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Reproductive performance of one-year-old Pacific abalone (*Haliotis discus hannai*) and its crossbreeding effect on offspring growth and survival

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ABSTRACT

The Pacific abalone (*Haliotis discus hannai*) is one of the most popular farming mollusks in China, where three- to four-year-old adults are used as broodstock for commercial seeding. However, no reports exist regarding the reproduction performance of one-year-old Pacific abalone and its potential use in commercial breeding. In this study, one line of one-year-old abalone (AO) and two lines of three-year-old abalone (BD and HD), which were selected for rapid growth for three or six generations, were adopted to establish three purebred groups (AO, BD and HD) and two crossbred groups (BDAO and HDAO). The performance of these five groups on embryonic development, growth and survival rate was evaluated. According to the results, the average yolk diameter of oocytes produced by one-year-old abalone was significantly smaller than that produced by three-year-old individuals. The fertilization rate of the AO group was significantly higher than those of the other four groups, while the metamorphosis rate was the opposite. During the first 40 d, the average shell growth rate of the AO group was slower than those of the other four groups, while the average shell length of this group was greatest after 120 d. The AO group also showed a significantly higher survival rate than those of other groups during the period from 38 d to 160 d post fertilization. No significant differences were found in the embryonic development parameters or shell growth rates between the purebred and crossbred groups that shared the same dam line. However, the survival rates of the crossbred groups were significantly higher than those of the purebred groups. These results suggest that one-year-old Pacific abalone could be used as broodstock for reproduction and that an accelerated breeding cycle may enhance commercial genetic improvement programs.

1. Introduction

The annual world production of farmed abalone is increasing, with most of the increases coming from China (Gordon and Cook, 2013), where the Pacific abalone (*Haliotis discus hannai*) accounts for most of the annual production. Increasing output is inseparable from improving the technology of breeding and aquaculture. Taking advantage of hybrid vigor by crossbreeding is a typical approach to yield improvement in commercial abalone breeding (Nie and Wang, 2004; Zhang et al., 2004). Luo et al. (2006) conducted intraspecific hybridization between *H. sieboldii* and *H. discus discus* and observed hybrid vigor in the shell growth rate. Although the fertilization rate between *H. discus hannai*♀ × *H. fulgens*♂ was significantly lower than in purebreds, their hybrids showed hybrid vigor for growth and survival, as well as for tolerance to summer high temperature (You et al., 2015). However, the most widely used commercial crossbreeding approach is intraspecific hybridization between different geographic populations. Intraspecific crosses of wild *H. discus hannai* between Chinese ♀ × Japanese ♂ populations exhibited significant heterosis for both growth and survival (Deng et al., 2007; Deng et al., 2010). The F_1 hybrids of these two populations have become the most favored seeds in the abalone farming industry in China since 1997 (Zhang et al., 2004). Unfortunately, wild sources of the Pacific abalone in China are declining, thus limiting the output of F_1 hybrids. As a result, increasing numbers of cultured broodstocks have been used in abalone seed production. Although genetic diversity may decline during domestication (Dixon et al., 2008; Rhode et al., 2014; Taris et al., 2007), domesticated animals may

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perform better than their wild counterparts in artificial cultivation environments, as domesticated lines are likely to possess advantages in local adaptation (Nhan et al., 2009).

Independent domesticated lines of mollusks for conducting cross-breeding were studied in shellfish, such as the Pacific oyster (Hedgecock and Davis, 2007; Hedgecock et al., 1995) and sea scallops (Zhang et al., 2007; Zheng et al., 2011; Zheng et al., 2006). These studies revealed that crossing between different lines may be a reliable way to acquire hybrid vigor, or heterosis, in breeding mollusks. However, this approach was not found in studies of abalone breeding. In addition, broodstocks of Pacific abalone used in commercial seeding are usually three to four years old, which is a relatively long time interval for selective breeding. Much more time is required to realize genetic improvements in abalone by simple selection compared to oysters and scallops, which can be used for reproduction at an age of one year (Wang et al., 2012; Zheng et al., 2011). No studies have been performed on the reproductive performance of one-year-old abalone or their use in commercial breeding.

The abalone's gonad can reach maturity at a young age while the shell length is much smaller than in adults. Wild-caught female and male donkey's ear abalone (*H. asinina* Linné) could reach maturation at a shell length of 40.6 mm (Capinpin et al., 1998). Awaji and Hamano (2004) found that Pacific abalone could reach full maturity when the shell length was approximately 40 mm. In this research, we studied the reproduction performance of one-year-old Pacific abalone (shell length approximately 57 mm) and evaluated the embryonic development, growth and survival performance of offspring from different selected lines and their F_1 hybrids.

2. Materials and methods

2.1. Broodstocks

Three lines of Pacific abalone, BD, HD and AO, were used as broodstocks (Table 1) to produce purebred and crossbred offspring. The BD stock, which originated from the wild population in China, has undergone six generations of mass selection for rapid growth since 1999. The HD stock, which originated from F_1 hybrids of the wild population (♀) in China × the wild population in Japan (♂), has also undergone six generations of mass selection for rapid growth since 1999. The AO stock, which has the same genetic origin as the HD stock, has undergone intensive mass selection for rapid growth at an age of one year for three generations.

2.2. Experimental design and offspring cultivation

Broodstocks from these three lines were transferred to the brood conditioning center at the National Engineering Research Center of Marine Shellfish in the city of Weihai in December 2014. Broods were fed kelp (*Laminaria japonica*) and conditioned there for four months before spawning. Over 50 female and 30 male broods with well-developed gonads from each line were induced to spawn in UV-irradiated seawater. Only one individual was put into each hatching incubator (10-liter food-grade polyethylene tank) to make sure that the number of spawned female and male broods exceeded 50 and 30, respectively. Eggs and sperm from each line were collected separately. Eggs from the BD and HD lines were equally divided into two samples; one sample was then fertilized by sperm from its own line and the other sample was fertilized by sperm from the AO line. All of the AO eggs were fertilized by sperm from their own line. Thus, three purebred lines, BD, HD and AO, and two crossbred lines, BDAO and HDAO, were established.

The hatched larvae were incubated at 22 °C in the hatchery room for 3 days, after which they were transferred to 4 m^3 cement ponds that contained pre-conditioned plastic plates coated with benthic diatoms. Larvae completed metamorphosis in these ponds and fed on benthic diatoms for 34 days, growing to a length of approximately 3 mm. Subsequently, juvenile abalone were detached from the plastic plates and resettled in the ponds, which were covered with tiles on the bottom (30 cm × 40 cm). Thereafter, juvenile abalone were fed an artificial diet (Golden Brand, Yantai, China) until the end of the experiments. The initial stock density of each line was adjusted to the same level. At 75 d and 110 d, some of the juveniles were removed from the ponds to reduce the stock density. The removed juveniles were not included in later survival rate calculations. Three replicates were carried out for each experimental group.

2.3. Data collection

Approximately 50 eggs from each line were collected for egg envelope and yolk diameter measurements using an ocular micrometer fitted to a compound optical microscope. One-and-a-half hours after fertilization, the numbers of fertilized (having completed the first cell division) and unfertilized eggs were counted and used to calculate the fertilization rate. To measure the metamorphosis rate, 100 fully shelled veligers (24 h post hatching) were transferred into separate beakers that contained pre-conditioned plastic plates (10 cm × 10 cm) coated with benthic diatoms. One week later, the individuals that completed metamorphosis were counted and used to calculate the metamorphosis rate.

The shell length was measured at 15 d using an ocular micrometer fitted to a compound optical microscope. After 40 d, the shell length was measured with a vernier caliper. The wet weight at 150 d and survival rate during the artificial diet feeding period (from 38 days to 160 days post fertilization) were also measured. 100 individuals were randomly sampled in each group and used for shell length and/or wet weight measurement. Based on these data, the mid-parent (H_M) and single-parent heterosis (H_D, relative to the dam line) of the two hybrid groups for the average shell length (ASL), average wet weight (AWW) and survival rate were calculated as follows:

$$H_M\% = \frac{F-P}{P} \times 100$$

$$H_D\% = \frac{F-P_D}{P_D} \times 100$$

where F = the average phenotypic value of the hybrids, P = the average phenotypic value of the two parental groups and P_D = the phenotypic value of the purebred dam line.

Table 1
The average shell lengths, wet weights and ages of three lines of broodstocks sampled before spawning.

Lines	Average shell length (mm)	Average wet weight (g)	Age (month)
AO	57.29 ± 5.69	24.42 ± 6.83	12
HD	87.31 ± 9.64	85.39 ± 15.30	36
BD	86.67 ± 9.35	84.20 ± 14.16	36

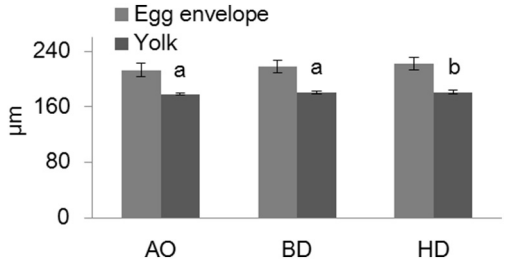

Fig. 1. The average egg envelope and yolk diameters of oocytes spawned by three lines of female abalone. The different letters above the dark grey bars indicate significant differences ($P < 0.05$) in average yolk diameters.