

# Application of Molecular Techniques for Sustainable Management of Inland Fisheries: the Experience of Indonesia

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Indonesia embraces large areas of inland water resources with potentials for development of its inland capture fisheries. To obtain the optimal and sustainable benefits from these resources for the benefit of present and future generations, an appropriate and systematic fisheries management effort is necessary. Application of the molecular approach through the DNA mitochondrial sequencing techniques could be used as basis for the management of the fishery resources in inland waters. For example, in an attempt to manage the fisheries habitat at the Merang Peat Swamp, information on the eleven freshwater fish species that spawn in this unique ecosystem was first established using molecular technique. The findings led to the identification of the fish species that inhabit the area based on the analysis done during the early stages of the life cycles of the species. These findings also imply that peat swamps are also important habitat of various fish species, especially during spawning, despite having extreme water conditions that might not even be ideal to sustain fish life. Results of the study on the use of molecular approach also provided the information needed for the management of belida fish or giant featherback (*Chitala lopis*). Specifically, the findings indicated that management of this fish species must be focused regionally because of the existence of more than one population in one river, which is not the same as in the case of the semah fish or mahseer (*Tor tambroides*). Nonetheless, the analysis made on these two species using molecular markers has led to the information on the presence of genetic diversity in belida and semah fish species in two separate rivers in Sumatra, a phenomenon which should be considered in the conservation efforts of these two fish species.

As defined, inland waters are those parts of the earth's surface that are permanently or periodically flooded with water (aquatic ecosystems), like fresh, brackish or salty water, formed naturally or artificially, and do not belong to any individual nor business entities. Moreover, the topography of inland waters is distinguishable from those of mainland waters or private ponds and marine waters (Anon, 2010), and according to some definitions provided by experts (Nontji *et al.*, 1996; Hartoto, 2005), inland waters are all forms of water bodies located above the lowest tide line to the mainland.

It has been reported that the inland waters throughout Indonesia comprise various types of ecosystems, namely: lakes, reservoirs, rivers, and estuaries, with a total area of about 54 million ha (Manggabarani, 2005; Sukadi, 2005). There are also a total of 5,590 major rivers with streams having a total area of 1.5 million km<sup>2</sup> (Husnah *et al.*, 2008) with total biomass potential which is assumed to be about 15,336

metric tons (Anon, 2010). The potentials of large inland waters are very important for the life of a nation and if managed appropriately, could be used optimally and sustainably for its people's wellbeing. However, appropriate resource management requires knowledge that underlies the biological principles, especially the ecology of the resource (Yusron, 2005). Nevertheless, the rapid development of molecular biology technology provides the biological information that could be used as basis for efforts to manage the fishery resources, specifically the application of mitochondrial DNA sequencing techniques that serves as guide in the management of the fishery resources in inland waters.

## Identification of species during the early stages of the fish life cycle

An activity to identify fish species during the early stages of their life cycles was carried out at the Musi River (**Figure 1**) in South Sumatra, Indonesia. Musi River flows from South-West to North-East, from Barisan Mountain range forming the backbone of Sumatra, in Kepahiang, Bengkulu, to the Bangka Strait that extends to the South China Sea. After flowing through Palembang, the capital City of South Sumatra, this 750 km-long river joins with the several other rivers, including the Banyuasin River, forming a delta near the city of Sungsang. Musi River is host to at least 233 species of fish (Utomo *et al.*, 2007; Husnah *et al.*, 2008) with a total annual fish production estimated at 12,500 metric tons (Utomo, 2006; Anon, 2010). An attempt was made to use DNA barcodes to



Figure 1. The Musi River of Sumatra Island, Indonesia



Figure 2. The Merang Peat Dome in South Sumatra, Indonesia

identify the fish larvae from the South Sumatra black water peatland forest, *i.e.* the Merang Kepayang Peat Dome (Figure 2) as the results could also be used to correctly identify the fish larvae specimens collected from Musi River at species level. After the activity, only about 66% of the samples could be analyzed, which could be due to the inappropriate barcode approach adopted or because of the insufficient number of reference sequences for the ichthyofauna study of this peat swamp.

This activity which is meant to identify the fish species during the early stages of their life cycles, is a pioneering study of the ichthyofauna in the lowland eastern part of Sumatra Peatland using the molecular approach. The samples used in the study were extracted based on a total of 72 internal sequences of COI (~ 376 bp) from 35 fish larvae, and 13 adult samples with sequential reference sequences and 24 NCBI Genbank and BOLD database for species identification.

The Merang Kepayang Peat is located precisely between the Medak and Kepahiang Rivers. The nearest village to the Dome forest is Muara Merang Village, which is 225 km or about 4-5 hours by car or boat from Palembang. These Rivers are the main access used by the villagers to go to neighboring villages, the nearest small city or to the forest. The Merang Peat Swamp Forest with an area of about 150,000 ha, is the last contiguous peat swamp forest in South Sumatra Province. It is part of a larger peat swamp area which is linked to the Sembilang National Park in the eastern part of Sumatra, the Muaro Jambi Peat Swamp Forest in the northern part next to Jambi Province, and Berbak National Park in the northwest.

The initial analysis indicated that at least eleven fish species complete their life history in this unique ecosystem. These are: the eyespot rasbora *Rasbora pauciperforata*, *Rasbora dorsiocellata*; kissing gourami *Helostoma temminckii*; three-spot gourami *Trichogaster trichopterus*; rasbora *Rasbora cephalotaenia*, snakeskin gourami *Trichogaster pectoralis*, croaking gourami *Trichopsis vittata*; climbing perch *Anabas testudineus*; and other species of gourami *Pectenocypris*

*korthusae*, *Parosphromenus deissneri*; and the Asian redtail catfish *Hemibagrus nemurus*. The species sequence reference of adult fish in the Merang Kepayang Peat represents 57% of the known species of the area.

The result of the study also explains the importance of the peat swamp ecosystem, *i.e.* in Merang Kepayang Peat, as a major part of biodiversity and their main role in maintaining the existence of at least eleven species of fish. Knowledge of the early stages of life cycles of fishes from fish eggs to larvae is very important for fisheries management. However, data interpretation problems could continue to occur, mainly because of the limited availability of key identification and rapid changes of morphological characters in the development of the initial larval phases, *i.e.* from preflexion to postflexion to the prejuvenary phase, contributing to the main challenges for species identification (Figure 3). The DNA barcoding approach that has a valid sequential reference sequence can increase the taxonomic resolution of larval identification at species level. This makes the existence of accurate and reliable sequences of DNA sequencing libraries for freshwater fish species very important in species-rich regions, such as Sumatra Island of Indonesia, which has around 285 species of freshwater fishes (Husnah *et al.*, 2008). Scientific efforts to provide the sequence for the sequential libraries of the Sumatran freshwater fish species had been made but the results are still spatial and uncoordinated.

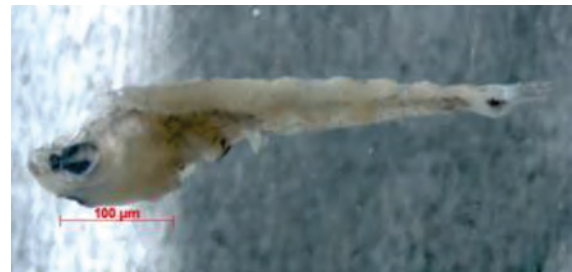


Figure 3. Unidentified fish larvae found in Merang peat dome, South Sumatra

Results from the DNA barcode study focusing on freshwater fish species in the Danau Laut Tawar System of Aceh Province, using the cytochrome oxidase subunit I (COI) gene mitochondrial DNA and 30 nucleotide species-specific sequence, have been compiled and together with a collection of at least 12 species of fish, are already made available in the Province' GenBank Database (Muchlisin, unpublished). Some information on reference sequences is also available for 20 Sumatran freshwater fish species (Wibowo, unpublished).

The first real effort to study DNA barcode applications for fisheries management in Sumatra was carried out by Wibowo *et al.* (unpublished) and intended for the conservation of the fishery resources. The local people catch freshwater fishes on location by small-scale fishing operations and the catch is sold at very low prices in local markets. Fish originating from the waters of the black aqueous peat forest are not brought to



other areas as these are mainly used for domestic consumption, notwithstanding the very high commercial potential of the fishes in the aquarium fish trade (Ng *et al.*, 1994). This situation is common in most black water peat swamp forest areas that are located in very remote or inaccessible places. With abundant fishery resources and as the ornamental fish trade flourishes and becomes very profitable and lucrative business, Indonesia now ranks as the second highest exporter of ornamental fishes, contributing at least 7% of world's ornamental fish trade (Lim and Ling, 2005). In 2003, this trade exceeded US \$298,000,000 and although some ornamental fish species are cultured, a big portion still comes from the wild (Ng, 1991 in Ng *et al.*, 1994), and thus, could be affected by the conversion of many black swamp land areas that has reached an alarming stage. In fact, the loss of peat swamp forests in the lowlands of Sumatra and Kalimantan has been estimated at 70% during the period 1990-2005 (Hansen *et al.*, 2009).

### Belida fish management strategy based on a regional approach

Morphological approaches have limitations when used in detecting species variations especially for those species with vague taxa, for example in the case of belida fish or giant featherback (*Chitala lopis*), which has very high economic and socio-cultural value in Indonesia (Wibowo, 2011). This fish is very popular not only for its meat which is delicious but also for its high fat content making it nutritious (Sunarno, 2002), especially its high protein and Vitamin A contents (Mno, 2005). The belida fish, a very exclusive freshwater fish, commands a price that is quite expensive (more than Rp. 50,000/kg or US\$ 3.50/kg). Traders classify the price of belida fish according to weight, with the lowest price for belida fish weighing less than 1.0 kg/tail, medium price for fish between 1.0 to 2.0 kg/tail, and the highest price for fish weighing more than 2.0 kg/tail. In Palembang, South Sumatra, Indonesia, the market price of belida fish in traditional fish markets is Rp. 200,000/kg (US\$ 14.27/kg) for the highest price category and becomes even more expensive if the fish is alive and traded as ornamental fish. The uncontrolled catching of large-sized belida fish from the wild puts much pressure on the sustainability of this fishery resource.

Currently, the demand for belida fish by the processing industries is estimated to be about 200 kg/day and for the ornamental fish trade and human consumption, as much as 40 kg/day. Although fishers could only supply less than 2% of the market requirement (Anon, 2003), the population of belida fish in nature is decreasing due to fishing and human activities. The annual production of belida fish has declined not only at the national level but also at the regional scene. For example, in Kampar River, Riau Province, the catch of belida fish had been decreasing: from 8,000 mt in 1991 to 5,000 mt in 1995, and to 3,000 mt in 1998 (Directorate General of Fisheries, 2000). The annual production of belida

fish in Kampar River had decreased further from 50.2 mt in 2003 to 7.6 mt in 2007 (Department of Marine and Fisheries, 2008). This condition is likely to continue because of the high demand for this fish. As a consequence, an appropriate fisheries management strategy to maintain and conserve this species is really necessary.

Belida or chitala fish (**Figure 4**) belongs to the Class Actinopterygii (ray fishes), Order Osteoglossiformes (bony tongues), Family Notopteridae (knife fish), Genus *Chitala*, and species *Chitala lopis* (Nelson, 1976; Kottelat *et al.*, 1993). At present, there are 4 types of notopteridae in the Genus *Chitala*, namely: *Chitala lopis*, *C. blanci*, *C. ornate*, and *C. chitala* (Inoe *et al.*, 2009).



Figure 4. Belida Fish (*Chitala lopis*)

There are no valid taxonomic records for chitala fish in Indonesia, but Kottelat and Widjanarti (2006) initially mentioned that the chitala fish in Indonesia belong to the species *C. hypselonotus*, *C. borneensis*, *C. lopis*, and *Chitala* sp., while *Chitala hypselonotus* and *C. borneensis* are found in Sumatra. Wibowo (2011) identified *Chitala lopis* in Sumatra Island based on partial mitochondrial DNA sequences. However, the mitochondrial sequence analysis, based on 12 bi-directional sequences (600-721 bp) from COI, 15 bi-directional sequences (496-1147 bp) from cytochrome b and 51 bi-directional sequences (566-936 bp) from non-coding (control region), shows that *Chitala lopis* in Indonesia can be distinguished by at least four groups of cryptic species (Wibowo, 2011; Wibowo and Farajallah, 2014 in press). This analysis was based on samples taken from rivers, reservoirs, lakes, swamps, ditches, and ponds, comprising almost all the distribution areas for belida fish in their natural habitat in Indonesia.

Although the result might not be comprehensive enough, but it indicated that the existence of belida fish in Indonesia is still poorly understood because some unidentified species tend to represent the evolutionary units and biological characters of the belida fish. The latest information from genetic population and life history studies however indicated that there are several belida fish populations that live in integrated rivers (Wibowo, 2011). Belida fish is a slow swimmer as can be seen from its shape. The fish inhabits the lakes, oxbow swamps, ditches, and ponds (Rainboth, 1996) and the eggs are not easily spread because belida fish eggs are usually attached to some submerged vegetations (Talwar and Jhingran, 1991).

As a consequence, gene flow will be inversely proportional to the local conditions. Therefore, for belida fish, a regional management strategy would be necessary.

For example, with a panmictic population structure, belida fishing in one area may not affect the subsequent recruitment in that area because new tillers (recruits) will come randomly from the parents originating from other areas. However, in the case of local populations, such as in the Kampar River (Wibowo, 2011), each local population tends to have certain migration circles and recruitment processes that almost none had occurred in the other regions. Overfishing of belida fish in Kampar River might have real influence on the recruitment of returning fish to certain areas by each local population. The loss or degradation of habitats together with overfishing can lead to the extinction of the local belida fish, so that management of belida fish in Indonesia, where the life history of most species is still not fully understood, must be seriously considered.

## Semah fish management

Another iconic fish species besides belida is the semah fish (*Tor tambroides*), belonging to the important group of freshwater cyprinids. Semah fish (**Figure 5**) is classified under the Class Actinopterygii, Order Cypriniformes, Family Cyprinidae, Genus *Tor*, and species *Tor tambroides*. The synonyms for semah fish in Indonesia are *Labeobarbus tambroides* and *Barbus tambroides* (Kottelat *et al.*, 1993). Semah is one of the most popular freshwater fishes in Indonesia both for domestic consumption and for traditional cultural functions. This fish is also sold for around Rp. 50,000/kg (US\$ 3.50/kg) in the local markets.



Figure 5. Semah fish (*Tor tambroides*)

Local fishers prefer to catch the large size fish compared to small size because of the higher price for bigger fish. The market price of semah fish in Malaysia ranges from Rp. 200,000 to 750,000/kg (Rachmatika and Haryono, 1999). Reports have indicated that the abundance and distribution of semah fish in their natural habitat has substantially decreased due to overfishing, pollution and deforestation (Kottelat *et al.*, 1993). *Tor tambroides* usually inhabits the fast-flowing waters, has a long flat and large torso with long lobes and small head, green brown and large scales, and this fish is sexually mature when the abdomen enlarges and the silvery fin color is yellow,

orange, pink or pale red (Pollar *et al.*, 2007). It has also been reported that domestication and breeding of the semah fish is difficult to undertake and reproduction in captivity is also a challenge. In spite of such constraints, artificial propagation through induced ovulation and spawning using hormonal treatment techniques had been tried (Ingram *et al.*, 2005). Also, the life history strategies and migration patterns of semah fish in river systems are not widely studied and not widely known.

Furthermore, efforts to study the genetic population of plants in Sumatran watersheds were also carried out by Wibowo and Husnah (2012); Wibowo (2012); Wibowo (unpublished) to study the ecology of watersheds with respect to the fishery resources, *e.g.* belida and semah fishes, that these areas possibly host. Results of the study in Sumatra River for example, revealed that the morphological characters could not clearly show the variations in character due to geographical and ecological variability in and between the Sumatra River, while DNA barcoding was based on 87 sequences of sequential bi-directional sequences (~ 654 bp) providing sufficient information on various types of gene profiles COI of these fish species.

The results also indicated that each population in the River system consists of a single panmictic population but has different genetic characteristics among the other rivers in Sumatra. Semah fish management strategies must therefore ensure that the semah fish in each local river must be protected so that the undiscovered biodiversity from these economically important freshwater fish can be conserved.

## Conclusion

The molecular approach through the application of DNA mitochondrial sequencing techniques can be used as basis for managing the fish resources in inland waters. Specifically, the use of such technique would provide information on the importance of peat swamp habitat as place for fish spawning despite having extreme water conditions that might not be ideal for the fish survival in the long run. Based on the results of the adoption of the molecular approach, the management of belida fish (*Chitala lopis*) should be focused regionally considering the existence of more than one population in one river, which is not true for the semah fish (*Tor tambroides*). As a result of the analysis using molecular markers, the genetic diversity in belida and semah fishes could be detected because most rivers in Sumatra are not interconnected.

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