Artificial propagation of empurau, *Tor tambroides* and semah, *Tor douronensis,* two species of commercial and conservation value to Sarawak, Malaysia

Guidelines for genetic management and conservation

SEPTEMBER 2007

Acknowledgements

Preparation of this material was coordinated by Prof. Sena De Silva, Director General of the Network of Aquaculture Centre in Asia-Pacific (NACA), Honorary Professor of Deakin Univeristy, Australia with assistance from other Project Team members: Dr. Thuy T. T. Nguyen and Dr. Sih Yang Sim of NACA, Dr. Brett Ingram and Mr. Geoff Gooley of the Primary Industry Research, Victoria (PIRVic), Australia, Dr. Stephen Sungan and Mr. David Tinggi of the Indigenous Fisheries Research and Production Centre (IFRPC), Sarawak, Malaysia.

This material was produced as part of the report for the project *Artificial Propagation of empurau, Tor tambroides and semah, T. douronensis, two species of commercial and conservation value to Sarawak, Malaysia*; funded by the State Government of Sarawak.

Special thanks are due to the former Minister of Agriculture and Food Industries, State Government of Sarawak, Dato Sri Haji Adenan Haji Satem; - the Director of Agriculture, Datu Hj Mohd. Sepuan Bin Hj Anu, for their continued interest and support; Mr. Joseph Wei - the Head of Inland Fisheries Division, Department of Agriculture, and Mr. Boniface Anat Litis. A special thank to Mr. Chong Ee Hook the former Head of Inland Fisheries Division for initiating this project. The help of all staff at the IFRPC, Tarat is also gratefully acknowledged. Finally, our thanks are also due to Prof. Uthairat Na-Nakorn for permitting the use of the facilities of the Genetics Laboratory, Faculty of Fisheries, Kasetsart University, Bangkok, Thailand, and Ms. Srijanya Sukmanomon for technical assistance. Kind help from Dr. Helen McPartlan, Dr. Mathew Baranski and Ms. Meghan Rourke from the Animal Genetics and Genomics Platform, PIRVic, Australia in microsatellite techniques as well as assistance for sample collection from Peninsular Malaysia and Sabah, from Mr. Ng Chee Kiat and Mr. Jephrin Wong, are gratefully acknowledged.

The project team: From left – Dr. Sih Yang Sim, Dr. Thuy Nguyen, Dr. Brett Ingram, Prof. Sena De Silva, Mr. Geoff Gooley, Mr. David Tinggi, Dr. Stephen Sungan

Table of contents

List of Tables and Figures

1. Introduction

The mahseer species, *Tor tambroides* and *T. douronensis*, are often referred to as *empurau* and *semah*, respectively in Sarawak, Malaysia. The two species are indigenous to the State with an aquaculture potential and of conservational value. *Empurau* and *semah* are well sought after due to high market value as well as being attractive sport fish. *Semah* is considered the State Fish of Sarawak, and juveniles of both species are also increasingly sought after by the aquarium industry (Ng, 2004). *Semah* are found in most major river systems of Sarawak, while *empurau* are restricted to some rivers, and both species often inhabit upper reaches of the headwaters. They also occur in Peninsular Malaysia and are distributed throughout southeast Asia from Indonesia to southern China (Kottelat *et al.*, 1993, Roberts, 1999, Zhou and Cui, 1996).

This document presents:

1. Current status on genetic diversity of *empurau* and *semah* in Sarawak, Malaysia; including taxonomic status;
2. A management guideline based on genetic data.

Many anthropogenic activities, including the recent developments in watersheds within the natural distribution of *empurau* and *semah*, as well as increased fishing pressure have led to depletion of their natural stocks. As such there is an urgent need to replenish such depleted stock as well as reducing pressure that affects the well being of natural populations of *empurau* and *semah*.

The Government of Sarawak, recognizing the importance of these two species, made an attempt to evaluate their aquaculture potential, including captive breeding using long-term pond-reared broodstock, commencing in the 1990s. However, limited success was achieved until the period 2002-2004 through an international collaboration, where researchers from Australia and Sarawak were able to breed both species using hormone induction techniques, popularly referred to as hypophysation, on long-term, pond-reared broodstock. Success in artificial propagation of *empurau* and *semah* would bring about significant developments in term of aquaculture and conservation. On the one hand, fish produced from aquaculture can be used to replenish the wild stocks – the practice often known as stock enhancement, and on the other hand, fishing for food fish will also be reduced due to the availability of cultured fish.

However, it is important to note that aquaculture and stock enhancement could be counter-productive if genetic aspects of broodstock management are not taken into account or broodstock are not properly managed. Detrimental genetic impacts of poorly or inappropriately managed fish breeding programs for both aquaculture and stock enhancement have well documented over the last two decades (Waples, 1991). When fish are removed from the natural environment and placed in a cultured environment and domesticated, random genetic drift and domestication effects (new and greatly different selective forces act upon fish in the domestic environment compared to the natural environment) alter the gene frequencies and reduce genetic variation. Domestication reduces genetic variability in fish through both selective processes and random genetic drift. Such fish once released in to the natural waters could have potential impacts on altering or diluting natural gene pools, and such events have been documented for many species.

Hybridization between closely related species can have a detrimental affect on natural gene pools. Interspecific hybridization among other mahseer elsewhere has been reported. Because of the high level of morphological similarity between *empurau* and *semah* there is risk that inadvertent mixing of the two species, especially during breeding, may lead to hybridization. Therefore hybridization is an important threat to the genetic integrity of both species.

In order to avoid the above mentioned potential problems, it is crucial that a genetic management plan be developed with the aim to warrant the long-term maintenance of genetic diversity of cultured stocks, as well as to minimize potential adverse effects on the genetic integrity of the wild populations through proper stock enhancement practices. Surveys on current status of genetic variability of *empurau* and *semah* are reported herein, and the results from which are used as baseline data for development of a genetic management plan.

Further, this document represents the first example in Asia of a comprehensive genetic management plan that was developed at the inception of industry development and commercialization, and that takes into account both commercial aquaculture of fish species as well as the conservation and management of wild populations. As such it is imperative that the State Government of Sarawak publicises this exemplary event and brings it to the notice of the rest of Malaysia.

2. How would molecular genetic data help?

2.1. On systematics

Systematics is the study of the kinds and diversity of organisms and the relationships among them. It encompasses the description, classification and naming of organisms (known as taxonomy), as well as reconstruction of their evolutionary history (known as phylogenetics).

Morphological comparison has been the major method used for recognising, describing and classifying new species, but this approach is hampered by several drawbacks. First, morphological characters can be plastic or subject to convergent evolution. This plasticity of morphological characteristics has the potential to lead to errors in classification, and flawed inferences concerning evolutionary histories. The use of molecular based methods in taxonomy has allowed a more complete understanding of systematic relationships, and provides an alternative view of evolutionary history.

Figure 1. *Empurau* (bottom) and *semah* (top) look very similar in terms of external characters

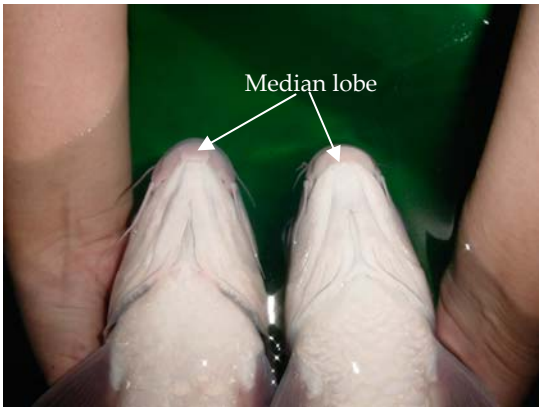

Figure 2. Median lobe – the main character used to distinguish *empurau* and *semah*, is more developed in *empurau* (left) and shorter in *semah* (right).

Taxonomic status of species within the genus *Tor* has been highly contentious, due to plasticity of many external morphological features. Low levels of differentiation between some species plus high levels of variation within a species in terms of morphological characters such as shape and colour make it difficult if not impossible to construct unambiguous identification keys for species (see Figure 1 and Figure 2). The natives, particularly the Ibans, reported about 3-4 varieties each of *empurau* and *semah*. The shape, size and length of the median lobe, the features that have often been used to distinguish species of *Tor* (Zhou and Cui, 1996), are highly variable (Roberts, 1999). In addition, the median lobe structure is also influenced by environmental factors, leading to confusion and as such its reliability as an indicator of species is questionable (Ng, 2004). In this regard, molecular data, in particular the mitochondrial DNA (mtDNA) sequences were used to genetically identify *empurau* and *semah*, and to study genetic relationships between the two species, as well as with other species in the mahseer group.

2.2. *On population structure*

Population genetics encompasses the description of the distribution of genetic variation within and between populations, thereby providing indirect information on population isolation or structure. More broadly, population genetics provides information on genetic diversity that can be used to measure levels of inbreeding, gene flow, population subdivision, and migration rates within and between populations.

Molecular genetics has proven useful in assessing the extent and patterns of population subdivision as well as for investigation of the forces that change population structure. The general advantages of molecular markers for the study of population structure are their ready availability, genetic simplicity, comparability across taxa, and ease of use with population genetic models. Various molecular genetic techniques have been employed to address population related issues, including allozyme electrophoresis and DNA-based markers, e.g. nucleotide data and microsatellite DNA which provide a source of highly polymorphic nuclear genes for the study of fine-scale population structure.

Devising methods of managing threatened species in order to maintain genetic variability requires the identification of evolutionary divergent populations, the estimation of genetic variability within and between populations and assessment of the conservation value of populations or areas from an evolutionary or phylogenetic perspective. Information about population structure can be obtained by determining levels of genetic diversity which can aid in the definition of management units. In the present context population genetic structure and genetic variability of *empurau* and *semah* were determined using mtDNA sequences and microsatellites.

3. Taxonomic status of empurau and semah

As mentioned earlier, the taxonomic status of the *mahseer* group is highly contentious. Distinguishing *empurau* and *semah* using external characters can be difficult. A study was conducted with an aim to better understand the genetic relationship between *empurau* and *semah*, and between these two species in Sarawak with other *Tor* species[1].

Attempts were made to collect samples of five *Tor* species, including *empurau* and *semah* from Sarawak, and five species of the closely related genus, *Neolissochilus* for genetic comparisons. Details on genetic analysis can be obtained from Nguyen (unpublished manuscript, Annex 1). Sampling localities are presented in Figure 3. Phylogenetic relationships amongst species were reconstructed based on nucleotide variation at three mitochondrial gene regions, and the results are summarized in Figure 4.

It can be seen from Figure 4 that *empurau* and *semah* are monophyletic and as such represent two separate species that can be identified using nucleotide sequences (up to 5.2% divergence). *Empurau* samples, though collected over a wide geographical range, showed very little variation, i.e. samples from Sarawak (Limbang River) are similar to those from Peninsular Malaysia (Kelantan and Nenggiri River in Kelantan, Lipis River in Pahang, and from Terrenganu, and samples from Yunnan, China; Jambi in Sumatra), as well as those from Thailand.

[1] This component utilised other funding sources and did not cost the project.

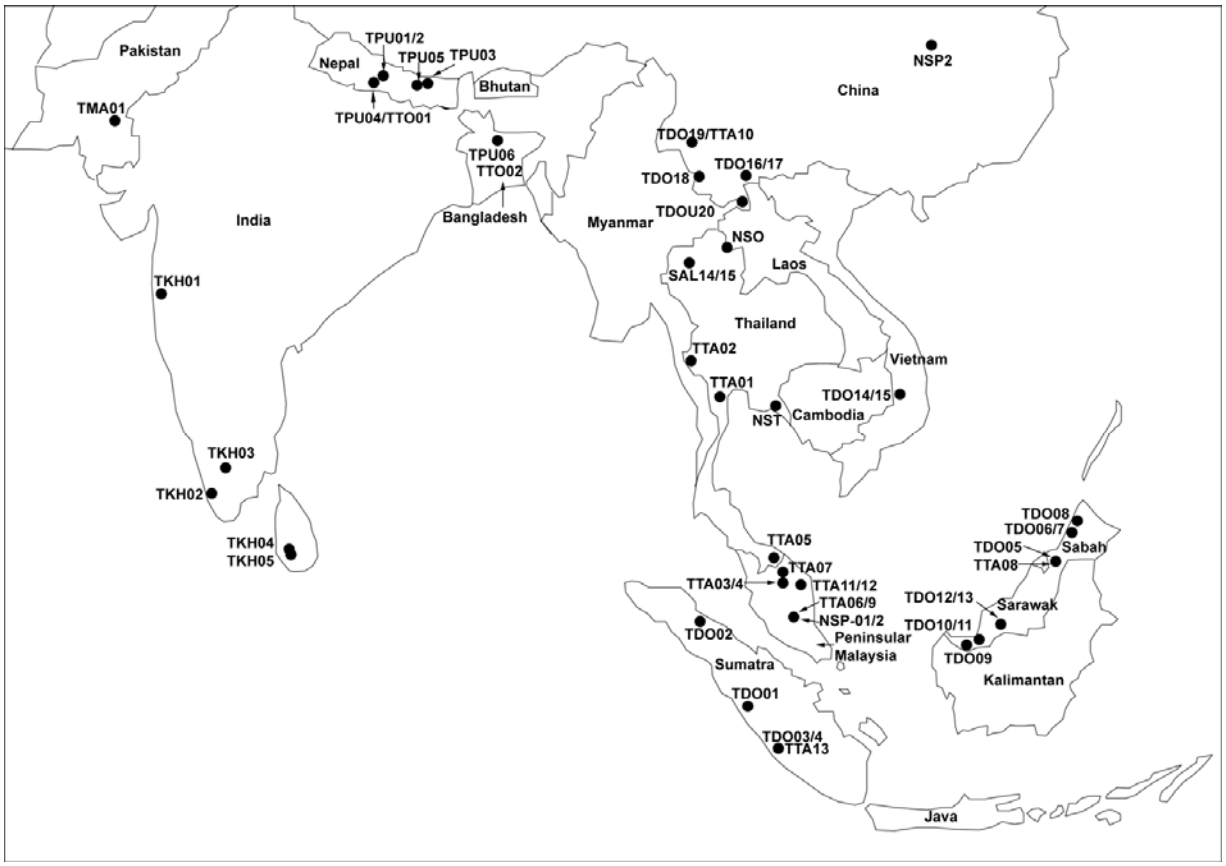

Figure 3. Sampling localities of mahseer used for phylogenetic analysis

Taxonomic status:

1. *Empurau* and *semah* are valid species with a high level of morphological similarity;
2. *Empurau* have little genetic variation throughout a wide geographical range;
3. There are three lineages of *semah* identified with high level of divergence: the Borneo Island, the Sumatra Island, and the Mekong River system. This suggests perhaps there are more than one species;
4. Within the Borneo Island, *semah* from Sabah are genetically different to *semah* from rivers in Sarawak.

In contrast to *empurau*, taxonomic status of *semah* is more complicated. Three genetic lineages of *semah* were observed – *Semah* samples from the Borneo Island are different to those of the Sumatra Island and the Mekong River System. Within the Borneo Island, two genetic forms of *semah* were found, representing samples from Sarawak (Limbang, Rejang, Layar and Bunnan rivers) and Sabah (Moyog and Wario rivers).

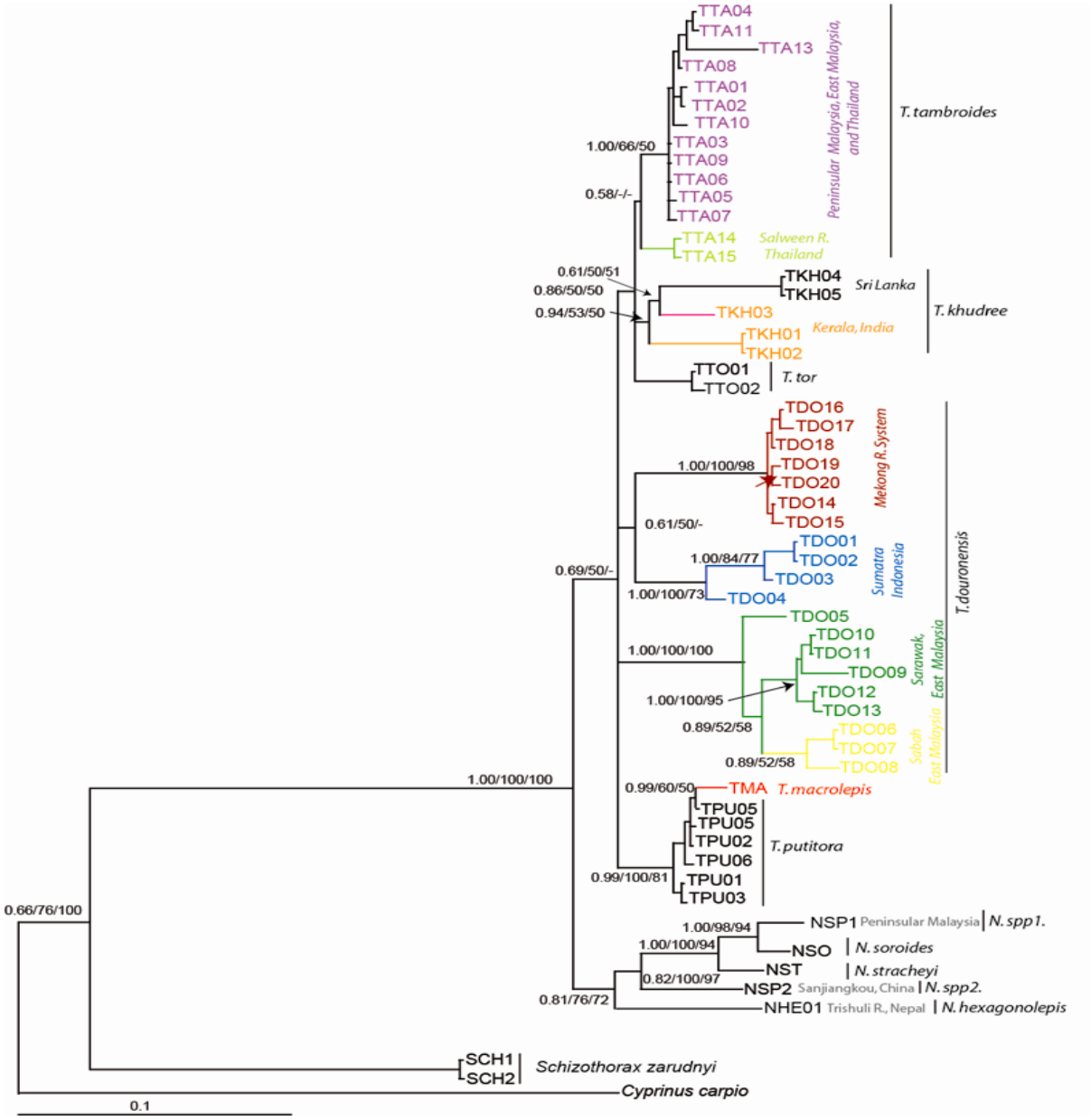

Figure 4. Phylogenetic relationships amongst 10 mahseer species based on nucleotide sequences of three mitochondrial gene regions

4. Genetic diversity of empurau and semah

Understanding levels of genetic variation and patterns of population subdivision would greatly benefit decision making processes with regard to identifying management and/or conservation units of species under concern. However, prior to the start of this project, this information was entirely lacking for *empurau* and *semah*.

Research has been undertaken as a part of the project to study genetic diversity of the two species and results were either published or submitted for publication (Nguyen *et al.*, 2007, Nguyen *et al.*, 2006, Nguyen, 2007) (see Annexes 2, 3 and 4). Information presented herein is largely extracted from these publications, and presented in a simplified manner.

4.1. *Empurau*

Broodstock samples of *empurau* were analysed using mtDNA and microsatellite markers. Majority of broodstock held at IFRPC (Tarat) came from Adang River, a tributary of Limbang River (179 individuals in 2004, held at Special Effect Pond 2 [SEP2] area), collected between 1989 and 1995; and a small number came from Indonesia (9 individuals, at Old Production Area [OPA]) with exact locality and time of collection unknown, and 18 individuals from Rejang River (collected between 1990 and 1993, maintained at OPA area).

Genetic diversity of *empurau* and recommended actions:

1. *Empurau* from Adang River, Indonesia and Rejang River have a relatively high level of genetic variation;
2. There are no significant genetic differences between *empurau* from the above localities;
3. A private haplotype was found in the Rejang *empurau* and as such it is recommended to maintain fish from this location separately.
4. Due to the low number and unknown origin of *empurau* from Indonesia held at the IFRPC, these fish should be removed from the centre to avoid possible genetic contamination with other stock.
5. All individuals of *empurau* from the Adang River maintained at IFRPC should be used for breeding in order to avoid genetic changes in captive stocks.

Analysis of mtDNA nucleotide sequences six unique haplotypes in all samples, with one haplotype dominant throughout all samples. One haplotype was only found in *empurau* from the Rejang river, with a frequency of 20%. Overall, *empurau* maintained in IFRPC have relatively high levels of within population variation but limited divergence among populations. However, the high percentage of a private haplotype observed in *empurau* from the Rejang River probably warrants the decision to keep this population separately from others. Furthermore, it is important to note that the isolation of small populations such as that from the Rejang River and from Indonesia may lead to subsequent effects of genetic drift and inbreeding, and it is recommended that fish from Indonesia should be removed in order to avoid genetic contamination if any, and more fish should be collected to supplement the present stock from the Rejang River to boost the breeding population so as to reduce inbreeding and genetic drift.

Further genetic analysis was undertaken using microsatellite markers. Similar to the results obtained from mtDNA sequences, a relatively high level of microsatellite variation was found in *empurau* broodstock from the Adang River held at IFRPC. Out of 14 loci screened, 10 were polymorphic with an average 2.86 alleles per locus. Deviation from Hardy-Weinberg equilibrium was found in one locus. It is recommended that all available individuals of *empurau* from the Adang River maintained at IFRPC be used for breeding in order to avoid genetic changes in captive stocks. More specific recommendations will be provided in later sections.

4.2. *Semah*

At the time this project commenced, *semah* broodstock held at IFRPC were mainly from the Adang River and Rejang River. Fish from the Adang River comprises two batches, one collected between 1990 and 1995 (220 individuals present in 2004) and another collected in 2000 (482 individuals present in 2004). The Rejang River stock had 45 individuals present in 2004. Between 2004 and 2006, attempts were made to collect samples from many other natural populations of *semah* for comparison purposes (see Figure 5 for details of these locations). Finclips from one stock of unknown origin which are currently held in AP4, were also analysed.

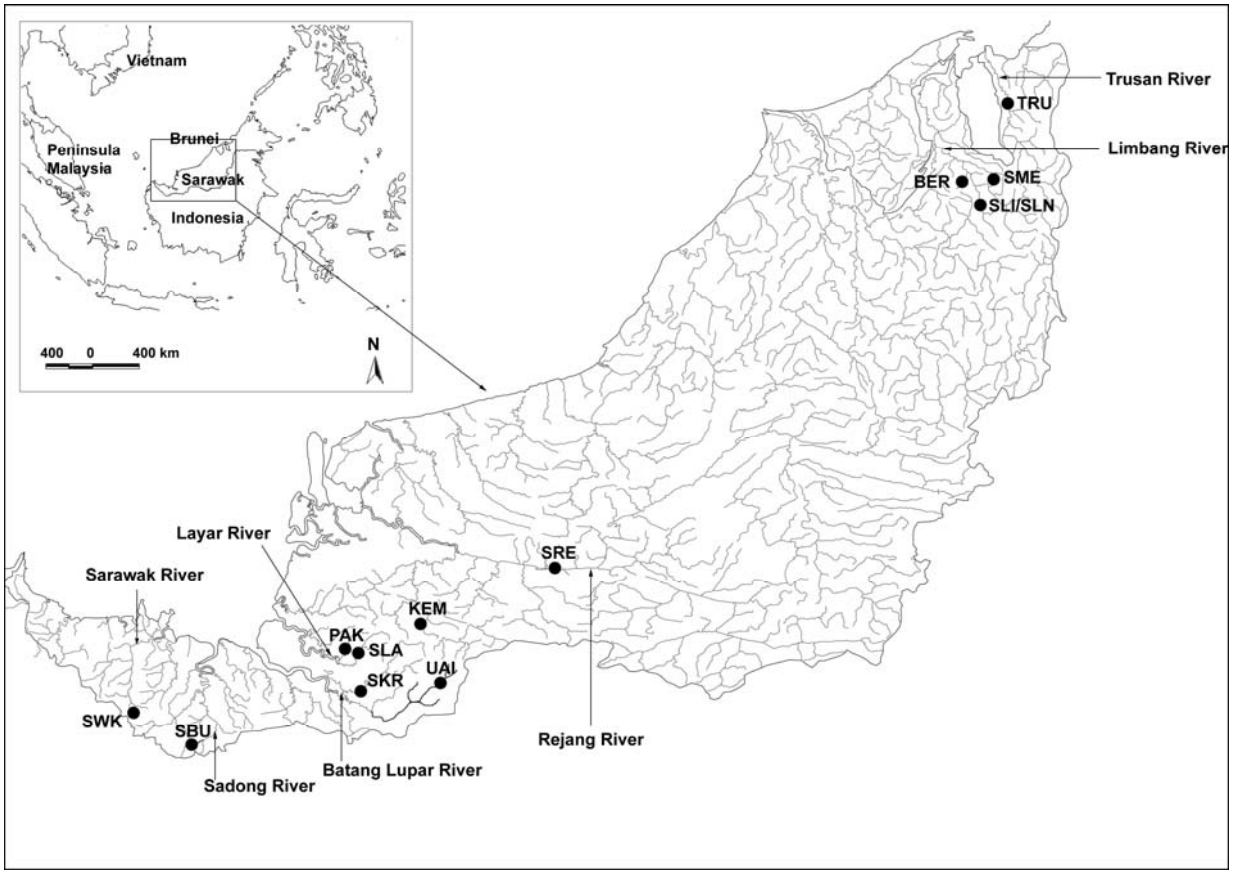

Figure 5. Localities from which *semah* samples were used for genetic analysis

Both mtDNA and microsatellite markers revealed deep subdivision of *semah* from the northeast and the southwest of Sarawak. The former includes those from the Limbang and Trusan river systems, and the latter includes samples from the Rejang, Layar, Batang Ai, Sadong and Sarawak river systems.

Further subdivision was observed within the northeastern cluster, i.e. significant genetic differentiation was found between populations from the Trusan River (TRU) and the Limbang River (BER, SME, SLI/SLN). Samples within the Limbang River showed no genetic differences, and the two batches (SLI and SLN) of *semah* collected from the Adang River also did not show any genetic difference.

Genetic diversity of *semah*

1. Deep population subdivision was found between the northeast and the southwest. Within each of these two clusters further subdivision was also observed.
   - Northeastern cluster
     - Trusan River
     - Limbang River
   - Southwestern cluster
     - Batang Lupar River
     - Sarawak River + Rejang River
     - Layar River
2. Samples with unknown origin currently held at the IFRPC are similar to those found in the Limbang River in the northeastern cluster.

Semah from the southwest are also further divided into three subdivisions, these being (1) Batang Lupar River (UAI and SKR); (2) Sarawak River (SWK) and the Kemalih branch and main stream of the Rejang River (KEM and SRE), and (3) Layar River. Samples from the Bunan River (SBU, Sadong River) appeared to be admixture of fish from the Trusan River, Layar River, and Sarawak River and/ or Rejang River. Reasons for this remain unknown and sample size of SBU is too small to warrant further interpretation.

Samples with unknown origin maintained at AP4, IFRPC fall well within the group of samples from the Limbang River.

5. Guidelines for genetic management and conservation

The economic importance of *empurau* and *semah*, their high commercial, recreational and conservational value necessitate proper management of fisheries for the species to ensure sustainability. A basic perquisite for managing biodiversity is the identification of population groups with independent evolutionary histories. The magnitude of genetic diversity and level of subdivision detected in the present project have significant implications for management and conservation of the two species in the long-term.

These guidelines are developed with an aim to ensure sustainable development of *empurau* and *semah* aquaculture in Sarawak, Malaysia; and at the same time to ensure the conservation of genetic diversity of the two species in the wild. More specifically, the guidelines provide recommendations for:

- Wild fisheries management and stock enhancement strategies – effects of hatchery-reared fish on wild counterparts and potential problems associated with mixing between stocks should be minimized.
- Aquaculture management – consider issues relating to broodstock genetics to avoid inbreeding and associated problems and possible genetic alteration in captivity.

There is a need for development of suitable management and conservation plans for *empurau* and *semah* because genetic variation and identity of wild populations are pivotal for species survival. Moreover, it is also important to note that irreversible biological damage may result from haphazard translocations and restocking.

5.1. *Management and conservation of wild populations*

5.1.1. Translocations

Translocation is the movement by humans of live aquatic organisms (including all stages of an organism's life cycle and any derived, viable genetic material) beyond their natural distribution, to areas that contain genetically distinct populations. Introduction of a different genetically distinct stock into a new environment may potentially result in replacement or dilution of the local stock.

Restocking of hatchery-produced fish into the natural environment also falls into the realm of translocation as it involves the movement of fish from one place to another. But in this document it is easier to separate the two activities, i.e. translocation referring to transferring fish from one wild population to another wild population, while restocking referring to stocking fish from a hatchery into natural waters, for conservation purposes or otherwise..

In the case of *semah*, there is strong evidence that there are at least five distinct stocks of this species present in different river systems in Sarawak. It is important to treat these stocks as separate management units, i.e. moving fish from one river system into another is not recommended as genetic integrity of the local population may be altered and/or replaced by the introduced one(s).

Movements of *empurau* or *semah* from one river system into another are not recommended, and could be detrimental to the genetic integrity of wild stock in the long-term.

As for *empurau*, there is no strong evidence of population subdivision, maybe because only a limited number of localities were examined, and small sample sizes due to lack of abundance. However, the presence of a different genetic form in the Rejang River but not in the Limbang River indicates that movements of *empurau* stocks between these two river systems are not recommended.

It is also important to note that translocation involves not only the target species (i.e. the fish being consigned and stocked), but organisms contained within the transport water (e.g. plankton, insects, amphibians, aquatic plants), organisms attached to the fish (e.g. parasites), or organisms within the fish (e.g. endoparasites). Some translocated aquatic organisms can create ecological problems that are extremely difficult to manage. These may involve competition for food and space, predation, habitat alteration, introduction of pathogens and diseases, which may result in reduction in the size of local populations, eventually leading to a reduction in genetic diversity through inbreeding and genetic drift in the long term.

5.1.2. Restocking

Breeding programs for conservation have different goals to those of commercial aquaculture programs. Fish stocked to replenish depleted populations may mix with existing populations and subsequently contribute to natural reproduction in the wild. Inbreeding and loss of genetic diversity are major concerns in conservation programs. Inbreeding has deleterious

effects on reproduction and survival, and loss of genetic diversity reduces the ability of populations to adapt in response to environmental change (Frankham *et al.*, 2002). The hatchery production of fish can cause the genetic hazards of extinction, loss of within population variation, loss of between-population variation and domestication (Miller and Kapuscinski, 2003). Consequently, conservation programs must endeavour to maximise variation in broodstock and progeny, and minimise effects of domestication. Captive breeding and restocking programs must ensure that the wild, endemic populations are not "swamped" by large numbers of fingerlings that are siblings or closely related, providing the opportunity for inbreeding and a rapid decrease in the reproductive fitness of the wild population.

In general, recommendations for management procedures for fish breeding programs for conservation and stock enhancement aim to maximise effective population size (N_e) – the number of broodstock that contribute genetic material to the next generation, minimise inbreeding depression and loss of genetic variation, reducing adaptation to captivity, preventing the swamping of the wild gene pool, and maintaining the genetic identity of the wild populations. These can be achieved through a number of techniques which are outlined in the box below.

Breeding for stock enhancement (restocking) of *empurau* and *semah* should ensure the following:

1. Use only F_1 offspring of wild broodstock for restocking,
2. Restocking should be undertaken within the region of broodstock origin,
3. All broodstock should be tagged. Broodstock from different origins should be kept separately. If there is limited space, they should be appropriately tagged if they are kept in the same pond/ tank,
4. Randomly collect and maintain a sufficient number of broodstock (at least 100 fish) for captive breeding ,
5. The male : female ratio of captive broodstock should be 1 : 1,
6. Minimise mortality in the hatchery,
7. Stock equal number of offspring from each breeding pair.
8. Ideally about 20% of broodstock should be replaced each year.
9. Return broodstock to the river and location where originally captured: ensure they are disease-free before restocking.

The breeding program for each species must be linked to a restocking program for each river/population to satisfy genetic guidelines. Fingerlings from a minimum of 10 pairs of broodstock must be stocked into each river over 5 consecutive years to maintain genetic variation and to achieve an N_e of 100. Fingerlings from 50 pairs of broodstock will achieve an N_e of 50 in 1 year. An N_e of 100 can be achieved by using the numbers of broodstock presented in Table 1.

Broodstock should be recruited on a regular basis. Ideally about 20% of broodstock should be replaced by wild caught fish each year. Due to the lack of abundance of *empurau* and *semah* in the wild, these could be done in 3-4 year period. Broodstock being replaced should be release back to location where they were originally captured. Disease-free status should be ensured before restocking.

Table 1. Number of broodstock or breeding pairs required to achieve an effective population size (N_e) of 100 or 200 in breeding/ stocking programs over one to 5 years

Number of years of program	Total number of broodstock each year		Number of different pairs each year	
	N_e = 100	N_e = 200	N_e = 100	N_e = 200
5	20	40	10	20
4	25	50	13	25
3	34	68	17	34
2	50	100	25	50
1	100	200	50	100

Breeding program for stock enhancement of *empurau* and *semah*:

1. If there is uncertainty with regard to species identification of broodstock, tag the fish, collect 1 cm^2 of finclip, and preserve in 95% ethanol then send for DNA analysis. Fish with unknown/ uncertain taxonomic status should not be used for breeding until the result of DNA analysis is confirmed.
1. Use at least 10 different pairs of *empurau* and *semah* broodstock each year to produce each batch (i.e. destined for a particular river) of larvae: *to maintain genetic identity, maximise genetic variation and achieve an N_e of 100.*
2. Inject a sufficient number of pairs each of *empurau* and *semah* broodstock for spawning to produce each batch of larvae: *to ensure that at least 10 pairs spawn, because in each group of broodstock there are often fish that don't undergo normal gonadal development, or don't spawn, or spawn poor quality eggs*.
3. Randomise matings with respect to fish size and appearance, do not just choose the big fish only: *to maintain genetic variation and achieve N_e. Broodstock with abnormalities should not be used*.
4. Mate females with different males each season: *to maximise genetic variation and achieve N_e*.
5. Rotate broodstock in ponds every year or at least every 2 years: *to reduce the chance of same-pair matings over consecutive years; to achieve N_e*.
6. Stock eggs from each successful spawning in a separate incubator: *to assess hatch rate and manage progeny numbers for restocking*.
7. Discard excess larvae: *to maintain equal contributions from each pair of broodstock and to achieve N_e*.
8. Collect data and keep records on number of eggs, fertilisation rate, hatch rate and number of larvae from each spawning.
9. Stock similar number of larvae from each of the 10 or more spawnings of *empurau* and *semah*, into each larval rearing pond/tank: *fingerlings harvested from this pond/tank can then be stocked directly into the river from where the broodstock were collected, after about 24 hour quarantine*.
10. Do not grade or select fish in any way after harvest, prior to stocking: *to maintain genetic variation*.
11. Link breeding program with restocking program for each population over a 5 year period: *to maintain genetic variation and achieve N_e*.
12. Ideally replace about 20% of broodstock each year to ensure regular influx of genetic material and to maintain genetic diversity.

5.2. *Breeding program for commercial aquaculture*

The major aim of breeding programs for the commercial production of market-size fish is to produce fish with favourable traits such as fast growth and disease resistance; however, the maintenance of genetic variation is also an important factor. Breeding programs should commence with, and maintain the maximum amount of genetic variation available to maintain reproductive fitness, reduce the chances of inbreeding and maximise the potential for improvement.

At present, there is no intention to conduct a selective breeding program for *empurau* and *semah* just yet. As such, the first priority in a breeding program for commercial aquaculture of these species is to maintain genetic variation and avoid inbreeding and potential associated problems. This could be obtained through maximising N_e and use male : female ratio of 1 : 1.

Crossing between different strains of a species may produce hybrid vigour, i.e. F1 may perform better than their pure parents. However, if this practice is undertaken, F1 should not be used for releasing into open waters. Also, F1 offspring should be maintained separately in bio-secured facilities to avoid genetic contamination with other pure strains.

Essential criteria for breeding programs for commercial aquaculture:

1. Use broodstock of known origin, age and genetics.
2. If there is uncertainty with regard to species identification, tag the fish, collect 1 cm^2 of finclip, preserve in 95% ethanol and send for DNA analysis.
3. Establish domesticated lines using as many founder broodstock as possible.
4. Maintain N_e of 100 to 200 in order to maintain genetic diversity.
5. The male : female broodstock ratio should preferably be 1 : 1
6. Use genetic strains or crosses known to be superior for intensive aquaculture. These should be kept separately to void genetic contamination with pure strains.
7. Broodstock should be collected from local rivers.
8. Broodstock should be supplemented with wild-caught fish on a regular basis (4-5 years).
9. Ideally about 20% of broodstock should be replaced each year.
10. Return broodstock to the river and location where originally captured: ensure they are disease-free before restocking.

It is also important to note that natural disaster such as flooding may lead to escapement of fish from commercial farms, and the escapees may interbreed and cause the dilution of local natural gene pools. As such, it is recommended that commercial hatchery should use local broodstock, i.e. broodstock should be collected from adjacent rivers. Otherwise, fish should be kept in a bio-secured facility.

Domestication of broodstock results in:

1. A loss of genetic variation;
2. Selection for traits that are favourable in aquaculture, but may be detrimental in the wild.

Consequently, long-term domesticated broodstock should not be used in conservation and restocking programs. In addition, these broodstock should also be kept in bio-secured facilities.

5.3. Biosecurity recommendations

Biosecurity is a set of standard scientific measures, adopted to exclude pathogens from the culture environment and host and, more broadly, to limit the establishment and spread of pathogen. The principles of biosecurity should be considered to keep dangerous pathogens not only out of the farm/hatchery but also out of the country and regions, particularly where there are shared water bodies. Once dangerous pathogens enter and become established (endemic) it becomes practically impossible to keep them out of the farm/hatchery. Some concepts vital for ensuring biosecurity are identification of pathogen entry routes, quarantine and screening of hosts introduced to the farm/hatchery, disinfection at defined critical control point, and identification of risk factors for disease outbreak. A biosecure hatchery is one that is protected against introductions of pathogens and unwanted animals, and prevents escape of animals and pathogens to the surrounding environments. The following guidelines are:

5.3.1. Reduction of introductions to hatchery

Likely pathogen carriers include infected hosts (e.g. seed, broodstock, vectors, intermediate hosts, reservoir hosts), non-host biological carriers (e.g. birds, dogs, insects, other predators, human beings) and fomites (e.g. water, vehicles, buckets, shoes, nets, clothing). The carriers could enter the hatchery system through waterborne, airborne and overland transport routes.

Regardless of the water source, water should be physically screened (preferably less than 0.5 mm) to prevent unwanted animals entering the facility, and ideally sterilized (ozonated, UV irradiated) to eliminate water borne pathogens.

To ensure that pathogens are not introduced with new stock, all fish being moved onto the facility, regardless of their origin, must be quarantined. The Quarantine facility should be separate from the rest of the facility. New stock should be quarantined for at least 2 weeks before introducing to the production facility. During that time, fish should be checked regularly for signs of disease, and if necessary be given therapeutic treatments to kill pathogens.

5.3.2. Reduction of escape from hatcheries

- Locate facilities away from flood prone land (i.e. above 1 in 100 year flood level),

- Maintain hygienic conditions and an active health monitoring/management program to reduce the incidence of pathogens on the facility.
- Physically screen discharge water from the facility to prevent escape of animals to receiving waters and escape of sick fish to receiving waters
- Develop procedures for proper disposal of sick and dead fish.

5.4. A list of do-nots

It is critical that breeding activities do not cause negative genetic impacts on *empurau* and *semah*, in particular:

1. Do not breed fish if there is uncertainty in species classification;
2. Do not hybridise *empurau* and *semah*;
3. Do not move fish from one location to another in the wild;
4. Do not release offspring to a water body that are different to their broodstock origin;
5. Do not release fish resulting from crossing between strains into open waters;
6. Do not just simply select fish with special characters to release; this should be undertaken in a random manner.

The guidelines entailed in this document have been developed based on stringent and prolonged scientific investigations. It is not very common, particularly in this region, to develop such guidelines hand in hand with artificial propagation and commercialization of the culture of a new fish species, as well for use for conservation purposes. Indeed it is the first such instance in all aquaculture developments in Asia. In the above regard the State Government of Sarawak has set an ideal example to such comparable developments in the region. This is even more significant taking into consideration the increasing impetus in the region to popularize the culture of indigenous fish species and gradually move away from dependence on exotic and or alien species.

References

Frankham, R., Ballou, J.D. & Briscoe, D.A. (2002) *Introduction to Conservation Genetics,* Cambridge University Press, Cambridge, UK.

Kottelat, M., Whitten, A.J., Kartikasari, S.N. & Wirjoatmodjo, S. (1993) *Freshwater Fishes of Western Indonesia and Sulawesi,* Periplus Editions, Hong Kong.

Miller, L.M. & Kapuscinski, A.R. (2003) Genetic guidelines for hatchery supplementation programs. In: *Population genetic principles and applications for fisheries scientists.* (Eds E.M. Hallerman), pp. 329-355. American Fisheries Society, Bethesda.

Ng, C.K. (2004) *King of the Rivers: Mahseer in Malaysia and the region,* Inter Sea Fishery (M) SDN BHD, Kuala Lumpur.

Nguyen, T.T.T. (2007) Population structure in a highly fragmented range of occurrence of *Tor douronensis* (Cyprinidae) in Sarawak, Malaysia revealed from microsatellite DNA markers. *Freshwater Biology,* **Submitted**.

Nguyen, T.T.T., Baranski, M., Rourke, M. & McPartlan, H. (2007) Characterization of microsatellite DNA markers for a mahseer species, *Tor tambroides* (Cyprinidae) and cross-amplification in four congeners. *Molecular Ecology Notes,* **7,** 109-112.

Nguyen, T.T.T., Ingram, B.A., Sungan, S., Gooley, G., Sim, S.Y., Tinggi, D. & De Silva, S.S. (2006) Mitochondrial DNA diversity of broodstock of two indigenous mahseer species, *Tor tambroides* and *T. douronensis* (Cyprinidae) cultured in Sarawak, Malaysia. *Aquaculture,* **253,** 259-269.

Roberts, T.R. (1999) Fishes of the cyprinid genus *Tor* in the Nam Theun watershed (Mekong Basin) of Laos, with description of a new species. *The Raffles Bulletin of Zoology,* **47,** 225-236.

Waples, R.S. (1991) Genetic interactions between hatchery and wild salmonids: lessons from the Pacific Northwest. *Canadian Journal of Fisheries and Aquatic Sciences,* **48,** 124-133.

Zhou, W. & Cui, G.-H. (1996) A review of *Tor* species from the Lancangjiang River (Upper Mekong River), China (Teleostei: Cyprinidae). *Ichthyological Exploration of Freshwaters,* **7,** 131-142.

Annex 1.

Nguyen, T. T. T., Na-Nakorn, U., Sukmanomon, S., ZiMing, C. (2007). A study on phylogeny and biogeography of mahseer species (Pisces: Cyprinidae) using sequences of three mitochondrial DNA gene regions. Submitted to *Molecular Phylogenetics and Evolution*.

Annex 2.

Nguyen, T.T.T., Ingram, B., Sungan, S., Gooley, G., Sim, S.Y., Tinggi, D., De Silva, S.S. (2006) Mitochondrial diversity of broodstock of two indigenous mahseer species, *Tor tambroides* and *T. douronensis* (Cyprinidae) cultured in Sarawak, Malaysia. *Aquaculture*, 253: 259-269

Annex 3.

Nguyen, T.T.T., Baranski, M., Rourke, M., McPartlan, H (2007). Characterisation of microsatellite DNA markers for the mahseer species, *Tor tambroides* and cross-amplification in other four congeners (*T. douronensis*, *T. khudree*, *T. putitora*, and *T. tor*). *Molecular Ecology Notes*, 7:109-112.

Annex 4.

Nguyen, T. T. T. (2007). Population structure in a highly fragmented range of occurrence of *Tor douronensis* (Cyprinidae) in Sarawak, Malaysia revealed from microsatellite DNA markers. Submitted to *Freshwater Biology*.