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Hybridization between "Haida No. 1" and Orange-shell line of the Pacific oyster reveals high heterosis in survival

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ABSTRACT

In order to assess the heterosis of the hybrid between the selected lines and develop a new hybrid variety of the Pacific oyster (*Crassostrea gigas*) with excellent survival and growth characteristics, a diallel crosses between selected "Haida No.1" (H, 12th generation) line and Orange-shell (O, 9th generation) line of *C. gigas* were carried out. Heterosis for growth and survival of two parental lines (HH, H♀ × H♂ and OO, O♀ × O♂) and their reciprocal hybrids (HO, H♀ × O♂ and OH, O♀ × H♂) were systematically assessed at both larval and grow-out stages. At larval stage, the two reciprocal hybrids exhibited heterosis in both survival and growth. Compared with HH, the survival rate of OH increased by nearly 23.00% at larval stage. Moreover, the heterosis in survival (average: 35.27%) was higher than that in growth (average: 8.03%) at larval stage. During grow-out phase, reciprocal hybrids showed excellent growth and survival traits at three commercial culturing areas. The high-parent heterosis for survival of hybrids at Rushan were higher than those at Rongcheng or Huangdao. Notably, the survival rate of OH increased by nearly 32.00% compared with HH at Rushan. Among three sites, the high-parent heterosis in survival varied from 31.18% to 141.18% at day 450. Overall, OH (Orange-shell line ♀ × "Haida No. 1" line ♂) exhibited superior growth and survival traits among four crosses, which could be prioritized as a new alternative variety for commercial oyster cultivation.

1. Introduction

The Pacific oyster (*C. gigas*) is one of the most economically important aquatic species in the world. In recent years, genetic improvement for economic traits of *C. gigas* have been widely conducted, such as disease tolerance (Divilov et al., 2021), growth (de Melo et al., 2016), yield (Langdon et al., 2003; Rawson and Feindel, 2012), and survival (Dégremont et al., 2010). Nonetheless, several problems remain, e.g., summer mass mortality of oysters (SMM). SMM have caused serious economic losses and have severely hampered the development of the oyster industry (Du et al., 2021; Pernet et al., 2012; Evans and Langdon, 2006; Azéma et al., 2015; Pernet et al., 2010). In France, 7-month-old oysters reared in Marennes-Oléron Bay suffered 50% mortality during 17 days in August 2009 (Dégremont, 2011). In northern China, the mortality rate of *C. gigas* was more than 60% in summer months (Yang et al., 2021). Moreover, SMM breaks out in oysters, regardless of ploidy levels and origins (Pernet et al., 2010; Azéma et al., 2016).

Crossbreeding combining favorable characteristics of both parents has been extensively applied in genetic breeding of organisms. In aquaculture, hybridization was commonly used to improve flesh quality, increase growth and survival rates, enhance disease and stress tolerance, as well as manipulate sex ratios, produce sterile animals (Bartley et al., 2001; Dang et al., 2011; Liang et al., 2014; Zhang et al., 2017a; Liang et al., 2018; Tan et al., 2020). For example, hybrids derived from two stocks of *C. hongkongensis* exhibited superior heterosis in survival and living weight (Zhang et al., 2017b). the survival and whole weight of *C. sikamea* could be significantly improved through crossbreeding between Chinese and American *C. sikamea* populations (Ma et al., 2022). Two hybrid strains with superior growth and survival could be obtained by hybridization between 4 geographical subpopulations of sea scallops (Wang and Côté, 2012). Additive genetic variation in yield traits has been accumulated by repeated selection. Non-additive genetic variation can be utilized in the offspring through crossbreeding. Therefore, hybridization between selected lines offer the possibility of exploiting both

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additive genetic variation accumulated within lines and non-additive genetic variation between lines (Han et al., 2020; Hedgecock and Davis, 2007). High heterosis in phenotypic characteristics was observed in crosses between selection lines of *Ruditapes philippinarum* (Zhang et al., 2014) and *C. gigas* (Kong et al., 2017; Han et al., 2020). Besides, the high-yield offspring was obtained through crossbreeding among inbred lines of *C. gigas* (Hedgecock et al., 1995; Hedgecock and Davis, 2007).

In 2007, two-year-old Pacific oysters from cultured stocks in Rushan (36.45°N, 121.42°E) in Shandong Province, China, were used to produce first-generation mass-selected line ("Haida No. 1", H) of *C. gigas* with the target of rapid growth (Li et al., 2011). By 2019, the 12th generation "Haida No. 1" has been successfully cultivated through mass selection, in which more than 50 male and female oysters were used as parents in each generation. Moreover, the shell of "Haida No.1" has no special color, with or without radioactive stripes on the left shell. In 2011, two male and two female *C. gigas* with solid orange left and right shells were accidentally identified in cultured population in Rushan, and been used to construct the first generation of Orange-shell line (O). Next two consecutive generations of family selection and six generations of mass selection were established to fix the shell color and improve growth from 2012 to 2019, thereby the Orange-shell line was bred (Han et al., 2019; Han and Li, 2020).

In this study, a complete diallel crosses between Orange-shell and "Haida No. 1" lines of *C. gigas* was carried out. The growth and survival performance of two parental populations and their reciprocal hybrids were systematically evaluated under three culturing environments. The objectives of this study were to compare the heterosis of four groups under three rearing conditions, and estimate the possibility of developing a hybrid variety with high survival through crossbreeding between selected lines.

2. Materials and methods

2.1. Experimental materials

In April 2020, one-year-old oysters of "Haida No. 1" line (12th generation) and Orange-shell line (9th generation) were collected from Rushan and transported to a shellfish hatchery in Shandong Province. Both broodstocks were maintained with conditioning water (temperature: 24.0–25.0 °C; salinity: 30 psu) until sexual maturity.

2.2. Experimental designs and larval rearing

In May 2020, gonadal mature oysters from "Haida No. 1" line (shell height: 107.17 ± 16.10 mm; total weight: 87.32 ± 15.50 g) and Orange-shell line (shell height: 90.31 ± 11.70 mm; total weight: 55.65 ± 17.59 g) were dissected. Then, 50 females and 50 males were selected from each line for mating. For each line, eggs of 50 females were pooled and divided equally into two 20-L buckets. After confirming no spermatozoon contamination under microscope, sperm were obtained from the two lines in the same way. Each container of eggs was then fertilized with a mixture of sperm from inter-line or intra-line at a sperm: egg ratio of 30–50. Thus, four genetic cohorts were produced, namely HH (H♀ × H♂), HO (H♀ × O♂), OH (O♀ × H♂) and OO (O♀ × O♂). Subsequently, the fertilized eggs from each group were hatched separately in a pool with 24 m^3 seawater.

Larvae and juveniles were reared according to the standard procedure described by Li et al. (2011). In brief, most fertilized eggs hatched into D-larvae approximately 24 h after fertilization. After hatching, some D-larvae of each group were collected and reared separately in three replicate 100-L polyethylene plastic buckets. The density of larvae in each bucket was initially set at 2–4 larvae ml^{-1} and decreased to 0.5 larvae ml^{-1} as larvae grew. The sea water was maintained at temperature of 23–25 °C and salinity of 30–31 psu, and fresh air was continuously pumped into each bucket. Larvae were fed with *Isochrysis galbana* during the D-larvae stage and appropriate *Platymonas* sp. was added at the umbo-stage and eyed-stage. When 50% of larvae reached eyed-stage, strings of clean scallop shells were hung in the bucket as substrates for larval settlement and metamorphosis. When all larvae had completed attachment, the settling substrates were transported to an outdoor nursery pond for a two-week temporary rearing. Subsequently, the spats from each group were transferred to three commercial culturing areas in Shandong Province: Rongcheng (37.11°N, 122.35°E), Rushan and Huangdao (35.35°N, 119.30°E). The average annual wave height, water temperature, and salinity is 0.30 m, 12.95 °C and 32.20 psu in Rongcheng; 0.50 m, 14.20 °C and 30.00 psu in Rushan; 0.32 m, 15.39 °C and 31.00 psu in Huangdao (Gao et al., 2012; Sun et al., 2014; Wang et al., 2021). After one month of long-line culturing, the spats were placed to 10-layer lantern nets with 30 spats per layer, and three lantern nets were set for each group.

2.3. Measurements of the growth-related parameters

At larval stage, growth-related parameters were measured on day 1, 5, 10, 15 and 20 after fertilization. Three 100-ml samples were collected randomly from each group to record the shell height and survival rate of larvae. The shell height of each group was measured according to Xu et al. (2019). In brief, the shell height of 30 individuals from each group were photographed by microscope (Olympus BX53) and measured using image analysis software (Image-ProPlus 6.0). Larval survival rate was calculated by the ratio between the living larvae on sampling day to larvae at the D-larvae stage.

During the grow-out stage, 30 oysters were randomly selected from each group on day 90, 180, 270, 360 and 450 to record the shell height and whole weight. The shell height of samples was measured using vernier calipers (0.01 mm accuracy). The living weight was measured using electronic scales (0.01 g accuracy). The survival rate was calculated based on the following formula (Jiang et al., 2021):

$$Z_t\ (\%) = (N_t/N_0) \times 100$$

Where Z_t is the survival rate of group at time t; N_t is the number of live oysters per lantern net at time t; N_0 represents the total number of oysters in each lantern net in July 2020.

2.4. Statistical analyses

The data are given as the means ± standard deviation (SD) and all analyses were performed using the SPSS 26.0. To improve the normality and homoscedasticity, we applied an arcsine transformation for survival rate and a logarithmic transformation with a base of 10 for the growth-related data prior to analysis. Differences in shell height, survival rate and living weight among four experimental groups were analyzed by one-way ANOVA and multiple comparison Tukey test. Differences were considered statistically significant if $P < 0.05$.

The mid-parental heterosis (M) was calculated using the following equation (Hallauer et al., 2010):

$$M_{F1}\ (\%) = [(F1 - MP) \times 100]/MP$$

Where F1 and MP represent the mean shell height (survival rate or living weight) of reciprocal hybrids and purebred groups, respectively. M_{F1} indicates the mid-parent heterosis of hybrids.

To further investigate the increasement in phenotype values of the hybrids compared with "Haida No. 1" line, the high-parent heterosis (H) was calculated using the following model adapted from Wang et al. (2011):

$$H_{(F1/HH)}\ (\%) = (X_{F1} - X_{HH}) \times 100/X_{HH}$$

Here, X_{F1} is the mean phenotypic value (shell height, survival rate, etc) of the hybrid F1 (HO or OH) cohorts, X_{HH} indicates the mean phenotypic value (shell height, survival rate, etc) of "Haida No. 1" line.

To determine the effects of the genotypes (hybrid groups vs. purebred groups) and environmental factors (Rushan vs. Rongcheng vs. Huangdao) on the survival and growth of *C. gigas* during juvenile and adult stage. A two-factor ANOVA was employed as the following model (Mallet and Haley, 1983; Cruz and Ibarra, 1997):

$$Y_{ijk} = \mu + G_i + E_j + (G \times E)_{ij} + e_{ijk}$$

Where Y_{ijk} indicates the mean phenotypic value (shell height, survival rate, etc) of the k replicate from the type i group in site j. G_i is the genotype effect on phenotypic value (shell height, survival rate, etc) (i = 1, 2), E_j is the environmental effect on the phenotypic value (shell height, survival rate, etc) (j = 1, 2, 3), $(G \times E)_{ij}$ represents the interaction effect of the group type and site, and e_{ijk} is the random observation error (k = 1, 2, 3).

3. Results

3.1. Growth, survival and heterosis at larval stage

No significant difference was observed in shell height between two reciprocal hybrids (Fig. 1A). The shell height of OO was lower than those of the other three groups at larval stage. From day 15, two reciprocal hybrids were significantly larger than two purebred lines in shell height ($P < 0.05$), with the order of OH > HO > HH > OO. On day 20, average shell height of HO (285.67 ± 35.87 μm) was slightly larger than that of OH (275.25 ± 24.75 μm). The shell heights of two hybrid groups were significantly larger than those of two purebred lines at day 20 ($P < 0.05$), with a high-parent heterosis $H_{(HO/HH)}$ of 15.51% and $H_{(OH/HH)}$ of 11.30% (Table 1).

On day 15, the mean survival rates of the hybrid groups were significantly higher than those of purebred groups ($P < 0.05$) (Fig. 1B). However, no significant difference was observed between the two hybrids. The OH group exhibited highest survival rate among the four groups on day 20, with a heterosis of 46.00% compared to HH (Table 1). Notably, the survival rate of OO group (37.66 ± 3.06%) was significantly lower than those of the other three groups ($P < 0.05$). Moreover, heterosis for survival in the hybrid groups (2.84–46.00%) gradually increased with larval growth (Table 1).

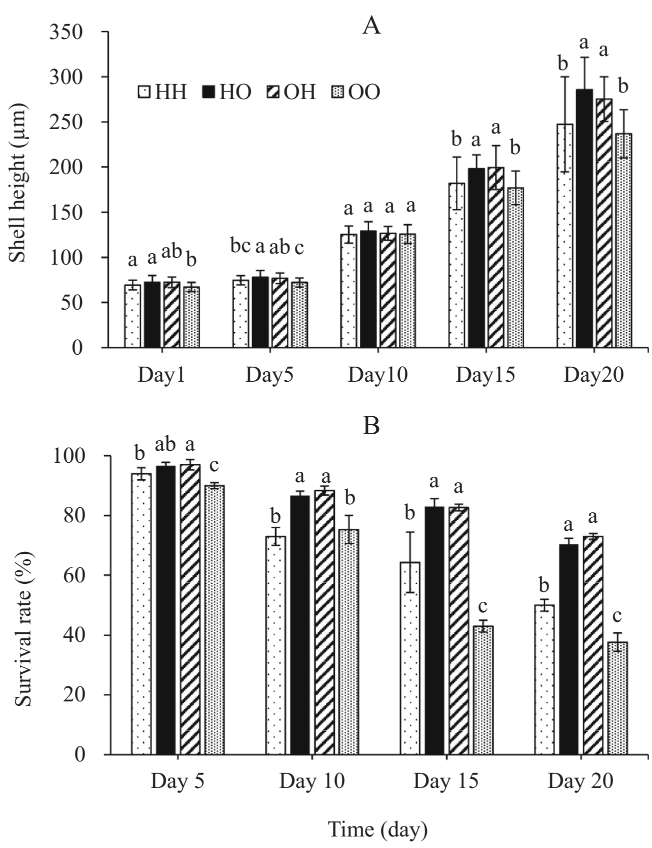

Fig. 1. Shell height and survival rate for two purebred groups (HH and OO) and two hybrid groups (HO and OH) at larval stage. A: the shell height of four groups; B: the survival rate of four groups. H and O indicate "Haida No. 1" line and Orange-shell line of the Pacific oyster, respectively. Two purebred groups: HH (H♀ × H♂), OO (O♀ × O♂); and their reciprocal hybrids: HO (H♀ × O♂), OH (O♀ × H♂). Different superscript letters in the same day indicate significant difference ($P < 0.05$) among four groups.

3.2. Growth, survival and heterosis at juvenile stage

Two-factor ANOVA showed that growth and survival traits for four experimental groups were significantly influenced by genotypes and environments (Table 2). The shell heights of four groups in Rushan were larger than those in Rongcheng and Huangdao (Fig. 2). Two hybrid groups exhibited heterosis in shell height compared to HH, with mid-parent heterosis ranged from 0.52% to 18.33% at three culturing areas. At day 450, significant difference in shell height was observed among four groups in Rongcheng ($P < 0.05$). Besides, the shell heights of two hybrid groups were larger than that of HH, with high-parent heterosis of 7.74% and 15.41% for HO and OH, respectively (Table 3). Moreover, the shell heights of OO were significantly smaller than those of the other three groups at three areas ($P < 0.05$), followed the order of OH > HO > HH > OO.

At day 450, the living weights of hybrid groups were significantly ($P < 0.05$) higher than those of purebred groups at Rongcheng (Fig. 2). Moreover, OH was significantly ($P < 0.05$) heavier than the other three groups, with values of high-parent heterosis at 18.44% and 22.00% in Rushan and Huangdao, respectively. Besides, the heterosis for living weight was higher at Rongcheng than at Rushan or Huangdao. While the living weights of four experimental groups at Rushan were highest among three environments (Table 4).

The mean survival rates of two hybrid crosses were higher than those of the two purebred groups at three environments (Fig. 2). At day 90, two hybrid lines in Rushan showed negative high-parent heterosis, with values at −3.07% for HO and − 1.15% for OH (Table 5). Similar results were also observed in Rongcheng and Huangdao. However, the survival rates of hybrid crosses (HO: 86.66%, OH: 85.66%) were higher than those of their parental groups (HH: 73.33%, OO: 64.00%) on day 180 in Rongcheng. Moreover, the survival rate of OO were significantly ($P <$ 0.05) lower than those of the other three groups, except on day 90 at three environments. Notably, on day 450, which indicates oysters had tolerated the summer for the second time, the survival rates of hybrid crosses were significantly ($P < 0.05$) higher than purebred groups at three culture areas. Meanwhile, OH group exhibited higher heterosis in survival than HO group, with high-parent heterosis ranged from 102.15% to 141.18% at three environments. Besides, the heterosis for

Table 1
Heterosis (*M* and *H*) for survival rate and shell height of "Haida No. 1" (H) line, Orange-shell (O) line and their reciprocal hybrids (HO and OH) at larval stage.

Items	Heterosis (%)	Day 1	Day 5	Day 10	Day 15	Day 20
Survival rate	M_{F1}	–	5.25	17.98	54.35	63.50
	$H_{(HO/HH)}$	–	2.84	18.72	29.02	40.67
	$H_{(OH/HH)}$	–	3.19	21.00	28.50	46.00
Shell height	M_{F1}	6.07	5.54	1.89	10.75	15.87
	$H_{(HO/HH)}$	4.47	4.62	3.06	8.87	15.51
	$H_{(OH/HH)}$	4.50	3.11	1.139	9.59	11.30

H and O indicate "Haida No. 1" and Orange-shell lines of the Pacific oyster, respectively. Two purebred groups: HH (H♀ × H♂), OO (O♀ × O♂); and two reciprocal hybrids: HO (H♀ × O♂), OH (O♀ × H♂). M_{F1} represents the mid-parent heterosis of the two hybrids; $H_{(HO/HH)}$ and $H_{(OH/HH)}$ means the high-parent heterosis of HO and OH, respectively.

Table 2
Two-factor analysis of variance (ANOVA) showing the genotype (G) and environment factor (E) effects for survival and growth of each experimental group at juvenile and adult stage.

Items	Source	df	Survival rate		Shell height		Living weight	
			MS	F-value	MS	F-value	MS	F-value
Day 90	E	2	0.154	<0.001***	0.002	0.747	2.319	<0.001***
	G	1	0.002	0.426	0.775	<0.001***	2.438	<0.001***
	G * E	2	0.007	0.146	0.049	0.001**	0.037	0.231
Day 180	E	1	0.035	0.020**	0.057	<0.001***	1.220	<0.001***
	G	2	0.650	<0.001***	0.682	<0.001***	2.784	<0.001***
	G * E	2	0.001	0.913	0.012	0.101	0.056	0.035*
Day 270	E	2	0.032	0.003**	0.171	<0.001***	1.795	<0.001***
	G	1	0.673	<0.001***	0.444	<0.001***	2.936	<0.001***
	G * E	2	0.002	0.691	0.000	0.932	0.067	0.006**
Day 360	E	2	0.052	0.003**	0.135	<0.001***	1.565	<0.001***
	G	1	0.628	<0.001***	0.027	0.003**	0.014	0.237
	G * E	2	0.001	0.903	0.226	<0.001***	1.209	<0.001***
Day 450	E	1	0.727	0.196	0.080	<0.001***	1.134	<0.001***
	G	2	<0.001	<0.001***	0.476	<0.001***	1.855	<0.001***
	G * E	2	0.011	0.981	0.017	0.002**	0.083	<0.001***

* indicates $P < 0.05$; ** indicates $P < 0.01$; *** indicates $P < 0.001$.

survival of hybrid groups were highest at Rushan.

3.3. Shell color of the hybrid cohorts

The 1000 hybrids from Rushan were classified by eye observation based on shell pigmentation of the left valve. Hybrid cohorts had two types on color patterns. The shell pattern of 58.4% of the progenies is the same as that of "Haida No. 1" line with or without radial stripes on left shell, while 41.6% of the hybrids exhibited purple colors (ranging from pale to dark purple) on both shells (Fig. 3).

4. Discussion

Improving yield traits such as growth rate and survival rate has been the main objective of genetic breeding for aquatic animals. Here, we compared the phenotypic values of the Pacific oyster "Haida No. 1" line, the Orange-shell line and their reciprocal crosses. The results showed that the four groups of oysters differed significantly in growth and survival at three environments.

4.1. Growth

The results of the study revealed that hybrid crosses exhibited heterosis in shell height, with values of mid-parent heterosis ranging from 1.89% to 15.87% at larval stage and 13.46% to 24.48% at grow-out stage. The positive association between growth and heterozygosity in marine mollusks is a common consequence of crossbreeding (Zouros and Mallet, 1989; Sheridan, 1981). Besides, the successful use of heterosis is attributed to an increase in heterozygosity (Mitton and Grant, 1984). Therefore, growth difference in this study may be interpreted as higher heterozygosity in the hybrids than in purebreds. In this study, hybrid crosses exhibited low heterosis in growth, and the similar results were also demonstrated in *C. gigas* (Kong et al., 2017; Han et al., 2020) and *Argopecten irradians* (Zhang et al., 2007; Wang and Li, 2010). However, high heterosis in shell height and living weight was found in reciprocal hybrids derived from *C. hongkongensis* and *C. sikamea* (Zhang et al., 2017a). The growth difference in hybrids may be caused by genetic differences between parents (Sheridan, 1997) and environmental conditions (Evans and Langdon, 2006). In addition, different degrees of inbreeding among parental populations may be another important cause of the above phenomenon (Rawson and Feindel, 2012). Moreover, the shell height and living weight were higher in HH than in OO. However, the heterosis for growth-related traits in OH were higher than that in HO. This phenomenon indicates the non-additive effects were critical for oyster growth (Hedgecock et al., 1995; Hedgecock and Davis, 2007).

4.2. Survival

In this study, the hybrid groups exhibited higher survival rate than purebred groups at both larval and grow-out stage. Especially, OH showed a significant survival advantage over the other three groups, with high-parent heterosis of 141.18% at Rushan, which was probably due to better adaptation of reciprocal hybrids than their parental counterparts under certain environmental conditions (Zhang et al., 2017b). On the other hand, heterozygosity and hybridization may have masked the deleterious or even lethal recessive survival-related genes in the parental lines (Wang and Li, 2010; Burke and Arnold, 2001). Moreover, "Haida No. 1" line originated from cultured stocks in Rushan, while the Orange-shell line was established based on only four *C. gigas*. Thus, genetic variation may exist between these two parental lines. The magnitude of heterosis between two particular lines depends on the square of the difference in gene frequency between crossed parents. Moreover, if no different gene frequency was existed between the parents crossed, there will be no heterosis. Nevertheless, the heterosis will be greatest when one particular trait-related loci was fixed in one parent and the other loci was fixed in the other parent (Falconer, 1981). Besides, high heterosis in survival suggested crossbreeding between selected lines is an efficient method to improve the survival of *C. gigas*.

The heterosis in survival was higher than that in growth throughout the life stage. This may be attributed to the fact that the two parental populations have been selected for generations with directional selection for growth rather than for survival. Thus, the survival-related loci of the two parents may be more diverse and complementary than growth-related loci (Han et al., 2020). On the other hand, fitness-related traits (survival rate, etc) are more likely to exhibit directional dominance compared to morphological-related traits (shell height, etc) (Lynch and Walsh, 1998), and the magnitude of heterosis depends largely on directional dominance of genes involved in target traits (Falconer and Mackay, 1996). Thus, the heterosis between two reciprocal groups is greater for survival than for growth.

4.3. Effects of generations and inbreed lines

The two reciprocal hybrids exhibited extraordinary performances in terms of survival and growth. Superior performance of hybrids could be partially explained by the combination of selection and crossbreeding. The Orange-shell line has been successively selected for 9 generations. Meanwhile, the "Haida No. 1" line has been successively selected for 12 generations with fast growth. Theoretically, when the parental populations have been selected for generations, the hybrid progeny will exhibit high heterosis due to the accumulation of plenty of different non-

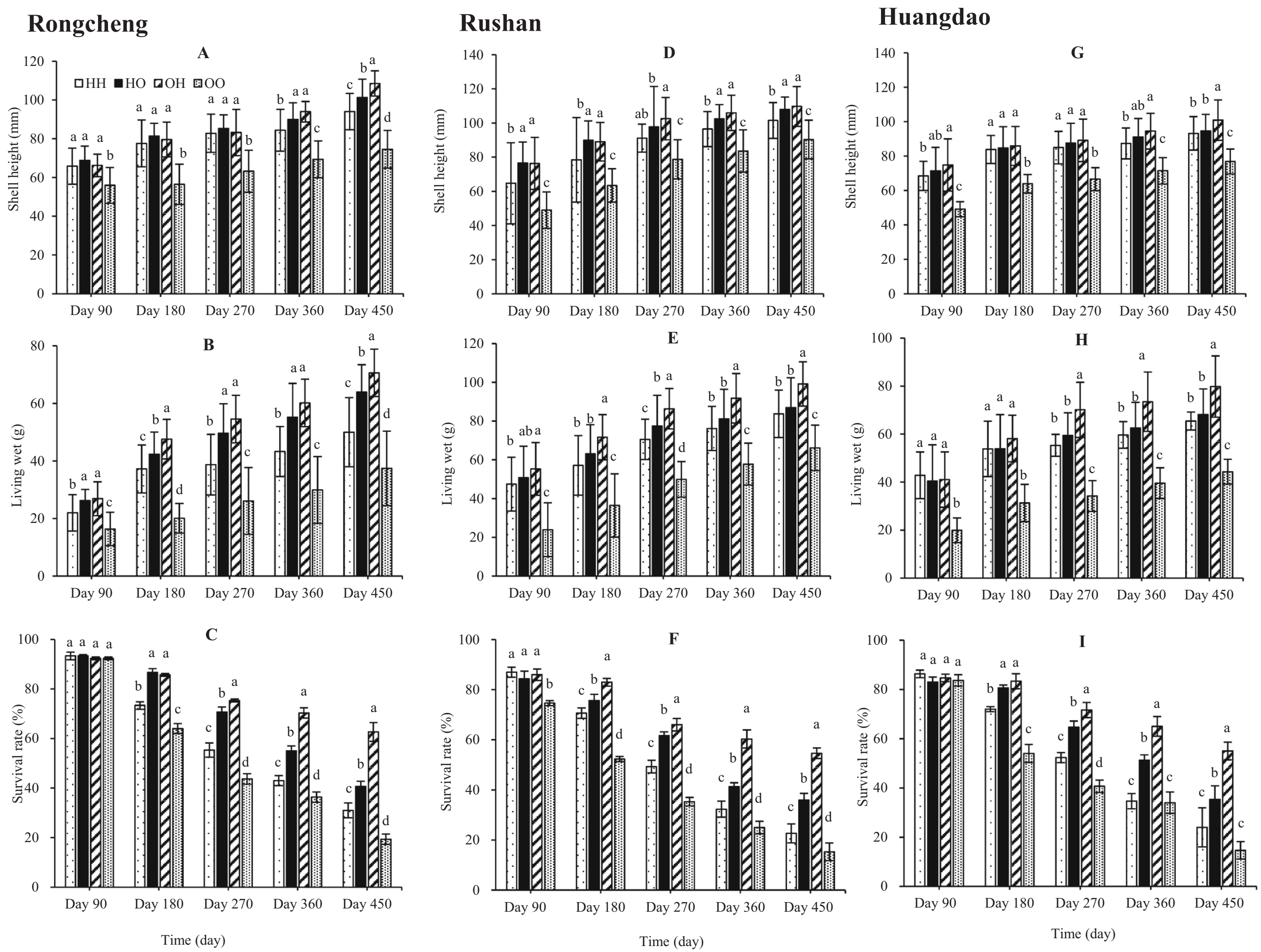

Fig. 2. Shell height, living weight and survival rate for two purebred groups (HH and OO) and two hybrid groups (HO and OH) among three sites. A: the shell height of four groups at Rongcheng; B: the living weight of four groups at Rongcheng; C: the survival rate of four groups at Rongcheng; D: the shell height of four groups at Rushan; E: the living weight of four groups at Rushan; F: the survival rate of four groups at Rushan; G: the shell height of four groups at Huangdao; H: the living weight of four groups at Huangdao; I: the survival rate of four groups at Huangdao. Different superscript letters in the same day indicate significant difference ($P < 0.05$) among four groups.

Table 3
Heterosis (*M* and *H*) for shell height in "Haida No. 1" (H) line, Orange-shell (O) line and their reciprocal hybrids (HO and OH) at grow-out stage in three culturing environments.

Heterosis (%)	Day 90	Day 180	Day 270	Day 360	Day 450
Rongcheng					
M_{F1}	10.91	20.09	15.41	19.66	24.48
$H_{(HO/HH)}$	4.53	4.89	3.03	6.61	7.74
$H_{(OH/HH)}$	0.63	2.62	0.52	11.41	15.41
Rushan					
M_{F1}	34.43	26.23	17.85	15.82	13.46
$H_{(HO/HH)}$	18.33	14.71	7.24	6.33	6.32
$H_{(OH/HH)}$	17.90	13.57	12.44	9.92	8.10
Huangdao					
M_{F1}	24.34	15.54	16.64	16.89	15.01
$H_{(HO/HH)}$	4.33	1.13	3.12	4.28	1.54
$H_{(OH/HH)}$	9.27	2.51	4.95	8.29	8.31

Table 4
Heterosis (*M* and *H*) for living weight in "Haida No. 1" (H) line, Orange-shell (O) line and their reciprocal hybrids (HO and OH) at grow-out stage among three culturing environments.

Heterosis (%)	Day 90	Day 180	Day 270	Day 360	Day 450
Rongcheng					
M_{F1}	38.62	56.87	60.67	57.69	53.85
$H_{(HO/HH)}$	19.52	13.74	28.11	27.60	27.76
$H_{(OH/HH)}$	47.89	73.78	73.46	69.93	66.38
Rushan					
M_{F1}	48.61	44.03	35.95	29.06	24.18
$H_{(HO/HH)}$	6.96	10.51	9.80	6.49	3.87
$H_{(OH/HH)}$	16.57	25.49	22.35	20.46	18.44
Huangdao					
M_{F1}	29.98	31.74	44.71	37.03	34.89
$H_{(HO/HH)}$	−5.49	0.18	7.38	4.76	4.19
$H_{(OH/HH)}$	−4.10	8.12	26.77	23.11	22.00

Table 5
Heterosis (*M* and *H*) for survival in "Haida No. 1" (H) line, Orange-shell (O) line and their reciprocal hybrids (HO and OH) at grow-out stage in three culturing environments.

Heterosis (%)	Day 90	Day 180	Day 270	Day 360	Day 450
Rongcheng					
M_{F1}	0.00	25.49	47.47	57.98	105.30
$H_{(HO/HH)}$	0.00	18.18	27.71	27.91	31.18
$H_{(OH/HH)}$	−1.07	16.82	36.14	63.57	102.15
Rushan					
M_{F1}	5.36	29.00	50.79	77.33	138.60
$H_{(HO/HH)}$	−3.07	7.08	25.00	27.84	58.82
$H_{(OH/HH)}$	−1.15	17.45	33.78	86.60	141.18
Huangdao					
M_{F1}	−1.37	30.16	46.59	69.42	133.62
$H_{(HO/HH)}$	−3.86	12.04	23.57	48.08	47.22
$H_{(OH/HH)}$	−1.93	15.74	36.94	87.50	129.17

additive genetic variation, and the more successive generations of the parents, the higher heterosis in the hybrids (Sheridan, 1997).

The orang shell line used in this study is an inbred line established based only on four *C. gigas*. When this inbred orange-shell line was crossed with "Haida No. 1" line as one parent, the hybrid groups exhibited favorable performances, especially in survival. This phenomenon probably due to the yield-related loci of these two parents may be more complementary (Han et al., 2020). Meanwhile, this result indicates inbred lines have great application potential in aquaculture (Hedgecock et al., 1995; Hedgecock and Davis, 2007). Nevertheless, inbreeding and crossing alone cannot produce any improvement unless the inbred lines derived from different foundation populations. Moreover, parental populations must be selected at some stage if any improvement is to be made (Gjerde, 1988).

4.4. Difference performances existed in different crosses and environments

The heterosis for survival of OH was greater than that of HO throughout the life stage. This indicated the importance of evaluating the performances of two reciprocal hybrids before choosing the paternal and maternal line for commercial seed production (Zhang et al., 2007). Asymmetric performance between reciprocal crosses were also found in the Kumamoto oyster *C. sikamea* (Ma et al., 2022), the Hongkong oyster *C. hongkongensis* (Zhang et al., 2017a), the bay scallop *A. irradians* (Zhang et al., 2007) and the boring giant clam *Tridance crocea* (Zhang et al., 2020). The performance variation between reciprocal hybrids were probably due to sex-linked genes, cytoplasmic inheritance, parental effects and extra-nuclear effects (Kong et al., 2017; Zhang et al., 2017a).

Both survival and growth traits of four groups varied among all three culturing environments. Notably, greater high-parent heterosis for survival and growth were found at Rushan. This difference may be caused by environmental condition, such as water circulation, salinity, temperature and food (Xu et al., 2019). Different performances among culture areas indicates the importance of evaluating the phenotypic traits of oysters among different environments to obtain greatest heterosis.

4.5. Application prospect of shell color variants

Aquatic organisms with special colors are not only of high scientific value, but also more favored by consumers in the market (Clydesdale, 1993; Alfnes et al., 2006). In this study, oysters with purple on both the left and right shells were unexpectedly found from the hybrid offspring. Therefore, it is necessary to employ them as parents to develop a new line with stable inheritance of purple shell color. The total left-shell pigmentation in *C. gigas* was under a high degree of genetic control and strongly influenced by additive genetic variation (Brake et al., 2004; Evans et al., 2009). Besides, the heritability estimates in growth-related traits of the Pacific oyster were medium to high (Evans and Langdon, 2006; Kong et al., 2015; Chi et al., 2021; Li et al., 2011). The relatively high heritability of traits recorded in oysters also indicated that artificial selection has a significant potential to improve the performance of this species (Kong et al., 2015; Zhang et al., 2019). Thus, selective breeding including family and mass selection could be prioritized for genetic improvement of purple-variant.

5. Conclusions

In summary, the growth and survival of "Haida No. 1" line, Orange-shell line and their reciprocal hybrids were systematically assessed under three commercial conditions. The reciprocal cross exhibited significant heterosis in terms of survival at both larval and grow-out stages. The high-parent heterosis of OH was 46.00% in survival at larval stage. Meanwhile, the survival rate of OH increased by nearly 32.00% compared with HH on day 450. Moreover, the heterosis for shell height and living weight of OH was higher than that of HO. Therefore, the results demonstrated that OH (Orange-shell line ♀ × "Haida No. 1" line ♂) has significant potential for application in the oyster aquaculture.

Credit author statement

Yuanxin Liang: Methodology, Software, Writing-original draft. **Guohan Zhang:** Data curation, Methodology. **Gaowei Jiang:** Writing-review. **Yiming Hu:** Writing-review. **Jiafeng Fang:** Writing-review. **Yong Chi:** Data curation, Software. **Qi Li:** Conceptualization, Formal analysis, Methodology, Writing-review. **Chengxun Xu**: Supervision. **Weiguo Liu:** Software. **Haijun Liu:** Software.

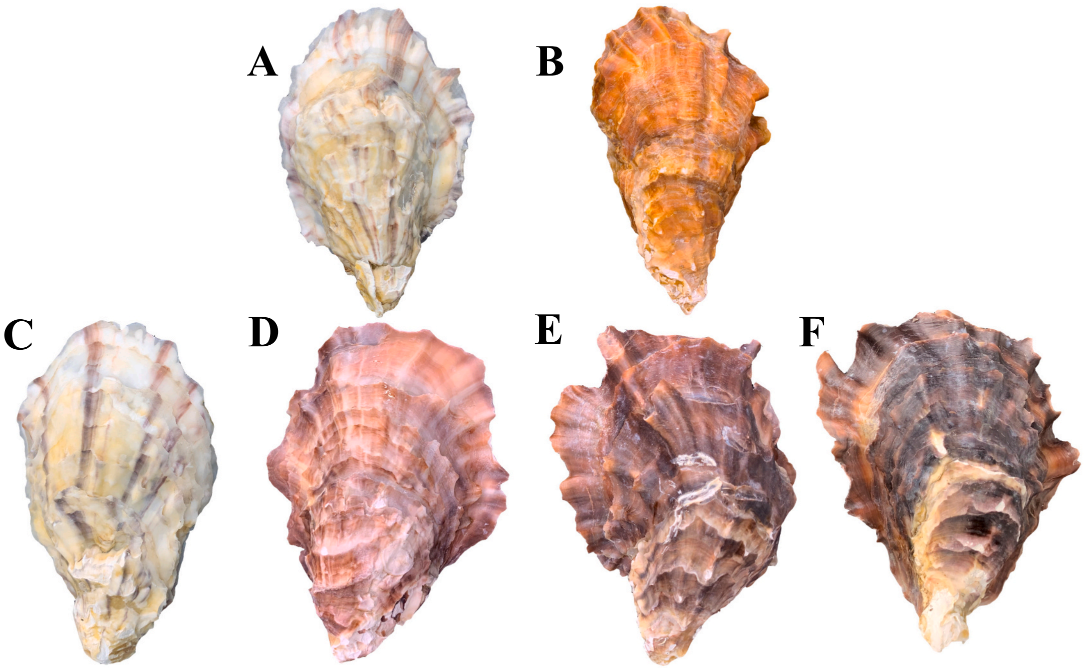

Fig. 3. Phenotypes of the two purebred groups and hybrid offspring. A: left shell for "Haida No. 1" line; B: left shell for Orange-shell line; C: left shell for hybrid offspring with natural shell color; D - F: left shell for purple-shell variant (ranging from pale to dark purple). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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