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Using of microsatellite DNA profiling to identify hatchery-reared seed and assess potential genetic risks associated with large-scale release of swimming crab *Portunus trituberculatus* in Panjin, China

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ABSTRACT

Many economically important species in demand in the market and with depleted populations have been artificial bred and their seed intensively released into the wild. As one of the most important fishery species in China, the swimming crab *Portunus trituberculatus* has been stocked in Panjin at a large scale since 2012. Nevertheless, no genetic profile considering genetic effects of these practices has been constructed. The present study traced the crab population enhancement in Panjin. In 2015, a total of 1671 crabs were captured in Panjin and among them 120 individuals were identified as being hatchery-raised, indicating that hatchery-raised seed contributed to the local resource. To address possible genetic effects from the hatchery stock, we compared the genetic characteristic among female broodstock, recaptured hatchery-raised, recaptured non-hatchery-raised and a wild control population based on six microsatellite markers. Results showed obvious reductions in gene diversity and effective population size (Ne) and increased relatedness in recaptured hatchery-raised crabs. Moreover, recaptured non-hatchery-raised – the local population in Panjin – also exhibited similar patterns of dramatic loss of Ne and increased degree of relatedness. The genetic homogeneity involving low Wright's fixation index (Fst), large percentage genetic variance among individuals and strong gene flow appeared in all sample locations distributed in the Bohai Sea and the Yellow Sea. Our data suggest that large-scale stock enhancement of *P. trituberculatus* presents strong potential genetic risks to the Panjin local population and even the whole waters surrounding the Liaodong Peninsula. Several approaches were proposed to gain better insight into *P. trituberculatus* enhancement practices in the future.

1. Introduction

Stock enhancement refers to release of hatchery-reared seed into the natural environment in order to supply the local population by increasing recruitment in fisheries, forestry and wildlife conservation (Blaxter, 2000; Bell et al., 2008). The purpose of stock enhancement includes increasing local population size, improving wild recruitment and promoting ecological balance (Blaxter, 2000; Lorenzen et al., 2010). Stock enhancement programs had applied to more than 180 kinds of marine species (Born et al., 2004). Recently, the number of countries carrying out mass-release programs in overexploited waters was greatly increased. In cultured marine commercial species, the genetic structure and the potential adaptive traits of populations could be significantly disturbed by human compared with natural selection (Frankham, 2008; Nakajima et al., 2013). Hence, there could be negative genetic effects of releasing hatchery-reared juveniles into native habitats (Araki and Schmid, 2010; Christie et al., 2012; Antognazza et al., 2016). Although such releases could improve fishery production to some degree (Kitada and Kishino, 2006; Wang et al., 2014; Kitada, 2016), they might also result in serious threats to genetic biodiversity and population structure, even over several generations (Kitada et al., 2009; Araki and Schmid, 2010; Laikre et al., 2010). To date, several studies attempted to document the genetic effects in stock enhancement programs.

The genetic interactions between hatchery-reared and wild individuals begins to be understood. First, hatchery-released juveniles are usually lack sufficient numbers of broodstock, as they only contain a small portion of the genetic information within the whole population.

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Thus, human selection and genetic drift occurring in the hatchery can lead to differentiation in first-generation stocks even using wild breeders (Matusse et al., 2016). In fish, loss of genetic variation, change in allele frequencies, increased relatedness and loss of rare alleles in hatchery-produced individuals were reported (Kitada et al., 2009; Selly et al., 2014; Bohling et al., 2016). Therefore, if juveniles with such a detrimental genetic background are released into the natural environment, interbreeding between released and wild individuals may alter the genetic composition of populations or even directly replace local wild fish due to the release of huge numbers (Araguas et al., 2008; Christie et al., 2012;). Second, due to the method of culture and costs, the effective population size (Ne) of hatchery-reared juveniles is commonly much lower than that of wild stocks. Supplementing wild populations with hatchery-born individuals can result in a reduction in their Ne, known as the Ryman–Laikre effect (Ryman and Laikre 1991; Waples et al., 2016). Ne is one important population parameter and can help in maintaining population biodiversity. A decrease in Ne will lead to breakdown of local genetic adaptation and this may subject the local population to adverse genetic effects (Christie et al., 2012; Baskett et al., 2013; Morvezen et al., 2016). Third, several studies found that fitness of hatchery and wild individuals might differ due to artificial domestication (Christie et al., 2014, 2016), especially adaptation to the hatchery rather than natural conditions (Ozerov et al., 2016). In some cases, significantly lower reproductive success of hatchery-reared individuals were reported. In salmonids, inbreeding between cultured and wild individuals might cause degeneration of reproductive ability (Araki et al., 2007; Christie et al., 2014). Aside from the adverse genetic effects that might result, environment-friendly seeding programs in marine stock enhancement were recorded: e.g. Red Sea bream (*Pagrus major*), Pacific herring (*Clupea pallasii*) and Honmoroko (*Gnathopogon caerulescens*) (Gonzalez et al., 2015; Kitada et al., 2009; Kikko et al., 2016). A number of empirical studies suggest that suitable stock enhancement should maximize the population gene-level biodiversity by using as many wild breeders as possible, renewing them every year and reducing the differences between hatchery-reared and wild individuals (Taniguchi 2004; Kitada et al., 2009; Morvezen et al., 2016). However, stock enhancement is a complex process – the released amount, juvenile body size, survival, location, environmental conditions and environmental capability may also relate to genetic background of the local population post-release (Bell et al., 2008; Kitada et al., 2009). All such factors should be considered carefully before composing guidelines for implementing enhancement programs.

It is noteworthy that the major efforts in genetic monitoring have focused on commercially important finfish, particular salmonids. However, crustacean species have distinctive genetic composition, life cycle and behaviors (such as multiple mating and storage of sperm in spermathecae of females) (Yue and Chang, 2010; Toonen, 2004). It is uncertain that stock enhancement of crustacean species would lead to the same genetic concerns as for finfish. Meanwhile, there is few reference material on crustacean post-release and very little information about large-scale crustacean enhancement programs. Consequently, genetic risks that may occur with crustacean stock enhancement should be monitored in order to optimize enhancement strategies for restoring sustainable local populations as well as sustaining fishery yields.

The swimming crab, *Portunus trituberculatus* (Crustacea: Decapoda, Brachyura) (Miers, 1876), is a widely-distributed species and inhabits the seafloor sand and pebble areas along the coasts of East Asia from Japan and Korea to China (Dai et al., 1986). This species is an important fishery resource and is considered a delicacy in China, where it is highly valued. Mainly because of high demand and market prices, *P. trituberculatus* populations dramatically collapsed in formerly productive regions of China, such as Panjin, Liaoning Province since in the early 1990s (Yang et al., 2005). Consequently, ministry of agriculture of China initiated crab supplementation programs through large-scale release of hatchery-reared juveniles, representing the second-highest released numbers for a marine species in China. In Liaoning Province, release of millions of hatchery-born *P. trituberculatus* seeds began in early 2012. Fertilized female crabs are caught as broodstock from the wild in April, then used to artificially culture and produce seeds. The female broodstock is renewed each year. The first-generation crabs are cultured until they reach the second crab stage (C_2), carapace width $\geq$6 mm, and then released in coastal waters in June in Liaoning Province. During 2012–2015, commercial hatcheries were implemented and more than 140 million crabs (C_2) were released in Liaoning Province. In particular, five million juveniles were released in Panjin in 2015. Although intensive stocking of this species has been implemented for several years, due to the limits of identification of wild and hatchery-released crabs, the genetic impacts of these activities have not yet been adequately assessed. Here, the term “wild” refers to crabs naturally produced in the wild, although the spawning population may include hatchery individuals, see Kitada et al. (2009). We traced the whole process of *P. trituberculatus* stock enhancement in Panjin in 2015, distinguished the hatchery-born crabs among captured samples and investigated genetic differences among each population according to their origins.

The present study aimed to evaluate whether hatchery-born crabs in Panjin contribute to the local *P. trituberculatus* resource as was expected for the stock enhancement program, whether such hatchery-rearing could maintain natural levels of genetic diversity and thus whether releasing hatchery-reared *P. trituberculatus* juveniles posed a potential risk to wild populations. Our results represented the first study monitoring the genetic effects of *P. trituberculatus* post-release using microsatellite DNA markers in China and would enable new insight into large-scale stock enhancement of crustaceans.

2. Materials and methods

2.1. Animal rights statement

All experiments in this study were approved by the Animal Care and Use Guideline of the Fishery Resources Enhancement Laboratory at Dalian Ocean University and were performed according to the regulations established by this laboratory.

2.2. Crab samples

In April 2015, 200 wild-fertilized female *P. trituberculatus* were obtained from Donggang waters (Fig. 1). During the artificial maturation period, they were fed nereis and bivalves to satiation. Water quality was constantly monitored throughout the holding period and maintained at temperature 20 ± 4 °C, salinity 30.0 ± 2.0‰, dissolved oxygen (DO) 5.3–7.4 mg/l, pH 7.4–8.2 and shaded. On 18 May, 50 egg-carrying females were chosen from the above 200 individuals. To avoid uncontrolled spawning, two females were placed in each of 25 15-m^3 spawning tanks and allowed to spawn overnight. After one-time spawning, all female crabs were removed and the hatched larvae were reared in the same tanks through four zoeal stages (Z_1-Z_4), the megalopal stage (M) and crab stages until they reached the second crab stage (C_2). The routine culture procedure included feeding with commercial pellets and yeast for Z_1-Z_4, and artemia for M-C_2. In addition, water quality was maintained at temperature gradually increase from 20 to 27 °C, but declining to 16 °C before final releasing, salinity 30.0 ± 2.0‰, DO 5.3-7.4 mg/l, pH 7.4-8.2 and photoperiod 14/10 h of light/dark. On 10 June, a total of 5.02 million first-generation offspring were released in Panjin. Shortly after, during July–October 2015, a total of 1671 *P. trituberculatus* samples were caught in Panjin coast waters during recapture investigations following release (Supplementary Table S1 for recapture sample information). Moreover, we also collected 44 samples in Changxing Island waters (carapace width at 120–176 mm), which were free from any crab releases as the wild control group in October 2014. Totally, 1765 crabs comprising 50 female broodstock samples, 1671 recaptured samples and 44 wild control