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Effects of dam structures on genetic diversity of freshwater fish *Sinibrama macrops* in Min River, China

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ABSTRACT

In order to explore the effect of different dam structures on the genetic diversity of freshwater fish, the genetic diversity of *Sinibrama macrops* in Min River was investigated using mitochondrial control region (D-loop) sequences. Populations of *S. macrops* at Jianyang and Shaowu rivers were separated from the rest of the system by low dams and the population at Gutian was isolated from the rest of the river by a high dam. The haplotype diversity in the samples analyzed varied from 0.577 to 0.933 and nucleotide diversity varied from 0.0031 to 0.00441. The lowest values of haplotype diversity and nucleotide diversity were obtained from Gutian. Indices of genetic differentiation between populations, gene flow, analysis of molecular variance and net genetic distance between populations showed widespread gene exchange among different populations, with the exception of Gutian and Yongtai. These results may be associated with the isolation of Gutian by the high dam. The result of neutrality test and mismatch distributions showed that the evolutionary history of Gutian population was different from others in the river system. Our results imply that high dams have a larger effect on *S. macrops*, leading to reduction of genetic diversity and genetic differentiation from other populations.

1. Introduction

Habitat fragmentation is a leading cause in the decline of biodiversity worldwide (Fahrig, 2003). In riverine ecosystems, dam construction is one of the primary causes for riverine fragmentation and can dramatically affect fish populations (Saunders et al., 1991). Dams prevent fish migration throughout a river system, particularly upstream migration, which isolates upstream populations (Gehrke et al., 2002). Such blockages reduce population size, alter life history features, and shift the spatial structure and/or genetic components of a population (Morita and Suzuki, 1999; Heggenes and Roed, 2006). Many studies have investigated the effects of dam construction on riverine fish populations, however, they most often focus on migratory fish, finding that population declines are primarily due to the change from a river to a lacustrine environment and the isolation of migratory fishes from their spawning and feeding grounds (e.g., Barthem et al., 1991; Ruban, 1997). So far, very few studies have focused on how dams affect residential populations. Resident freshwater fishes such as *Sinibrama macrops* G.

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1868, can complete their life history without migration, so would seem not to be affected by dams. However, even non-migratory residential riverine fish populations may often be divided into several smaller populations by dams, which may adversely affect their genetic diversity in freshwater.

Water-power (hydro-electric) designs are of two types, high dams or low dams, which are constructed in consideration of the needs of water storage regulation and the distribution of hydraulic resources. The high dam, also called an annual-regulating dam or multi-annual regulating dam, has a large drop and a strong capability of impoundment, which causes areas upstream to be submerged, forming a riverine impoundment (Li et al., 2007). The low dam, also called a daily regulating dam, has a small drop and has a weak capability of impoundment, which does not change the river as drastically (Huang and Yi, 2004). The different structures of the two dam types have differing influences on fish habitat and fish migration. Because the two kinds of dams have different effects on fish communities, they can be used to better understand the relationship between freshwater fish and habitat changes caused by human activities. Previous studies have found that riverine impoundments and reservoir-induced habitat fragmentation adversely affects the genetic characteristics of fishes inhabiting small streams (Fluker et al., 2014), but no study has focused on the genetic effects on fish living in the riverine impoundment and mainstream-inhabiting fishes after the construction of either dam type. For the resident freshwater fishes, dams can influence genetic diversity in two main ways. First, dams can prevent gene flow and fragment the population, and secondly, dams change the environment the fish inhabit by creating a riverine impoundment.

The Min River is located in southeast China and is the largest river in Fujian Province. It originates in the Wuyi Mountains, and flows into the East China Sea. According to the requirements of the distribution of water power resources, different types of dams are constructed in the three main tributaries to the Min River. The dam constructed on the Gutian River is an annual-regulating dam and forms the Gutian Reservoir. There are two daily regulating dams in the Jian River and Futun River. In previous investigation, *S. macrops* was a widely distributed fish in the Min River basin (Lian, 1988). In freshwater river ecosystems in East Asia, there are a large number of resident freshwater fish species similar to *S. macrops*. In this study, the fish samples were collected from *S. macrops* populations, both upstream and downstream of dams in the Min River and its tributaries, and used the mtDNA control region (D-loop) partial sequence genetic marker to explore genetic diversity and structure. The objective of our study were: To detect the different effects of dam types (high dam and low dam) on genetic diversity of resident freshwater fish.

2. Materials and methods

2.1. Sample collection

Samples of *S. macrops* (n = 160 specimens) were collected in April 2011 and November 2012 from six sites in the Min River Basin (Table S1), called (1) Jianyang (JY) in the Jian River, (2) Nanping (NP) in the mainstream of Min River Basin, (3) Shaowu (SW) and (4) Weimin (WM) in the Futun River, (5) Yongtai (YT) located in the Dazhang River, and (6) Gutian (GT) which is a reservoir in the Gutian River. The Gutian, Jian, and Futun rivers are the main tributaries of the upstream section of Min River. The Dazhang River is located in the downstream section of Min River. Samples of the GT population were collected from upstream of the high dam, and SW and JY were collected from upstream of the low dam (Fig. 1). A clipping of tail muscle was non-lethally removed from each individual, preserved in 100% ethanol separately and marked with the individual's code, then stored at 4 °C for DNA extraction.

2.2. DNA extraction and amplification

Total genomic DNA was extracted from small sub-samples (100–200 mg) of tissue using improved phenol/chloroform techniques (Sambrook and Russell, 2002). The extracted DNA was dissolved in sterile water and stored at −20 °C for subsequent utilization. Fragments of the mtDNA including the $tRNA^{phe}$ and $tRNA^{Pro}$ genes and a part of the control region, were amplified by polymerase chain reaction (PCR) using the following primers: the forward primer was DL1 (ACC CCT GGC TCC CAA AGC) and the reverse primer was DH2 (ATC TTA GCA TCT TCA GTG) (Liu and Chen, 2003). The final volume after amplification was 50 μl containing a final concentration of 1 × PCR buffer, 0.2 mM dNTP, 0.5 μM of each primer, 1.25 U Taq polymerase, and 375 ng DNA. PCR used the following conditions: 95 °C for 2 min, followed by 40 cycles of 94 °C for 30s, 50 °C for 30s, 72 °C for 1.5 min, followed by an extension at 72 °C for 5 min (Liu and Chen, 2003). The PCR product was run in 1% agarose gel electrophoresis to verify the amplified fragment length with a standard size marker and then visualized with ethidium bromide under ultraviolet light. The remaining PCR products were purified and sequenced by Sangon Biotech Co., Ltd (Shanghai). An approximately 1000 bp length in the variable position of the mtDNA control region was sequenced with an ABI 3730XL DNA Sequencer following the manufacturer's instructions. Both primers, DL1 and DH2, were used for the sequencing reaction. Sequences were edited using the program SeqMan in DNASTAR ver.7.1.0 Software suite and aligned manually using the program ClustalW in MEGA 5.0 Software (Tamura et al., 2011) to obtain the final 907 bp length sequences.

2.3. Data analysis

Specimens were assigned mtDNA haplotypes based on the discrete combinations of nucleotides at polymorphic sites with DnaSP 5.10 (Librado and Rozas, 2009). Population haplotype diversity (Nei, 1987), nucleotide diversity (Tajima, 1993), and