also in the Mekong drainage. Additional samples were collected from the Mae Klong and Chao Phraya catchments in Thailand for the purpose of placing levels of genetic divergence within the Mekong system into broader context (see Figure 2; Table 1).

### *Statistical Analysis*

The relationship among unique mtDNA genotypes (haplotypes) was determined by constructing a haplotype network for each species using the TCS program (Clement *et al.*, 2000) which gives a visual representation of the level of divergence among unique haplotypes (Figure 3a,b). The level of genetic relatedness among haplotypes is indicated by the number of lines separating nodes (unique haplotypes) in the network. Each line represents a single base pair (bp) mutation.

Stock structure was determined using a simulated annealing approach applied to the analysis of molecular variance using the SAMOVA program (Dupanloup *et al.*, 2002). This method groups sampling sites together so as to simultaneously minimise the component of total genetic variation within groups ( $F_{SC}$ ; among sampling sites within stock variation) while maximising the component of genetic differentiation among groups ( $F_{CT}$ ; among stock variation).

Table 1: Sampling sites and sample sizes for *H. siamensis* and *H. lobatus* used in the current study. Sites from tributaries of the Mekong are identified (TS = Tonle Sap; Mun = Mun River)

Site Name	Country and Drainage	Abbrev.	Sample Size	
			<i>H. siamensis</i>	<i>H. lobatus</i>
Chau Doc	Viet Nam - Mekong	CD	17	12
Phnom Penh	Cambodia - Mekong	PP	15	
Kampong Chhnang	Cambodia – Mekong (TS)	KC	11	2
Pursat	Cambodia – Mekong (TS)	PS		23
Battambang	Cambodia – Mekong (TS)	BB	21	
Siem Reap	Cambodia – Mekong (TS)	SR	5	1
Kampong Thom	Cambodia – Mekong (TS)	KT	20	
Stung Treng	Cambodia – Mekong	ST	17	7
Ubon Ratchathani	Thailand – Mekong (Mun)	UB	4	9
Mun/Mekong Conf.	Thailand – Mekong (Mun)	MR	40	
Nongkhai	Thailand – Mekong	NK	7	8
Chainard	Thailand – Chao Phraya	CH		6
Kanchanaburi	Thailand – MaeKlong	KB	10	

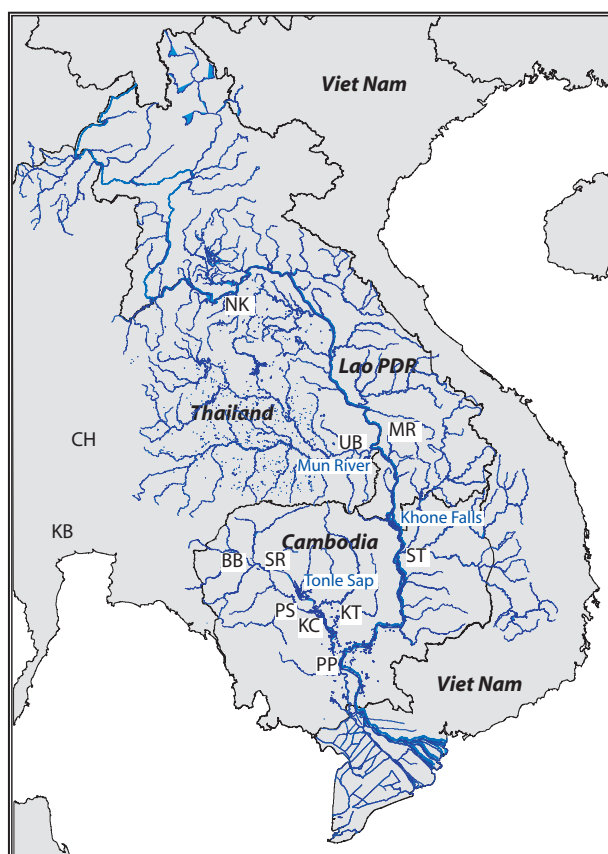


Figure 2. Map showing sampling sites for the present study.

DNA was extracted from fin tissue and a partial sequence of the *ATPase 6* and *ATPase 8* mitochondrial genes (mtDNA) was amplified using polymerase chain reaction (PCR). The resulting mtDNA fragment was directly sequenced resulting in a 630 base pair fragment used for further analysis.

The genetic relationship between *H. siamensis* and *H. lobatus* was resolved by reconstructing the phylogeny of the two species in combination with several other Cyprinids using the neighbour joining method (Saito and Nei, 1987) in the program MEGA2 (version 2.1) (Kumar *et al.*, 2001). Corrected distance was determined using the (Tamura and Nei, 1993) genetic distance model. The reliability of the phylogeny was determined with 500 bootstrap replicates.

## RESULTS

### *H. siamensis*—Genetic Variation and Stock Structure

DNA analysis of the 167 *H. siamensis* sampled for this study revealed 59 unique haplotypes with most haplotypes separated by a single or two bp differences among nearest neighbours in the haplotype network (Figure 3a). The analysis for *H. siamensis* revealed a relatively high haplotypic diversity of 0.35.

While the geographic distribution of these haplotypes is complex (i.e. several haplotypes are widespread while many others are confined to a single site), the SAMOVA analysis arranged the sampling sites into three groups (stocks) with approximately 22.5% of the genetic variation proportioned among these groups ( $F_{SC} = 0.010$ ,  $p = 0.410$ ;  $F_{CT} = 0.223$ ,  $p = 0.002$ ). The three groups were: 1) all sites below the Khone Falls (CD, PP, KC, BB, SR, KT, ST); 2) sites immediately above the Khone Falls (UB, MR); and 3) Nongkhai in the MRB and Kanchanaburi in the Mae Klong River (NK, KB).

### *H. lobatus*—Genetic Variation and Stock Structure

DNA analysis of the 68 *H. lobatus* sampled in this study revealed only 16 unique haplotypes (Figure 3b). While the sample size for *H. lobatus* was smaller than for *H. siamensis*, even so the level of genetic diversity at 0.026 was an order of magnitude lower than that seen for *H. siamensis*. Furthermore the degree of genetic divergence among haplotypes in the network was considerably greater.

The SAMOVA analysis identified three groups with approximately 99% of the genetic variation evident among groups ( $F_{SC} < 0.001$ ,  $p = 0.596$ ;  $F_{CT} = 0.985$ ,  $p < 0.001$ ). The three groups identified were: 1) all sites below the Khone Falls and the major channel of the Mekong River as far north as Nongkhai (CD, KC, PS, SR, ST, NK); 2) Ubon Ratchathani in the Mun River above the Pak Mun Dam (UB); and 3) Chainard in the Chao Phraya drainage (CH).

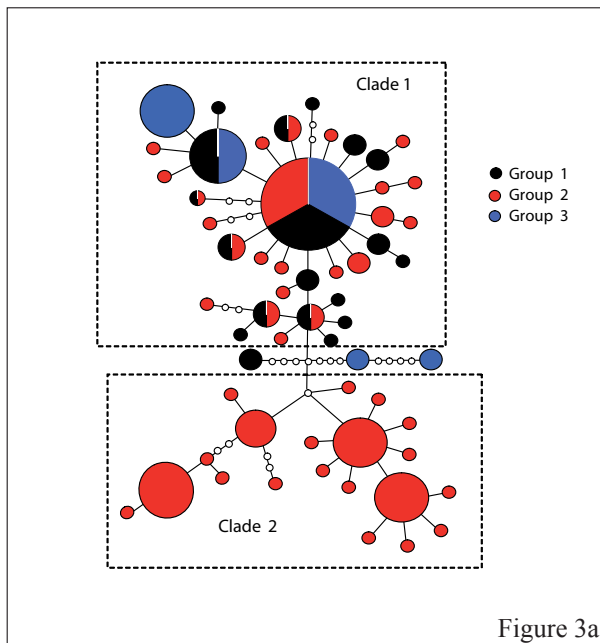


Figure 3a

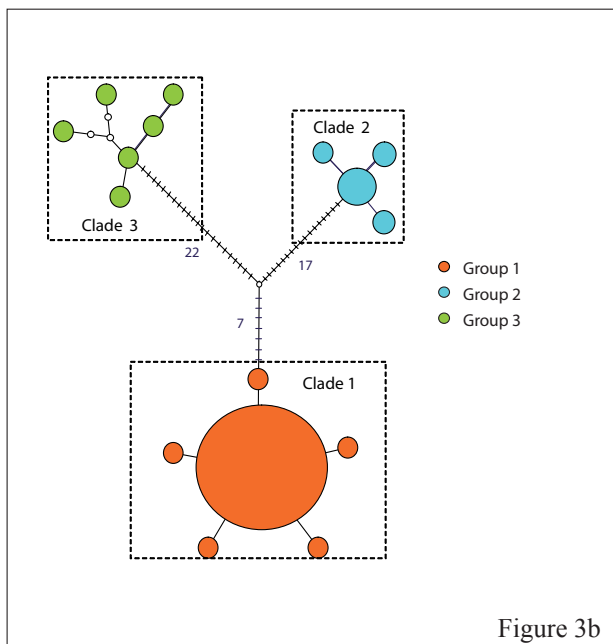


Figure 3b

Figure 3. Parsimony networks for (a) *H. siamensis* and (b) *H. lobatus*. Circles represent unique haplotypes, the size of the circle indicates the frequency of the haplotype in the total sample and the shading of the circles represents the group (i.e. groups of sampling sites that are genetically differentiated from each other as identified in the SAMOVA analysis) from which it was detected. Small open circles represent hypothesised haplotypes that were not detected in the sample. Lines joining neighbouring haplotypes represent a single base pair mutation. The dotted lines indicate clusters of haplotypes that are closely related to each other (clades). The hash marks in 3(b) represent numbers of base pair mutations among clades (total number of mutations are given).



Phylogenetic Relationship of *H. siamensis* and *H. lobatus*

Although *H. siamensis* and *H. lobatus* present as a monophyletic clade, the phylogenetic reconstruction of cyprinid species (Figure 4) clearly shows that the two *Henicorhynchus* species are not closely related and significant divergence is evident between the two species. The corrected distance of approximately 15% is the level of divergence that is more commonly detected among genera in this family based on the currently accepted systematics rather than among species within a genus.

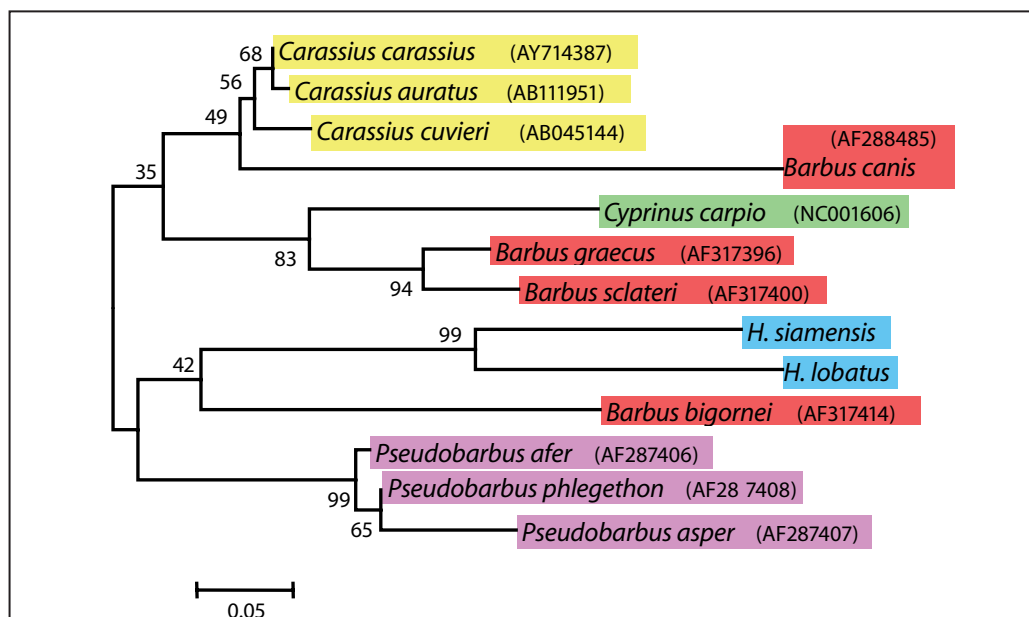


Figure 4: Neighbour joining tree depicting the relationship between *H. siamensis* and *H. lobatus* along with divergence levels among other Cyprinids. Bootstrap values represent the percentage of times a particular node was supported through 500 replicates. The scale bar represents 5% divergence. Colour coding highlights species from the same genus. (GenBank accession numbers are given for each species).

DISCUSSION

*H. siamensis* and *H. lobatus* are two of the most abundant and widespread fishes in the Mekong River Basin and represent a significant proportion of the annual catch. As they are difficult to distinguish morphologically and appear in large numbers at roughly the same time of year in the same locations, it is not surprising that they are generally grouped together. In fact they share the same common names respectively: *trey riel* in Cambodia; *ca linh* in Viet Nam; and *pla soi* in Thailand and *pa soi* in Lao PDR. Yet the results of this study clearly indicate that the two species are significantly different in several respects.

Firstly, they are not genetically closely related, no more so than what is expected among species from different genera in this family. The fact that they shared a common ancestor millions of

years ago suggests that they have been evolving independently for an extensive period of time and that they most likely have very different ecologies and perhaps different life history traits. This is highlighted by the fact that, while the analysis of the distribution of genetic variation across the sampled range revealed three groups or stocks for both species, the individual stock structures of *H. siamensis* and *H. lobatus* are markedly different.

#### *H. siamensis*—Stock Structure

The greatest amount of genetic variation was detected in the lower MRB. This is a common occurrence in freshwater systems due to unidirectional water flow. The pattern emerges because it is more difficult for new haplotypes arising downstream to disperse against the current than it is for new upstream haplotypes to move downstream with the water flow. *H. siamensis* shows a break above and below the Khone Falls, as may be expected if the falls has acted as an instream barrier to upstream dispersal. It is evident that there is some downstream dispersal (indicated by the sharing of Clade 1 above and below the falls; Figure 3a). Whether the downstream movement is part of the migration cycle or alternatively is the result of passive dispersal (i.e. moving downstream with the current) of larval or juvenile individuals from spawning events above the Khone Falls cannot be determined with the genetic marker used in the present study. There is a possibility that what appears to be a genetically diverse panmictic stock in the lower MRB is actually two sympatric but non-interbreeding stocks. A similar scenario has been recently detected for the sutchi catfish (*Pangasianodon hypophthalmus*) in the Mekong (So *et al.*, 2006) using nuclear markers. The data presented here however, suggest clearly that dispersal upstream beyond the Khone Falls is limited, if not absent (Clade 2 is found solely in the lower Mekong River), or alternatively, only individuals from Clade 1 are traversing the falls.

Interestingly, the third group contained sampling sites from Nongkhai in northern Thailand and the sample from Kanchanaburi (in the Mae Klong River system). These fish are not only geographically distant but inhabit discrete drainage systems currently totally isolated from each other with no freshwater avenues of connectivity for potential dispersal. These data suggest that either at some time in the recent evolutionary past (historically) there has been some dispersal among the drainage systems, or alternatively, fish have been actively translocated among drainage basins. Further sampling and analyses are required to resolve this question.

#### *H. lobatus*—Stock Structure

Compared to *H. siamensis*, *H. lobatus* shows a much more structured population. Group 1 (i.e. the whole of the Mekong and Tonle Sap) shows widespread movement, at least across the range of the study to date. However, due to the substantially lower level of genetic variation observed for this mtDNA marker and the smaller sample size for *H. lobatus*, there is insufficient power to confidently state whether extensive gene flow occurs at this geographic scale. In contrast to *H. siamensis*, there is no evidence to suggest that the Khone Falls acts as a barrier to dispersal for *H. lobatus*. It is



interesting to note, that reports based on observational data suggests that while both species move upstream (beyond the Khone Falls) during migrations at the end of the wet season (Baird *et al.*, 2003), far greater numbers of *H. lobatus* (up to 10 times more than *H. siamensis*) attempt to traverse the falls. The genetic data in the present study support these observations, suggesting that the Khone Falls may not act as an instream barrier for either species, but rather the higher level of genetic differentiation seen for *H. siamensis* above and below the falls is a function of the species migratory behaviour.

The most striking aspect of the genetic pattern for *H. lobatus* is the considerable level of divergence among clades in the network (Figure 3b). The level of divergence found between Chainard in the Chao Phraya drainage (Clade 3) and other sampling sites in the Mekong River is what might be expected from freshwater fishes from isolated river catchments. That is, a relatively high level of genetic divergence among drainages with reciprocal monophyly (all individuals within a drainage are more closely related to each other than to any individual from a neighbouring drainage). However, the divergence of the samples from the Mun River and all other samples from the Mekong River is difficult to explain as there appears to be no significant barrier to dispersal between the Mun and Mekong Rivers. Although the Pak Mun Dam (completed in 1994) represents a significant barrier to upstream dispersal, the time of its construction is not concordant with the divergence levels detected in this study. However, the construction of the dam led to the flooding of significant rapids which may have acted as a natural barrier for a considerable length of time in the past.

Naturally the small sample from the Mun River prevents making conclusions regarding whether the Mekong group (Group 1, Clade 1) are present in the Mun River or not. However it appears that the Mun River fish do not disperse to the main channel of the Mekong suggesting that the full life cycle including associated migrations occurs solely within the Mun River for this stock.

## IMPLICATIONS FOR MANAGEMENT

Although *H. siamensis* and *H. lobatus* are numerous and widespread throughout the Mekong and surrounding drainages, their economic importance to the region will necessitate management to allow continued sustainable harvesting. The results of the present study strongly indicate that, although both species appear similar in some respects, management strategies should be developed for each species separately.

Analysis of *H. siamensis* revealed a high level of genetic variation with three independent stocks in the Mekong River (one in northern Thailand, and one each above and below the Khone Falls, although two discrete stocks may exist below the falls). As a result, these data have provided the spatial scale at which management should be focussed for this species.

In contrast, the analysis for *H. lobatus* shows a very different picture. Firstly, the level of genetic variation present for this species is extremely low, especially considering the current abundance

of this species in the MRB. These results suggest that the population as a whole has undergone a dramatic reduction in size in the recent evolutionary past. As a consequence, the reduced level of variation may compromise the 'genetic health' of the population (it is generally accepted that low variation is associated with low evolutionary potential) and thus may affect its long term viability. Effort should be directed to ensuring the level of genetic variation that currently exists for this species is not eroded further.

Secondly, the highly divergent clade detected in the Mun River suggests that this stock has been evolving independently from the Mekong stock for a considerable length of time. Thus from a management perspective, the data suggests that it would be unwise to mix these two stocks as there is a potential for outbreeding depression to occur or one of the stocks could go extinct. Outbreeding depression is essentially where the resulting hybrid offspring of genetically divergent parents have a lower fitness than both parental stocks. This may be due to the disruption of co-evolved gene complexes or by driving locally adapted phenotypes from their optimum.

The data presented here, which are the result of an ongoing study, highlight the utility of a molecular approach to stock delineation for fisheries management. Analysis at a finer spatial scale is currently being undertaken to address some of the questions raised here. Furthermore, it should be stressed that the outcomes of this study should be used in conjunction with ecological data when formulating management plans in the future.

#### REFERENCES

- Baird, I.G., Flaherty, M.S., Phylavanh, B., 2003. Rythms of the river: lunar phases and migrations of small carp (Cyprinidae) in the Mekong River. *Nat. Hist. Bull. Siam Soc.* 51, 5-36.
- Clement, M., Posada, D., Crandall, K.A., 2000. TCS: a computer program to estimate gene genealogies. *Mol. Ecol.* 9, 1657-1659.
- Dupanloup, I., Schneider, S., Excoffier, L., 2002. A simulated annealing approach to define the genetic structure of populations. *Mol. Ecol.* 11, 2571-2581.
- Gyllensten, U., 1985. The genetic structure of fish: differences in the intraspecific distribution of biochemical genetic variation between marine, anadromous, and freshwater species. *J. Fish Biol.* 26, 691-699.
- Kumar, S., Tamura, K., Jakobsen, I.B., Nei, M., 2001. MEGA2: Molecular Evolutionary Genetics Analysis software. Arizona State University, Arizona, USA, Tempe.
- MRC, 2003. Fish Migrations in the Mekong River basin: Interactive CD-ROM.
- Roberts, T.R., 1997. Systematic revision of the tropical Asian labeoin cyprinid fish genus *Cirrhinus*, with descriptions of new species and biological observations on *C. lobatus*. *Nat. Hist. Bull. Siam Soc.* 45, 171-203.

- Roberts, T. R., Baird I.G., 1995. Traditional fisheries and fish ecology on the Mekong River at Khone Waterfalls in southern Laos. *Natural History Bulletin Siam Society*, 43: 219-262.
- Saito, M., Nei, M., 1987. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406-425.
- So, N., Maes, G.E., Volckaert, F.A.M., 2006. High genetic diversity in cryptic populations of the migratory sutchi catfish *Pangasianodon hypophthalmus* in the Mekong River. *Heredity* 96, 166-174.
- Tamura, K., Nei, M., 1993. Estimation of the number of nucleotide substitutions in the control region of mitochondrial DNA in humans and chimpanzees. *Mol. Biol. Evol.* 10, 512-526.
- Ward, R.D., Woodwark, M., Skibinski, D.O.F., 1994. A comparison of genetic diversity levels in marine, freshwater, and anadromous fishes. *J. Fish Biol.* 44, 213-232.