

COMPARING EFFECTIVE FEMALE POPULATION SIZE AND POPULATION HISTORY OF *Tor tambroides* FROM GENE COI (MTDNA)

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ABSTRACT

The mahseer (*Tor tambroides*) is an important commercially fishes in Indonesia. Here we analyze DNA sequence data from the Cytochrom Oxidase Subunit 1 (COI) of the mitochondrial genome to estimate genetic effective female population size (N_{ef}) of mahseer in Manna River, Bengkulu Province and Tarusan River, West Sumatra Province. The aim is to compare the effective female population size and population history of giant mahseer from two rivers which constitute different status of population and what could be fuel from the information. Samples obtained from the year of 2011-2012. The molecular data, a stretch of 628 nucleotides (Manna specimens) and 706 nucleotides (Tarusan specimens) of COI of mtDNA were used to investigate effective female population size (the formula $\theta = 2 N_{ef} \mu$) and population history of mahseer. Population history of mahseer was generate through growth module and neutrality test in DnaSP3.51 to yield *mismatch distribution* plot. Our results show that despite this reduction, the effective female population size of both populations is sufficient to survive from a genetic viewpoint. However, we have been able to show that protected population is much more likely to be higher N_e or individuals than the unprotected population. Therefore, ongoing conservation efforts remain worthwhile. The historic population expansion of the mahseer (inferred from this study) may indicate a non possible re-expansion in the future of the current population of the mahseer. It is possible for the mahseer to extinct if the mahseer population continues to decline and the necessary conservation actions are not taken.

Key words: *Tor tambroides*, effective female population size, mitochondrial DNA, population history

INTRODUCTION

The mahseer (*Tor tambroides*) is an important commercially fishes in Indonesia. This species can only be found in pristine freshwater rivers (Ismail *et al.*, 2011). Mahseer are endemic to Asia with natural distribution encompassing the trans-Himalayan region in the northwest to Sumatra and Borneo islands in the southeast, across a number of countries such as Nepal, Pakistan, India, Sri Lanka, Myanmar, China, Thailand, Laos, Cambodia, Vietnam, Indonesia and Malaysia. These species are large-scaled barbels that live in upstream, clear, running waters (Ng, 2004). However, these species have undergone decline in distribution and abundance due to significant degradation of their natural habits from deforestation, overfishing and agricultural development (Ismail *et al.*, 2011).

Some local and government have been recognising the importance of these species, made a concerted effort to evaluate their sustainable potential and to implement general policy for integrated management of mahseer species such as in Batang Tarusan River, West Sumatra with involves conservation of particular well-defined areas. Others did not have a strategy of mahseer conservation such as the Manna River, Bengkulu that lead to a dramatically decline in Mahseer populations.

In this regard, the genetic data of the present population is valuable in providing baseline data for monitoring the condition of two rivers on the population genetic structure of the mahseer. Moreover, we pursued the questions on the effective female population size and historic population changes. Effective population size is the size of an ideal, Wright–Fisher model population having the same rate of genetic drift as the observed population (Hartl & Clark 1989). Sharp declines in spawning stock resulting from natural variation in recruitment and/or over-harvesting are expected to reduce values of N_e from historical levels (Vucetich *et al.* 1997); N_e thus can provide important baseline data for monitoring stock size.

In this study, we analyze DNA sequence data from the Cytochrom Oxidase Subunit 1 (COI) of the mitochondrial genome to estimate genetic effective female population size (N_{ef}) of mahseer in Manna and Tarusan River. The aim is to compare the effective female population size and population history of giant mahseer from two rivers which constitute different status of population and what could be fuel from the information.

MATERIALS AND METHODS

A sum total of 72 mahseer were collected from two rivers in the western Sumatra River (Figure 1). Specimens were taken by a variety of methods including gill net, cast net and hook and line. Muscle tissues were removed from individual fish, preserved in absolute ethanol solution and returned to the laboratory where they were stored at -4°C . Individual mahseers were assayed for variation in mtDNA polymorphic sites via sequences methods.

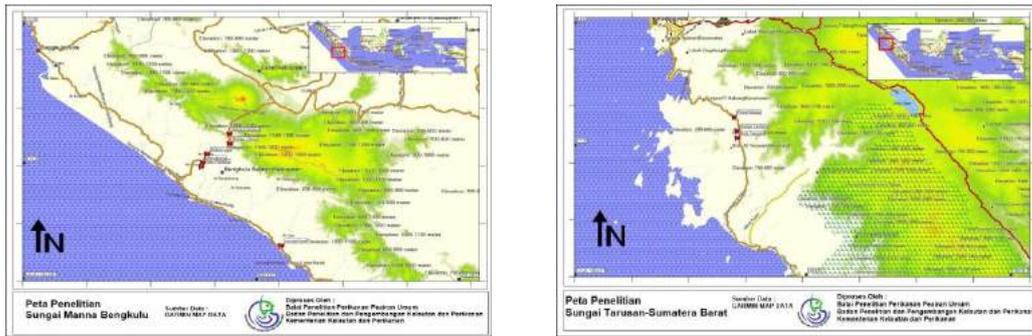


Figure 1. The geographic locations relating to the mahseer (*T. tambroides*) (Manna and Tarusan River).

DNA was extracted using the Extraction Kit procedure 'DNeasy Blood & Tissue' (Geneaid). Partial fragments of the mitochondrial gene Cytochrome c Oxidase Subunit I were amplified using universal primers described by Ivanova et al. (2007):

Fish-COI-F (5'-ACT TCA AAC TTC CAY AAA GAY aty GG-3) and
COI-Fish-R (5'-TAG ACT TCT GGG TGG CCR AAR Aay CA-3 ').

Polymerase chain reaction (PCR) amplifications were made in a 50 μ L of reaction volume consisted of 5 μ L DNA samples, 16 μ L double distillate water, 2 μ L of each primer and 25 μ L of PCR ready mixture solution (KAPPA). PCR cycling parameters included an initial denaturation phase at 95°C for 10 min, followed by 35 cycles at 94°C for 1 min, 48°C for 1 min and 72°C for 1.5 min and ended with a final extension at 72°C for 7 min. Finally, all amplicons were automatically sequenced in both directions at First Base, Singapore (www.firstbase.com).

Estimation of effective female population size

DNA sequence alignment was done using Clustal X (Thompson *et al.* 1997). Considering the fact that the effective female population size (N_{ef}) is maternally inherited, it allows us to compute N_{ef} values based on formula $(\theta) = 2N_{ef} \nu$ (Tajima, 1993). The infinite-site equilibrium relationships between numbers of segregating sites are used to produce theta θ (per DNA sequence) employing similar distance method in DnaSP3.51 software (Rozas and Rozas, 1999). In the equation $\nu = m\mu$, m is the sequence length, and μ is mutation rate per generation, which was acquired by multiplying generation time (14 years for the female mahseer) by mutation rate per

year (λ). In this study, we used the animal mitochondria nucleotide substitution rate ($0.05 - 0.1 \times 10^{-7}$ substitutions/ nucleotide site/year) (Harrison, 1989). We estimate of N_e to a benchmark value ostensibly important for conservation and management of mahseer in the western Sumatra River.

We also calculated the population history of masheer and generated a *mismatch distribution* plot (Rogers, 1995) using DnaSP3.51 software (Rozas and Rozas, 1999). Mismatch distributions are graphs/histograms showing the pattern and the frequency distribution of observed number of differences between pairs of haplotypes/nucleotide. This distribution is usually multimodal in samples drawn from populations whose size has been constant over a long period, but it is usually unimodal in populations having passed through a recent demographic expansion (Slatking and Hudson, 1991). Detecting population expansion, past population growth or stability based on DNA sequences can be estimates employing a range of neutrality statistics (Ramos-osins and Rozas, 2002) such as Fu and Li's D^* test, Fu and Li's F^* , Fu's FS test (Fu and Li, 1993) and Tajima's D test (Tajima, 1989). All of calculations were computed using DnaSP 3.0 software.

RESULTS AND DISCUSSION

We have compiled information on each specimen, a stretch of 628 nucleotides (Manna specimens) and 706 nucleotides (Tarusan specimens) of COI of mtDNA was PCR amplified and sequenced. Analysis found that the all data set comprised 4 haplotypes.

Based on our estimate of theta (θ) at 0.451 in Manna River, the effective female population size (N_{ef}) was calculated to be 2,560 to 5,130. Given that effective population size inferred from the mitochondrial DNA is half of overall effective population size, and then we assume the effective population size (N_e) would be in between of 1,280 to 2,560. Meanwhile in Tarusan River, the effective female population size (N_{ef}) was calculated of 9,140 to 18,280, with N_e of the overall population approximately in a range of value of 4,570 to 9,140, the value is based on theta (θ) esimation at 1.607.

It seems that the N_e value of both populations is sufficient to survive from a genetic viewpoint. The relatively high N_{ef} reduces the probability of inbreeding

among mahseers, potentially allowing the population to recover quickly (Zhang *et al.*, 2003). The generally accepted view is that a N_e of less than about 50 is vulnerable to the immediate effects of inbreeding depression. Although populations might occasionally decline to numbers on this order without adverse effects, maintenance of adaptive genetic variation over longer periods of time (e.g., centuries) probably will require N_e averaging more than 500. These numbers have been generally applied as criteria for determination of conservation status among taxa (Mace and Lande, 1991). If we accept the general guidance of the 50/500 criteria, an average of 1,000 or more (i.e., $1000 \times 0.5 = 500$) adults spawning annually would be necessary to maintain genetic variation indefinitely. Those criteria might be relaxed if there were clear evidence that the adult population is larger than the number of fish spawning in any year (because all females do not spawn in all years), or if more precise estimates of other life history parameters and variation were available.

Theoretical studies suggest that the ratio of genetic effective population size to adult census size should range between 0.25 and 0.75 over a wide spectrum of life histories (Nunney & Elam 1994). From this point of view may refer the estimation of N_e in Manna River would be 1,700 to 10,000 individuals and Tarusan 6,000 to 36,000 individuals. Since information on the interval of repeated spawning, number of spawning events in a lifetime, and population structure is largely ambiguity for the mahseer species, it is very difficult to get an exact number of the census population size. Clearly, the present data needs further calibration.

However, we have been able to show that protected population is much more likely to be higher N_e or individuals than the unprotected population. Therefore, ongoing conservation efforts remain worthwhile. We emphasize, that our analysis is simply an approximation, built on other approximations. Without detailed information, managers should acknowledge this uncertainty and recognize that the guidelines we have provided are conservative minimums and not goals that will assure the viability of any population.

The population history of Manna and Tarusa River population was examined using two different methods. A pairwise distance matrix was constructed with the 72 sequences of the COI of the mtDNA. The mismatch curve is bell-shaped (Figure 2),

graphs observed mismatch distributions were compared with the expected ones. In the upper graphs expected curves were drawn by assuming constant population size whereas in the lower graphs expected curves were drawn under the assumption of population growth. Because the fit between the observed and expected distributions was better in the lower half of the figures especially, these distributions suggest that all haplogroups experienced population growth / expansion as expected (Slatkin and Hudson, 1991; Rogers and Harpending, 1992).

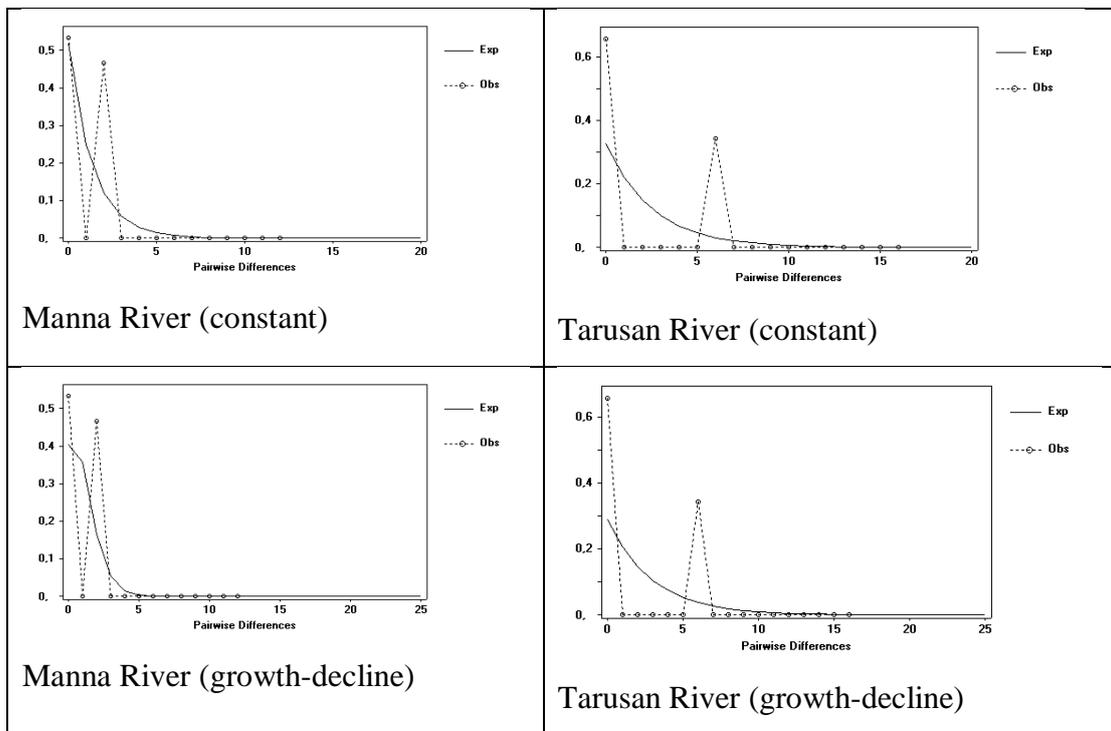


Figure 2. Observed and expected mismatch distributions for mahseer mtDNA COI sequences from Manna and Tarusan River under both constant (upper graphs) and population expansion (lower graph)

The means of the distributions were disparate, recommending different time spans for the origin and dispersal of each haplogroups/populations. The mean difference values for haplogroup Manna was 0.934 and for haplogroup Tarusan was 2.065. The mean difference value was considerably high in haplogroup Tarusan which represented the oldest date of descent for this haplogroup, then Manna.

More sensitive tests Fu's F_s test of neutrality (Fu, 1997) was applied to further test the possible occurrence of population expansion. Results are presented in

Table 1. However the Tajima's D were positive in both populations and the index of Fu's F_s test, was not significant at the 5% level for both population for the pooled dataset. This is a weak indication of population expansion (Aris-Brosou and Excoffier, 1996). Perhaps both haplogroups did not experience population growth or because of the few haplotypes observed in mahseer were not enough to detect the true demographic history of both haplogroup. The high sensitivity of neutrality test indicated that the population of the mahseer had not undergone a historic expansion.

Table 1. Neutrality calculates for for mahseer mtDNA COI sequences from Manna and Tarusan River. Not significant values in the data are indicated

	<i>Manna River</i>	<i>sig</i>	<i>Tarusan River</i>	<i>sig</i>
Tajima's (1989) D	1,930	ns	0,863	ns
Fu & Li's (1993) F*	1,276	ns	1,305	ns
Fu & Li's (1993) D*	0,751	ns	1,232	ns
Fu's (1993) FS	3,160		5,851	
Generation time	14			
Not significant $P > 0,10$				

The historic population expansion of the mahseer (inferred from this study) may indicate a non possible re-expansion in the future of the current population of the mahseer.

CONCLUSION

Despite this reduction, the effective female population size of both populations is sufficient to survive from a genetic viewpoint. However, we have been able to show that protected population is much more likely to be higher N_e or individuals than the unprotected population. Therefore, ongoing conservation efforts remain worthwhile. The historic population expansion of the mahseer may indicate a non possible re-expansion in the future of the current population of the mahseer. It is possible for the mahseer to extinct if the mahseer population continues to decline and the necessary conservation actions are not taken.

ACKNOWLEDGMENTS

We deeply thank the following person for support in the collection of the samples: Siswanta Kaban, Mirna Dwi, Rosidi and Farid. Funding was provided by grants from the Research Institute for Inland Fisheries (RIFF), Project year 2012.

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