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Genetic differentiation between cultured and wild populations of *Paralichthys olivaceus* based on AFLP markers

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ABSTRACT

Paralichthys olivaceus is a warm-temperate benthic species distributed in the western Pacific Ocean from the Kuril Islands, Japan to the South China Sea. In the present study AFLP (amplified fragment length polymorphism) marker was employed to examine the genetic differences between cultured and wild populations of *P. olivaceus*. The results showed that the gene diversity and Shannon's information index of cultured populations ($h = 0.130–0.150$; $I = 0.201–0.236$) were similar with wild populations ($h = 0.123–0.136$; $I = 0.199–0.220$). Pairwise F_{st} and AMOVA revealed significant genetic differentiation among them, indicating cultured populations exhibited distinctive heterogeneity with wild populations. The results of present study suggested that broods of this study should not be released into natural sea areas due to its genetic alterations.

1. Introduction

Japanese flounder, *Paralichthys olivaceus*, is a benthic and economical flatfish in the family Bothidae, which distributes throughout the western Pacific Ocean from the Kuril Islands, Japan to the South China Sea (Li and Wang, 1995; Masuda and Tsukamoto, 1998). This species is endemic to the western Pacific Ocean, and is the most common flatfish species raised in China, Japan and Korea due to its rapid growth, delicious quality and good adaptability. With the heavy exploitation of the fishery resource, market landings of *P. olivaceus* began to decline since 1970 s, while the aquaculture productions have shown a trend of sustained growth since 1980 s, exceeding fishing productions (FAO, 2008). In order to protect and recover the natural population of *P. olivaceus*, more and more cultured individuals has been released into sea areas (Tomiyama et al., 2008). In Shandong Province of China, the number of released individuals varied from less than 200,000 individuals at 2005 to more than 18,000,000 individuals at 2014.

Some studies have proved that hatchery-reared individuals may have a negative effect on the variability of recipient wild populations (Goodman, 2005; Jonsson and Jonsson, 2006). Hatchery-reared individuals may compete for food, space with native, and even spread parasites and diseases (Jonsson et al., 1991; Bakke et al., 1990; Jonsson and Jonsson, 2006). When hatchery-reared broods are released into nature, their genetic effect on wild populations has also attracted great attention. Reduced genetic diversity of cultured fish has been reported (Kohlmann and Kersten, 1999; Skaala et al., 2004), which can cause a reduction in fitness because mean phenotypes could be shifted by inbreeding (Lynch, 1991). When these hatchery-reared individuals are released into natural waters, they may change the genetic variability of recipient populations

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through reproduction behavior (Bert et al., 2007). So, it is very important to monitor the genetic diversity of released broods to avoid the genetic alteration of recipient wild populations.

Until now various molecular markers have been successfully applied to survey genetic influence of stock enhancement. For example, Araki et al. (2007) found that even a few generations of domestication may have negative effects on natural reproduction in the wild by reconstructing a three-generation pedigree with microsatellite markers and the results suggested that the repeated use of captive-reared parents to supplement wild populations should be carefully reconsidered. Comparative studies on the genetic differences of *Paralichthys olivaceus* between cultured and wild population have been conducted by different molecular markers until now. The screening of genetic variations by earlier allozyme markers revealed significant genetic differentiation between wild and cultured-released populations but no reduced genetic diversity were obtained (Liu et al., 1997). However, recent analysis by allozyme markers, mitochondrial DNA and microsatellite DNA have detected obvious reduced genetic diversity of cultured *P. olivaceus* (Yoshida et al., 2000; You et al., 2001; Sekino et al., 2002).

Amplified fragment length polymorphism (AFLP) analysis is a PCR-based, muti-locus fingerprinting technique that combines the strengths and overcomes the weaknesses of the RFLP and RAPD methods (Vos et al., 1995). It is an effective molecular maker and widely used to study interspecific genetic diversity because it allows fast and efficient generation of a large amount of genetic data (Wang et al., 2000; Liu et al., 2009). It also has been employed to detect loss of the genetic diversity in cultured populations of various fish species, (Wang et al., 2002). Xu et al. (2006) evaluated the genetic diversity of four wild geographical populations of Japanese flounder by AFLP marker and a certain extent of differentiation among them was detected. Moreover, Zhang et al. (2004) found smaller number of total loci for cultured populations and no genetic differentiation between wild and farmed populations by AFLP marker. In the present study, two wild and four cultured populations were collected from Chinese coastal waters to examine the present population genetic diversity and variability of *P. olivaceus*. The results of the present study will reveal the genetic structure and diversity of this economically important species and provide vital information for sustainable exploitation, aquaculture and management of its natural populations.

2. Material and methods

2.1. Fish samples

Two wild populations (Qw and Ww) were collected from nature waters and four cultured populations were collected from hatchery during December 2007 to April 2009. Moreover, one cultured population Rc was obtained from random sampling of cultured stock for releasing in Rongcheng at May 2008 (Fig. 1 Table 1). All individuals were identified based on morphological characteristics, and a piece of muscle tissue was obtained from each individual and preserved in 95% ethanol or directly extracted from frozen samples.

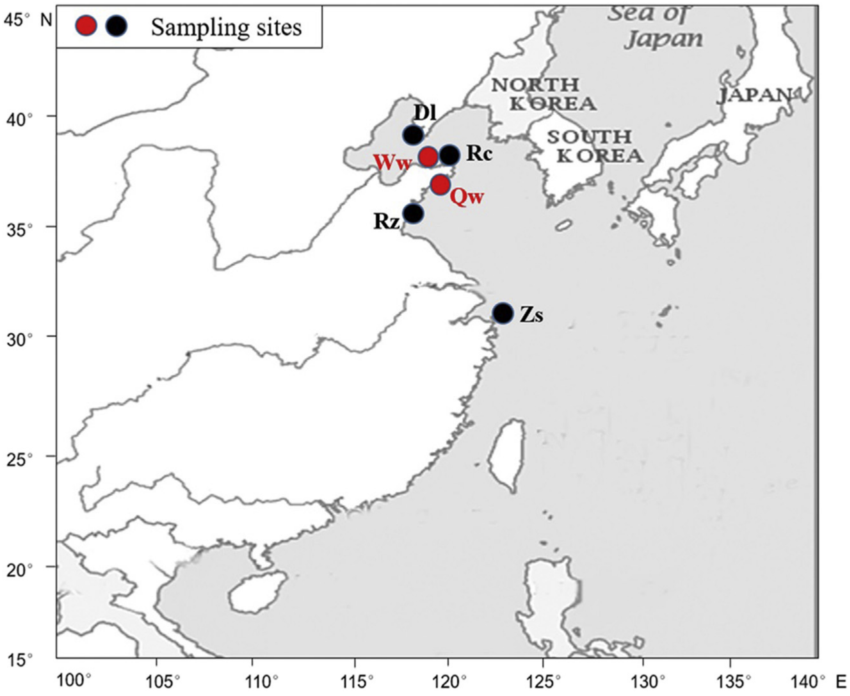

Fig. 1. Map showing sample locations of *P. olivaceus*. Dl: Dalian, Zs: Zhoushan, Rc: Rongcheng, Rz: Rizhao, Ww: Weihai, Qw: Qingdao.