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Inbreeding Depression on Growth and Survival of Full-Sib Family of Manila Clam (*Ruditapes philippinarum*)

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Abstract In present study, the inbreeding depression (*ID*) of growth and survival of Manila clam (*Ruditapes philippinarum*) was investigated at larval and juvenile stages. Nine inbred families (A_2, B_2, C_2, D_2, E_2, F_2, G_2, H_2 *and* I_2) were established by mating within nine full-sib families with expected inbreeding coefficient of 0.25. Inbred families showed significant differences in shell length and hatching rate of D-larvae (straight-hinged larvae). The larvae of the nine inbred families grew slower than those of control group (*CG*), and their *ID* value ranged from 0.81%±6.09% to 16.10%±1.49%. The *ID* value of larval survival rate varied between 27.47%±9.36% and 70.50%±13.66%. The *ID* was also detected for juvenile growth in A_2, B_2, C_2, and D_2, which ranged from 4.60±2.21 to 17.71±7.73. The A_2 family maintained the highest juvenile survival rate, whereas the other inbred families exhibited *ID* values varying between 62.79%±4.54% and 96.14%±0.87%. The linear relationship of estimated *ID* between growth and survival was negatively correlated ($R=-0.434$, $P<0.05$). The results of this study suggested that the *ID* of growth was common at the larval stage but was less prevalent at juvenile stage. In contrast, the *ID* of survival increased from larval to juvenile stage. A better understanding of the effect of inbreeding may aid to selective breeding of Manila clam.

Key words *Ruditapes philippinarum*; growth; survival; inbreeding depression

1 Introduction

Manila clam (*Ruditapes philippinarum*) distributes mainly along the coasts of the Pacific and Indian Oceans and is now cultured for commercial purposes in a number of European, American, and Asian countries (DOF, 2014; Huo *et al*., 2014; Nie *et al*., 2015b). In China, the production of this species accounts for 73% of the total mudflat shellfish, and more than 3 million tons are produced each year (Nie *et al*., 2015a; Zhu *et al*., 2015).

Research on inbreeding effect is helpful for a better understanding of the genetic breeding and evolutionary biology. Commonly, inbreeding leads to two main opposite results, inbreeding depression (*ID*) and inbreeding purification. *ID* reduces the fitness relative to random-bred individuals. In contrast, inbreeding purification, a favorable aspect of breeding, has been used in agricultural breeding to 'purify' the breed by concentrating the good genes and increasing the uniformity of offspring (Taris *et al*., 2007).

Harmful effects of inbreeding have been pervasive in numerous animal and plant species in many centuries. Most studies examining the effects of inbreeding among oyster, scallop, and clam species have evaluated progeny of one or more generations of full-sib crosses with expected inbred coefficients of 0.0625, 0.203, 0.25, and 0.5 (Longwell and Stiles, 1973; Mallet and Haley, 1983; Beattie *et al*., 1987; Bierne *et al*., 1998; Hedgecock *et al*., 1995; Bucklin, 2002; Langdon *et al*., 2003; Zheng *et al*., 2008). These studies reported significant *ID* of growth and/or survival at larval and adult stages. However, not all inbred populations experience *ID* (Lannan, 1980; Beattie *et al*., 1987; Falconer and Mackay, 1996). The effects of inbreeding have become more apparent as shellfish breeders attempt to develop selected lines from purebred stocks via inbreeding (Saavedra, 1997; Hedgecock and Sly, 1990; Hedgecock *et al*., 1995), yet the genetic bases of these phenomena remain poorly understood (Husband and Schemske, 1996; Charlesworth and Charlesworth, 1999). Little is known about the effects of inbreeding on Manila clam at different developmental stages. The present study is the first of examining the effects of inbreeding on growth and survival in Manila clam.

The goal of this study was to quantify the effects of inbreeding of Manila clam on its production traits, growth, and survival within full-sib families. A better understand-

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ing of the effects of inbreeding on growth and survival traits among inbred families will permit designing selective breeding programs for this species.

2 Materials and Methods

2.1 Experimental Design and Treatments

The present study was conducted using nine full-sib families (A_1, B_1, ···, I_1) at the age of one year, which were produced by employing the single mating design with parental breeders sampled from nature. The control brood stock was obtained from natural population. After being dried for 8 h, parents from the nine families each (50 clams each) and from the control group (*CG*, a natural population, 50 clams) were placed separately in 100 L buckets full of fresh seawater at 25℃, salinity 28, and pH 8.0 to reproduce. Ultimately, nine inbred families were produced and named A_2, B_2, ···, I_2, respectively (Fig.1).

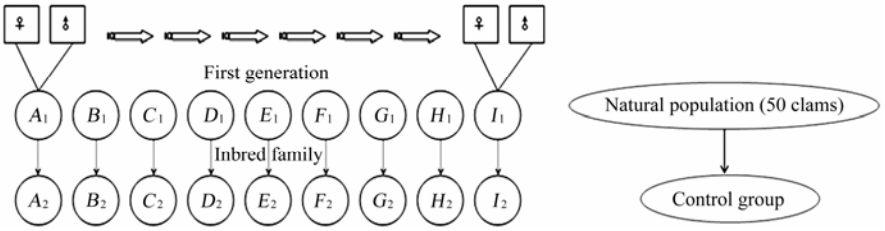

Fig.1 Mating strategy of full-sib families and control group of Manila clam.

The fertilized eggs each group were hatched at an initial density of 40–50 eggs mL^{-1}. After about 24 h, the fertilized eggs developed into D-larvae (straight-hinged larvae). D-larvae each group were separated into three portions (replicates). Larvae and juveniles of all inbred families and *CG* were reared at the same density, temperature and salinity, and fed the same diet. Larval rearing, juvenile nursery, and grow-out conditions were the same as those previously described by Yan *et al*. (2006). Initially, the D-larvae density each replicate bucket was 5 or 6 larvae mL^{-1}. This was reduced with larval growth to 2 or 3 larvae mL^{-1} after metamorphosis. During the larval rearing, 100% of the water was exchanged with sand-filtered seawater every day, and the temperature was maintained at 25–28℃. Salinity was kept at 28–29, pH at 8.00, and light intensity at 500–1000 lux. The larvae were fed with *Isochrysis galbana* during days 1–3, and with a mixture of *I. galbana* and *Chlorella* spp. (2:1 w/w) from day 4 to juvenile stage. The feeding ration was increased with larval development. Larvae were fed a ration of 2000–10000 cells mL^{-1} d^{-1} on days 1–3, and 30000–60000 cells mL^{-1} d^{-1} from day 4 to juvenile stage. This was to ensure the same density condition which was adjusted every three days.

After 60 d, the juveniles were transferred to nursery ponds (775 m × 200 m × 1.2 m) and placed in bags with a mesh size of 700 μm. The stocking densities were adjusted every 15 d, starting with 100–150 clams per bag and ending with 30–50 clams per bag. During this period, the water temperature was 19–22℃, salinity was at 26–28, and pH was at 7.80–8.00.

2.2 Sampling and Measurement

To evaluate growth, 30 individuals per group at D-stage were killed using 4% formaldehyde on days 3, 6, 9, and 30, and then the shell length (longest anterior-posterior dimension) was measured using a microscope (100×). At days 60 and 100 after hatching, 30 live individuals per group were sampled and the shell length was measured using a Vernier caliper (0.02 mm accuracy). The hatching rate was estimated as the ratio of the number of normal D-stage larvae to that of fertilized eggs. The survival rate was calculated as the ratio of the number of larvae or juveniles to that of larvae at D-stage on days 3, 6, 9, 30, 60, and 90. At each time point, the number of fertilized eggs, normal D-stage larvae, and planktonic larvae was counted in three 1 mL samples (D-stage larvae had been killed using 4% formaldehyde) using a microscope (20×); the average of the three samples was calculated and used to extrapolate to the total volume. For juveniles, on days 30, 60, and 90, sieves with a mesh size of 300 μm were used to gather the juveniles from each family into 500 mL beakers. Moisture was blotted from the surface of the juveniles with absorbent paper. The gross weight was measured using an analytical balance (0.0001 g precision), and 1 g of juveniles was then removed. The number of juveniles in the 1 g sample was counted using a stereomicroscope (20×) and was used to extrapolate to the total volume. Three replicates were used to determine the growth and survival.

2.3 Statistical Analysis

Statistical differences in shell length, hatching rate, and survival rate among the ten experimental groups were analyzed using one-way analysis of variance (ANOVA) and multiple comparison tests (Student-Newman-Keuls (SNK) method). Differences in the magnitude of *ID* for all traits between the nine inbred families were analyzed by *t*-test, respectively.

In this study, based on the mean phenotypic value of inbred families and the *CG*, the magnitude of the effect of *ID* on the growth and survival of inbred families was estimated (Cronkrak and Roff, 1999). The formula is as follow:

$$\delta_X\% = 1 - \frac{S_x}{P_x} \times 100,$$

where δ_χ% is the estimate of the magnitude of *ID* for inbred family χ; S_x is the phenotypic value of inbred family χ; and P_x is the phenotypic value of the *CG*. All statistical analyses were conducted using SPSS 16.0 software, and the significance for all analyses was set to $P<0.05$ unless noted otherwise.

3 Results

3.1 Hatching Rate and Growth

D-larvae size and hatching rate differed among the experimental groups (Fig.2). The D-larvae size of F_2 was significantly larger than that of the other experimental groups ($P<0.05$). The SNK comparisons showed that the D-larvae size of *CG* was significantly smaller than that of inbred families ($P<0.05$), except for D_2 ($P>0.05$).

The hatching rate of A_2 (91.67% ± 1.53%) was the highest, and that of *CG* was the lowest. The SNK comparisons showed that the difference in hatching rates was not significant among inbred families A_2, B_2, C_2, D_2, F_2, G_2, and I_2 ($P>0.05$). However, the hatching rate differed significantly between *CG* and these seven inbred families A_2, B_2, C_2, D_2, F_2, G_2, and I_2 ($P<0.05$), but not between *CG* and H_2 ($P>0.05$). The hatching rate of E_2 was significantly lower than that of A_2, B_2, and G_2 ($P<0.05$) but significantly higher than that of *CG* ($P<0.05$). The difference in hatching rates was not significant among inbred families C_2, D_2, E_2, F_2, and I_2 ($P>0.05$).

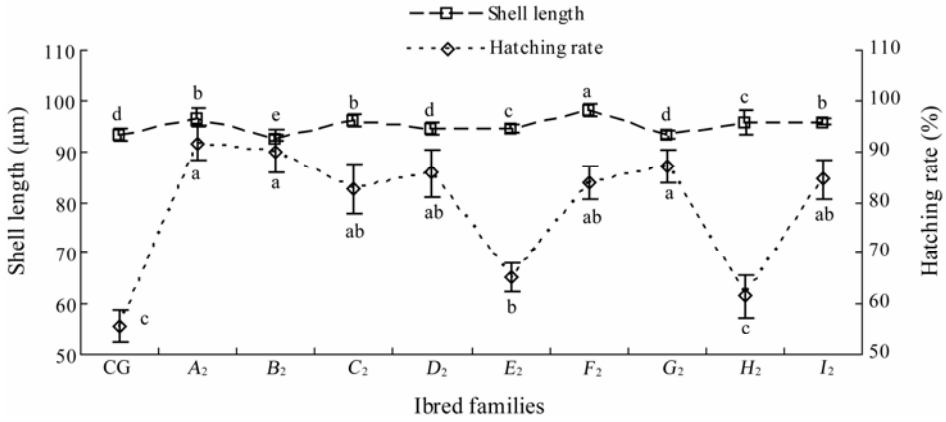

Fig.2 D-larvae size and hatching rate of nine inbred families and control group. Within each line, means with the same letter are not statistically different ($P>0.05$).

In Table 1, the growth data for the larvae of nine inbred families and *CG* are summarized. On days 3 and 6, the larval shell length was significantly larger in B_2 than that in other groups, and the larval shell length was significantly smaller in D_2, I_2, and A_2 than that in *CG* ($P<0.05$). On day 9, the larval length was larger in *CG* than that in the inbred families. The SNK comparisons showed that the difference in larval shell length was statistically significant between *CG* and inbred families ($P<0.05$).

Growth differences for the juveniles are summarized in Table 1. On day 30, the juvenile shell length of inbred family A_2 was significantly smaller than that of *CG* ($P<0.05$). On day 60, the shell length of I_2 was larger than those of other groups. The SNK comparisons showed that the difference in juvenile shell length was not significant among families I_2, F_2, G_2, B_2, D_2, E_2, and H_2 ($P>0.05$), but was significant between I_2 and *CG* ($P<0.05$). On day 90, the shell length of I_2 was still larger than those of other experimental groups. The SNK comparisons showed that the difference in juvenile shell length was not significant among groups I_2, H_2, F_2, G_2, D_2, *CG*, and E_2. In addition, juvenile shell length of A_2 was always the smallest among all experimental groups. The SNK comparisons showed that shell length of A_2 juveniles was significantly smaller than that of *CG* throughout the whole juvenile stage.

Table 1 One-way ANOVA results for growth of inbred families ($Fx=0.25$) at different ages

Family	Shell length at larval stage (μm)			Shell length at juvenile stage (mm)		
	Day 3	Day 6	Day 9	Day 30	Day 60	Day 90
A_2	102.33 ± 12.28^{g}	128.00 ± 26.22^{e}	172.67 ± 21.12^{d}	0.50 ± 0.09^{e}	1.32 ± 0.45^{e}	2.57 ± 1.18^{d}
B_2	123.83 ± 10.50^{a}	160.83 ± 14.77^{a}	186.33 ± 26.22^{d}	0.62 ± 0.12^{c}	1.48 ± 0.55^{ab}	2.69 ± 1.29^{cd}
C_2	120.00 ± 8.09^{b}	143.83 ± 12.01^{bcd}	190.67 ± 22.43^{bc}	$0.59\pm0.13c^{d}$	1.39 ± 0.59^{cd}	3.05 ± 1.79^{cd}
D_2	112.33 ± 6.91^{f}	134.17 ± 25.80^{d}	178.33 ± 33.74^{d}	0.55 ± 0.12^{de}	1.43 ± 0.54^{abc}	3.39 ± 1.32^{ab}
E_2	114.67 ± 11.50^{e}	148.17 ± 21.18^{bc}	175.67 ± 37.09^{d}	0.63 ± 0.15^{c}	1.62 ± 0.66^{abc}	3.54 ± 1.82^{bc}
F_2	114.83 ± 4.31^{d}	142.5 ± 14.11^{bcd}	172.33 ± 30.51^{d}	0.53 ± 0.20^{de}	1.81 ± 0.91^{ab}	4.18 ± 1.49^{a}
G_2	118.67 ± 9.64^{cd}	135.17 ± 13.8^{cd}	182.00 ± 26.18^{bcd}	0.62 ± 0.15^{c}	1.78 ± 0.87^{ab}	4.06 ± 2.11^{a}
H_2	112.83 ± 9.53^{e}	137.83 ± 22.43^{bcd}	208.00 ± 20.41^{b}	0.82 ± 0.25^{a}	1.58 ± 0.62^{abc}	4.25 ± 2.15^{a}
I_2	107.33 ± 7.4^{f}	125.33 ± 10.74^{e}	176.50 ± 26.17^{d}	0.74 ± 0.16^{b}	1.85 ± 0.68^{a}	4.87 ± 1.59^{a}
CG_2	119.51 ± 6.20^{bc}	154.10 ± 11.21^{b}	208.00 ± 22.41^{a}	0.57 ± 0.08^{cd}	1.54 ± 0.52^{bcd}	3.50 ± 1.03^{ab}

Note: Different letters within each line indicate that the means are significantly different ($P<0.05$).

3.2 Survival

Table 2 shows the survival results for the larvae and juveniles. The survival rate of A_2 was always significantly higher than that of *CG* throughout the larval and juvenile stages. From day 6, the survival rate of inbred families B_2, C_2, D_2, E_2, F_2, G_2, H_2, and I_2 were always significantly lower than that of *CG*.

Table 2 One-way ANOVA results for survival of inbred families ($Fx=0.25$) at different ages

Family	Days after hatching					
	Day 3	Day 6	Day 9	Day 30	Day 60	Day 90
A_2	94.54 ± 3.62^{a}	87.80 ± 5.80^{a}	69.05 ± 5.50^{a}	54.42 ± 4.16^{a}	45.17 ± 2.65^{a}	36.74 ± 1.53^{a}
B_2	91.00 ± 3.55^{a}	30.50 ± 1.20^{e}	19.13 ± 1.32^{c}	12.47 ± 2.92^{e}	7.69 ± 3.51^{e}	3.82 ± 1.52^{c}
C_2	35.90 ± 5.15^{g}	15.50 ± 4.50^{d}	10.50 ± 2.50^{c}	8.07 ± 1.94^{b}	6.00 ± 1.53^{c}	3.00 ± 1.00^{c}
D_2	48.00 ± 1.20^{f}	21.15 ± 1.45^{d}	8.88 ± 1.15^{de}	5.43 ± 1.00^{d}	3.71 ± 1.53^{d}	1.74 ± 0.53^{d}
E_2	85.76 ± 4.40^{b}	21.95 ± 7.60^{f}	12.07 ± 1.94^{de}	8.78 ± 2.62^{c}	6.38 ± 1.00^{c}	3.21 ± 1.53^{c}
F_2	66.67 ± 1.40^{d}	29.33 ± 2.40^{d}	16.84 ± 2.36^{d}	11.22 ± 4.01^{d}	7.63 ± 1.53^{d}	3.61 ± 1.08^{d}
G_2	47.11 ± 1.40^{f}	12.78 ± 3.50^{f}	2.67 ± 1.38^{f}	1.42 ± 0.52^{f}	0.82 ± 0.22^{f}	0.34 ± 0.11^{f}
H_2	36.50 ± 2.85^{g}	24.56 ± 5.40^{c}	8.33 ± 1.26^{de}	4.95 ± 1.34^{e}	3.13 ± 0.52^{e}	1.51 ± 0.52^{cd}
I_2	55.56 ± 1.65^{e}	9.22 ± 1.72^{g}	2.81 ± 2.33^{e}	1.55 ± 0.02^{e}	0.95 ± 0.03^{e}	0.45 ± 0.03^{cd}
CG_2	78.62 ± 1.12^{c}	59.22 ± 3.24^{b}	38.00 ± 2.12^{b}	29.38 ± 1.48^{a}	22.53 ± 1.64^{b}	10.89 ± 1.36^{b}

Note: Different letters within each line indicate that the means are significantly different ($P<0.05$).

3.3 *ID* of Growth and Survival

Results of *ID* of growth are shown in Fig.3. The *ID* value ranged from 0.81%±0.09% to 16.10%±1.49% for nine inbred families at larval stage. In comparison, *ID* on juvenile growth was found only in families A_2, B_2, C_2, and D_2. The highest *ID* on growth occurred in A_2 throughout larval and juvenile stages.

The *ID* of survival is shown in Fig.4. *ID* of survival was not detected in A_2, but *ID* values in other inbred families ranged from 27.47%±9.36% to 70.50%±13.66% for larvae and 62.79%±4.54% to 96.14%±0.87% for juveniles. *ID* of growth and *ID* of survival (Fig.5) were negatively correlated significantly ($R=-0.434$, $P<0.05$).

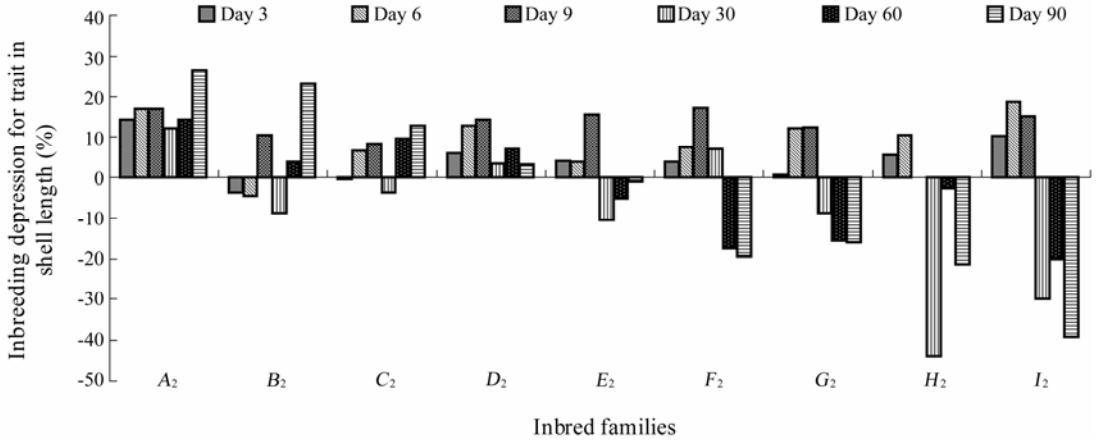

Fig.3 Comparisons of inbreeding depression of growth of inbred families at larval and juvenile stages.

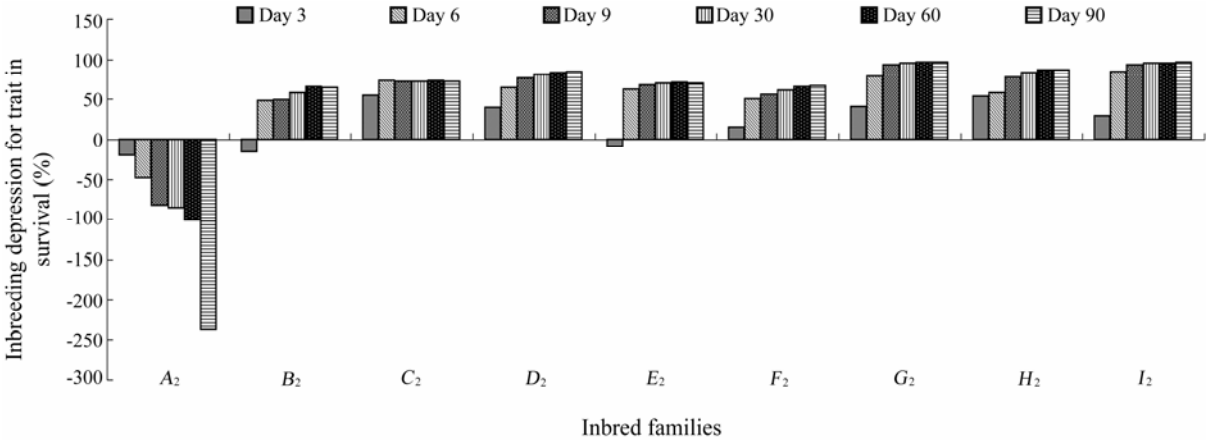

Fig.4 Comparisons of inbreeding depression of survival of inbred families at larval and juvenile stages.

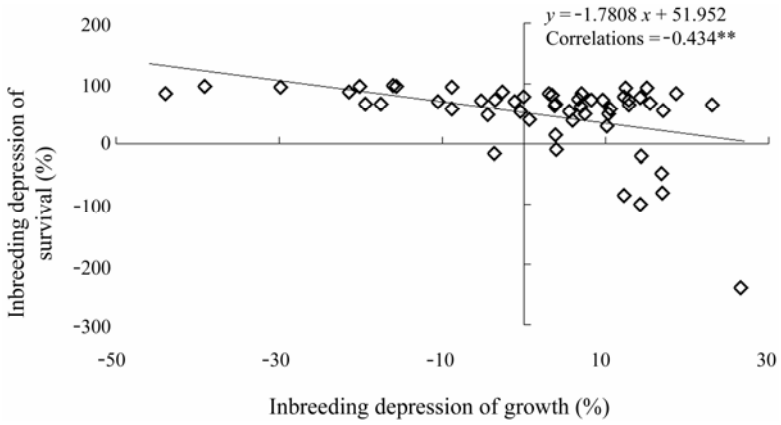

Fig.5 Correlations between inbreeding depression of growth and inbreeding depression of survival.

4 Discussion

In present study, the differences in D-larvae size and hatching rate among inbreeding families were observed. These differences are attributable mainly to maternal effects. For mollusk species, maternal effects on fitness traits such as growth and survival are common during the larval stage due to the variation of egg quality. The amount of energy reserves in the form of lipids in eggs has been shown to be a strong determinant of success at the early stages of larval development (Cragg and Crisp, 1991). Maternal effects have been observed in hybrid experiments in oyster *Crassostrea virginica* in which such effects were clearly observed on 6-day-old larvae (Newkirk *et al*., 1977). In addition, Cruz and Ibarra (1997) reported a significant maternal effect on the growth of scallop *Argopecten circularis*larvae on day 11. Deng *et al*. (2005) found that maternal effects were absent in Pacific abalone *Haliotis discus hannai* before day 10.

Harmful effects of inbreeding have been noticed in numerous animal and plant species for many centuries. *ID* arises both from the expression of partially recessive deleterious alleles and from the loss of heterozygosity. Understanding the importance of inbreeding in shellfish is essential to designing efficient long-term breeding strategies (Evans *et al*., 2004). *ID* is more likely to occur for traits related to growth and survival, as indicated by previous studies in *C. virginica* (Longwell and Stiles, 1973), *Pecten maximus* (Beaumont and Budd 1983), *Ostrea edulis* (Bierne *et al*., 1998), *H. discus hannai* (Deng *et al*., 2005), and *Crassostrea gigas* (Hedgecock *et al*., 1995; Launey and Hedgecock, 2001).

In present study, the larval growth in inbred families (F = 0.25) was significantly lower than that in *CG* on day 9, and the *ID* values ranged from 0.81% ± 6.09% to 16.10% ± 1.49% for the inbred families at larval stage. These results suggested that larval growth was strongly affected by the inbreeding within full-sib families.

ID of juvenile growth was found in families A_2, B_2, C_2, and D_2 but not in other five. This revealed that *ID* of growth was common at larval stage but was less prevalent at juvenile stage. These results were consistent with the reported (Evan, 2004) in Pacific oyster *C. gigas*, which revealed a small amount of depression of growth among families with F = 0.203 and F = 0.625 during two growing seasons because the lethal recessive alleles were eliminated during larval stage. Manila clam has a larval cycle that is roughly 10 d long, and it is possible that slow growing larvae may die before reaching metamorphosis because of the lethal recessive genes. In contrast, fast growing larvae with perfect developmental function likely are maintained and grow into juveniles.

The survival rate of inbred families was lower than that of *CG*, except for A_2 which had the highest survival rate at both larval and juvenile stages. *ID* occurred in the inbreeding families at values varying between 27.47% ± 9.36% and 70.50% ± 13.66% for larvae and 62.79% ± 4.54% and 96.14% ± 0.87% for juveniles. Survival is a trait closely associating with fitness (Falconer and Mackay, 1996). Several studies have evidenced that *ID* is a fitness trait and shows detrimental inbreeding effect including lower viability and survival, lower resistance to disease, and high abnormalities (Beaumont and Budd, 1983; Stiles and Choromanski, 1995; Bucklin, 2002; Zhang *et al*., 2003; Winkler and Estévez, 2003; Zheng *et al*., 2008). In addition, the *IDs* of growth and survival observed in this study were negatively correlated, which means that *ID* of survival will decrease as *ID* of growth increases.

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References

Beattie, J. H., Perdue, J., Hershberger, W., and Chew, K., 1987. Effects of inbreeding on growth in the Pacific oyster *Crassostrea gigas*. *Journal of Shellfish Research*, **6**: 25-28.

Beaumont, A. R., and Budd, M. D., 1983. Effects of self-fertilization and other factors on the early development of the scallop *Pectenmaximus*. *Marine Biology*, **76**: 285-289.

Bierne, N., Launey, S., Naciri-Graven, Y., and Bonhomme, F.,

1998. Early effect of inbreeding as revealed by microsatellite analyses on *Ostreaedulis* larvae. *Genetics*, **148**: 1893-1906.

Bucklin, K. A., 2002. Analysis of the genetic basis of inbreeding depression in the Pacific oyster *Crassostrea gigas*. PhD thesis, University of California Davis, Davis, CA, USA, 140pp.

Charlesworth, B., and Charlesworth, D., 1999. The genetic basis of inbreeding depression. *Genetic Research*, **74**: 329-340.

Cronkrak, P., and Roff, D. A., 1999. Inbreeding depression in the wild. *Heredity*, **83**: 260-270.

Cruz, P., and Ibarra, A. M., 1997. Larval growth and survival of two Catalina scallop (*Argopecten circularis* Sowerby, 1835) populations and their reciprocal crosses. *Journal of Experimental Marine Biology and Ecology*, **212**: 95-110.

Deng, Y. W., Liu, X., Zhang, G. F., and Guo, X. M., 2005. Inbreeding depression and maternal effects on early performance of pacific abalone. *North American Journal of Aquaculture*, **67**: 231-236.

Evans, F., Matson, S., Brake, J., and Langdon, C., 2004. The effects of inbreeding on performance traits of adult Pacific oysters *Crassostrea gigas*. *Aquaculture*, **230**: 89-98.

Falconer, D. S., and Mackay, T. F. C., 1996. *Introduction to Quantitative Genetics*. 4th edition. Pearson Education Ltd., Essex, England, 33pp.

Hedgecock, D., and Sly, F., 1990. Genetic drift and effective population sizes of hatchery propagated stocks of the Pacific oyster, *Crassostrea gigas*. *Aquaculture*, **88**: 21-38.

Hedgecock, D., McGoldrick, D. J., and Bayne, B. L., 1995. Hybrid vigor in Pacific oysters: An experimental approach using crosses among inbred lines. *Aquaculture*, **137**: 285-298.

Huo, Z. M., Yan, X. W., and Zhao, L. Q., 2014. Larval and juvenile growth performance of Manila clam hybrids of two full-sib families. *Journal of Ocean University of China*, **14**: 564-568.

Husband, B., and Schemske, D. W., 1996. Evolution of the magnitude and timing of inbreeding depression in plants. *Evolution*, **50**: 54-70.

Langdon, C., Evans, F., Jacobson, D., and Blouin, M., 2003. Yields of cultured Pacific oysters *Crassostrea gigas* Thunberg improved after one generation of selection. *Aquaculture*, **220**: 227-244.

Lannan, J. E., 1980. Brood stock management of *Crassostrea gigas*: I. Genetic and environmental variation in survival in the larval rearing system. *Aquaculture*, **21**: 323-336.

Launey, S., and Hedgecock, D., 2001. High genetic load in the pacific oyster *Crassostrea gigas*. *Genetics*, **159**: 255-262.

Longwell, A. C., and Stiles, S. S., 1973. Gamete cross incompatibility and inbreeding in the commercial American oyster, *Crassostrea virginica* Gmelin. *Cytologia*, **38**: 521-533.

Ma, D. Y., Hu, H. L., and Kong, J., 2005. Inbreeding and its impact on aquaculture. *Journal of Fisheries of China*, **29**: 849-856 (in Chinese with English abstract).

Mallet, A. L., and Haley, L. E., 1983. Growth rate and survival in pure population matings and crosses of the oyster *Crassostrea virginica*. *Canadian Journal of Fisheries and Aquatic Sciences*, **40**: 948-954.

Newkirk, G. F., Haley, L. E., Waugh, D. L., and Doyle, R., 1977. Genetics of larvae and spat growth rate in the oyster *Crassostrea virginica*. *Marine Biology*, **41**: 49-52.

Nie, H. T., Liu, L. H., Ding, J. F., and Yan, X. W., 2015a. Characterization of fourteen single nucleotide polymorphism markers in the Manila clam (*Ruditapes philippinarum*). *Conservation Genetics Resources*, **7**: 1-4.

Nie, H. T., Niu, H. B., Zhao, L. Q., Yang, F., Yan, X. W., and Zhang, G. F., 2015b. Genetic diversity and structure of Manila clam (*Ruditapes philippinarum*) populations from Liaodong peninsula revealed by SSR markers. *Biochem System Ecology*, **59**: 116-125.

Saavedra, C., 1997. Low effective sizes in hatchery populations of the European oyster *Ostrea edulis*: Implications for the management of genetic resources. *Journal of Shellfish Research*, **16**: 441-446.

Stiles, S., and Choromanski, J., 1995. Inbreeding studies on the bay scallop *Argopecten irradians*. *Journal of Shellfish Research*, **14**: 1.

Taris, N., Batista, F. M., and Boudry, P., 2007. Evidence of response to unintentional selection for faster development and inbreeding depression in *Crassostrea gigas* larvae. *Aquaculture*, **272**: 69-79.

Winkler, F. M., and Estévez, B. F., 2003. Effects of self-fertilization on growth and survival of larvae and juveniles of the scallop *Argopecten purpuratus* L. *Journal of Experimental Marine Biology and Ecology*, **292**: 93-102.

Yan, X. W., Zhang, G. F., and Yang, F., 2006. Effects of diet, stocking density, and environmental factors on growth, survival, and metamorphosis of Manila clam *Ruditapes philippinarum* larvae. *Aquaculture*, **253**: 350-358.

Zhang, G. F., Liu, S. X., Liu, X., Guo, X. M., and Zhang, F. S., 2003. Self-fertilization family establishment and its depression in bay scallop *Argopecten irradians*. *Journal of Fisheries Science of China*, **10**: 441-445 (in Chinese with English abstract).

Zheng, H. P., Zhang, G. F., and Guo, X. M., 2008. Inbreeding depression for various traits in two cultured populations of the American bay scallop, *Argopecten irradians irradians* Lamarck (1819) introduced into China. *Journal of Experimental Marine Biology and Ecology*, **364**: 42-47.

Zhu, D. P., Nie, H. T., Qin, Y. J., Li, J., Liu, L. H., and Yan, X. W., 2015. Development and characterization of 38 microsatellite makers for Manila clam (*Ruditapes philippinarum*). *Conservation Genetics Resources*, **7**: 517-520.

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