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Reciprocal hybrids derived from *Crassostrea gigas* and *C. angulata* exhibit high heterosis in growth, survival and thermotolerance in northern China

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ABSTRACT

The Pacific oyster is one of the most economically important species in global aquaculture. However, the production of *C. gigas* has been compromised by widespread and severe summer mortality events in recent years. To explore the possibility of obtaining a new hybrid line with rapid growth and high summer survival rate traits through hybridization, we performed a systematic comparative study on heterosis for growth and survival of *C. gigas* (GG), *C. angulata* (AA) and their reciprocal hybrids of *C. gigas* × *C. angulata* (GA) and *C. angulata* × *C. gigas* (AG) for 450 days in northern China. The fertilization and D-stage rates of reciprocal hybrids were lower than those of parental species, but obvious heterosis for growth, survival and metamorphosis were observed at larval stages. Meanwhile, the hybrid cohorts showed high heterosis for growth and survival at both juvenile and adult stages. Moreover, compared with growth, heterosis was higher in survival with a mid-parent heterosis ranged from 12.75% to 110.20% throughout the whole period. At day 450, the cumulative survival rate of hybrid GA increased by nearly 34% compared with GG, with a single patent heterosis $I_{(GA/GG)}$ of 191.14%. Notably, the final yield of hybrid crosses was significantly higher than that of parental lines, with a mid-parent heterosis of 182.72% and $I_{(GA/GG)}$ of 239.46%. To investigate the relationship between summer mortality and high temperature tolerance, the survivability of reciprocal hybrids and parental species through exposure to acute increasing temperature stresses for 96 h were compared. The reciprocal hybrids exhibited greater thermotolerance than the parental species, followed the order of GA > AG > AA > GG. Overall, our study confirmed that GA showed the greatest heterosis in terms of growth, survival and thermotolerance, which could be used as the preferred alternative variety in oyster farming in northern China.

1. Introduction

Recurrent Summer Mortality Syndrome (SMS) outbreaks have caused loss of production and economic hardship, and become a major problem for oyster farming worldwide. This syndrome was reported as early as 1915 in Japan (Mori et al., 1965) and again in the late 1950s in the United States (Beattie et al., 1980) and during the early 1980s in France (Berthelin et al., 2000). Since 2008, the severity of mass mortalities of oyster has dramatically increased in Europe but also other parts of the world. It affects both juveniles and adults, as well as both diploids and triploids (Azéma et al., 2016), and even offspring (Monserrat et al., 2016). One of the most striking examples of mortality outbreaks is the Pacific Oyster Mortality Syndrome (POMS), killing up to 70% of the spat and juvenile oysters every year in France (Azéma et al., 2015). Not only almost all farming areas were severely hit by POMS in France, but geographically extended to all its coastal regions (Atlantic, Channel, and Mediterranean) (Pernet et al., 2012; Petton et al., 2013). In other cases, farmers reported mortality of up to 100% of oysters in baskets harvested from Upper and Lower Pitt Water and Blackman Bay, Australia (Biosecurity-Tasmania, 2016), resulting in disruption or complete cessation of local oyster production (Evans et al., 2019).

China is the largest producer of oysters with a production about 5.1 million metric tons in 2018 (FAO, 2020). The Pacific oyster which originated from East Asia is dominant commercial species in northern China. The estimated production of *C. gigas* in 2019 was about 1.2 million tons (BOF (Bureau of Fisheries), 2020). However, Pacific oyster mass mortality events frequently have been found in Liaoning and Shandong provinces for decades in northern China, and the mortalities ranged from 40% to 80% in different farming areas (Mao et al., 2005; Lian et al., 2010; Yang et al., 2021), causing significant economic

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damage to the Pacific oyster industry. Different from those reported in Europe and other countries, mass mortalities associated with OsHV-1 infection in China were not found in Pacific oyster (Bai et al., 2015). A recent evidence suggests that *Vibrio alginolyticus* play a major role in Pacific oyster mass mortality events off the coast of Shandong Province and continuous high temperature promotes the proliferation of pathogens (Yang et al., 2021). Reducing the impact of this disease is important to maintain the viability of the Pacific oyster industry. Genetic breeding for oyster stocks that resist summer mortality is a promising way to reduce losses (Dégremont et al., 2016; de Kantzow et al., 2017).

Hybridization is a most effective methods for genetic improvement, and has been extensively applied in aquaculture. Interspecific hybridization is commonly used to combine advantageous characteristics of both parents, which often results in increased growth rates and survival rates, as well as enhanced disease resistance and stress tolerance (Hulata, 1995; Rahman et al., 2013; Xu et al., 2019). For example, Dorson et al. (1991) reported that crosses of coho salmon *(Oncorhynchus kisutch*) with rainbow trout (*O. mykiss*) had increased disease resistance to a variety of salmonid viruses. Hybridization of the white bass (*Morone chrysops*) ♀ and the striped bass (*M. saxatilis*) ♂ created the sunshine bass, which had higher survival rate and stronger temperature tolerance than parents (Liang et al., 2014). In shellfish, the “Pacific” scallop, produced by hybridizing the Weathervane scallop (*Patinopecten caurinus*) and Japanese scallop (*P. yessoensis*), is characterized by high growth rate and disease resistance (Elliott, 2000). The hybrids of Pacific abalone (*Haliotis discus hannai*) and Giant abalone (*H. gigantea*) showed heterosis in disease resistance than one or both parental species (Liang et al., 2018). Furthermore, an oyster strain with fast growth and enhanced salinity tolerance were obtained by artificial selection from the backcross progeny between *C. hongkongensis* ♀ and *C. gigas* ♂ fertile hybrids and parental species (Zhang et al., 2016). Fujian oyster *C. angulata*, a subspecies of *C. gigas*, is mainly cultivated in Fujian province in southern China (Wang et al., 2010). Compared to northern counterpart, Fujian oyster exhibits stronger tolerance to the high temperature of the subtropical zone (Ghaffari et al., 2019). Hybridization between the two subspecies may be beneficial for the transfer of high temperature tolerance from Fujian oyster to Pacific oyster, and a new strain with high survival in summer can thus be bred.

In this study, a two-by-two factorial cross between *C. gigas* from a fast-growing selected line and *C. angulata* was carried out. The fertilization, survival and growth performance were compared among parents and their reciprocal hybrids for 450 days in northern China. The survival was compared through exposure to acute increasing temperature stresses for 96 h. The purposes of this study were: (1) estimating the heterosis for growth and survival at the whole life stages; (2) evaluating the possibility of obtaining a hybrid line with high survival characteristic in summer through hybridization; (3) comparing high temperature tolerance among four groups in acute increasing temperature stresses. Our ultimate goal is to breed a new hybrid variety with fast growth and high summer survival rate in northern China.

2. Materials and methods

2.1. *Preparation and mating of broodstocks*

In May 2019, five hundred one-year-old *C. gigas* produced by 12 generations of mass selection with rapid growth performance was collected in Rongcheng, Shandong Province, China (Zhang et al., 2018), and five hundred one-year-old *C. angulata* were collected from a cultured population in Zhangzhou, Fujian Province, China (Fig. 1). Cytochrome oxidase I makers were used to identify each parent oyster before mating experiment (Wang and Guo, 2008). Parent oyster acclimation, larvae production and rearing occurred at a hatchery in Laizhou, Shandong Province, China (Fig. 1). In June 2019, gonadal mature *C. gigas* (shell heights: 101.32 ± 14.98 mm, total weight 53.04 ± 16.13 g) and *C. angulata* (shell heights: 65.52 ± 6.07 mm, total weight 24.76 ± 6.59 g) were dissected and 40 females and 40 males were chosen to mating. For each species, eggs from forty females were pooled and divided equally into two 10-L containers. Each container of eggs was fertilized with a mixture of sperm from forty *C. gigas* or forty *C. angulata* with a sperm: egg ratio of 30–40. Thus, a complete diallel cross was created producing four different combinations: GG (*C. gigas* ♀ × *C. gigas* ♂), GA (*C. gigas* ♀ × *C. angulata* ♂), AG (*C. angulata* ♀ × *C. gigas* ♂) and AA (*C. angulata* ♀ × *C. angulata* ♂). The fertilized eggs from each cross were

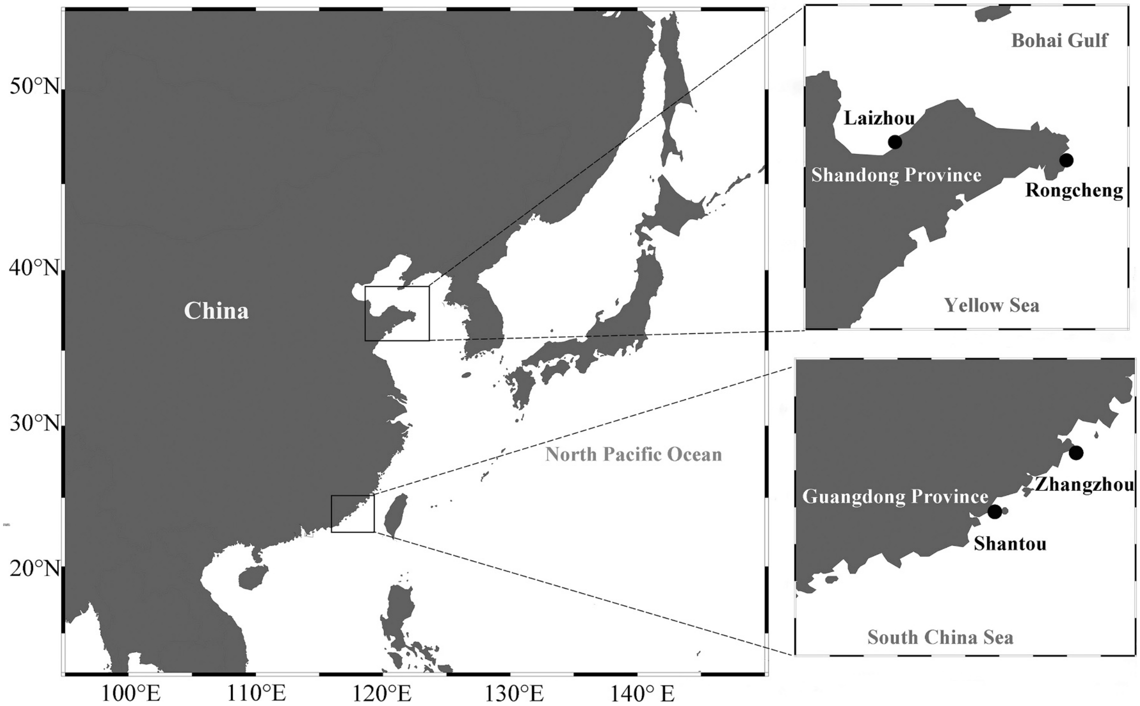

Fig. 1. Location of the broodstocks origin and experimental grow-out sites in China.

divided into three equal portions and maintained in three 100-L buckets for hatching.

2.2. Larval rearing and spat grow-out

Larvae and juveniles were reared according to the routine culture procedure described by Han et al. (2020). Briefly, hatched larvae from each combination were then separately reared in three replicate 100-L buckets. The larvae density was initially set to 2 larvae ml^{-1} and decreased with larval growth. The seawater was kept at 23.1–25.2 °C with a salinity of 30–31 psu. Larvae were fed with *Isochrysis galbana* in the D-larvae stage, and added on *Platymonas* sp. in the umbo-stage and eyed-stage. When 30% larvae appear eyespots, string of scallop shells were hung as spat collectors. Newly settled spats were transported to an outdoor pond for a 15 days temporary rearing. Then spats were transferred to Rongcheng for farming in July 2019 (Fig. 1). Three 10-layer lantern nets were set for each combination and 30 spats were put in each layer. As the oysters grew, spats in each lantern net were diluted monthly to keep a consistent volume and biomass in each replicate.

2.3. Growth-related parameter measurement

The fertilization rate was defined as the ratio of the number of fertilized eggs to the total number of eggs. The hatching rate was measured as the percentage of D-larvae among fertilized eggs. At larval stage, three 50-mL sample was collected on days 5, 10, 15 after fertilization to record the survival rate and the shell height. The shell height of 30 larvae randomly measured using microscope (100×) fitted with an ocular micrometer. Cumulative survival rates of larvae were defined as the ratio between the numbers of individuals at different days to that of D larval stage. Metamorphosis rate was calculated as the percentage of spats to the total number of pediveligers (Xu et al., 2019).

During grow-out stage, the growth-related parameters of each line were recorded on 90 d (summer), 180 d (autumn), 330 d (winter), 390 d (spring), and 450 d (summer). The incremental survival rate was calculated according to the follow formula (Qin et al., 2020):

$$S_{t+1}\ (\%) = (N_{t+1}/N_t) \times 100$$

where S_{t+1} is the incremental survival rate of oyster at time t + 1; N_t is the number of live oysters at time t; N_{t+1} is the number of live oysters at time t + 1; t + 1 was the next measurement time after time t.

The cumulative survival rate was calculated according to the follow formula (Qin et al., 2020):

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

where Z_t is the cumulative survival rate of oyster at time t; N_t is the number of live oysters at time t; N_0 is the total number of oysters per lantern net in July 2019.

The shell height of 30 individuals was randomly measured using a vernier caliper (0.01 mm). On day 450, the total weight (TW) was measured from a random sample of 30 oysters per lantern net using an electronic scale (0.01 g). Yield was calculated with the following formula (Rawson and Feindel, 2012):

$$Y = Z_{450} \times N_0 \times TW$$

where Z_{450} is the cumulative survival rate of oyster on 450 d; N_0 and TW were as defined previously.

2.4. Statistical analyses

All data were analyzed using the SPSS 26.0 software. Differences in shell height and survival rate among hybrid and inbred cohorts were analyzed with one-way analysis of variance followed by multiple comparison Tukey test. To improve the normality and homoscedasticity, we used an arcsine transformation for hatching rate and survival rate and a logarithmic transformation for shell height and yield. Differences were considered statistically significant if $P < 0.05$.

The formula to calculate mid-parent heterosis (*H*) was taken from Cruz and Ibarra (1997):

$$H\ (\%) = [X_{F1} - (X_{GG} + X_{AA})/2] \times 100/(X_{GG} + X_{AA})/2$$

where X_{F1} is the mean shell height (yield or survival rate) of the hybrid F1; X_{GG} and X_{AA} are the mean shell height (yield or survival rate) of the Pacific oyster and the Fujian oyster.

The single patent heterosis (*I*) was calculated by the two formulas (Wang et al., 2011):

$$I_{(F1/GG)}\ (\%) = (X_{F1} - X_{GG}) \times 100/X_{GG}$$

$$I_{(F1/AA)}\ (\%) = (X_{F1} – X_{AA}) \times 100/X_{AA}$$

where X_{F1} indicates the mean shell height (yield or survival rate) of the hybrid F1 cohort (GA or AG), X_{GG} and X_{AA} indicates the mean shell height (yield or survival rate) of Pacific oyster and Fujian oyster, respectively.

2.5. Measurement of high temperature tolerance range

The measurement of high temperature tolerance was conducted according to a previous study (Wang et al., 2017). Before the experiment, adult oysters were kept in 20 °C seawater for two weeks. The seawater temperature was increased at a rate of 0.5 °C d^{-1} to reach the desired temperature. The temperature was set at 24, 26, 28, 30, 32, 34, 36 and 38 °C in plastic tanks (450 L) equipped with temperature controlling units. For each temperature group, twenty oysters were deployed in each of 3 replicates held in plastic bucket (5 L) for 96 h. Oysters were considered dead when they did not respond to any external stimulus and were removed immediately.

2.6. Annual water temperature data

The annual water temperature data in coastal areas of Shandong Province and Guangdong Province in 2019 was downloaded from National Marine Data Center, National Science & Technology Resource Sharing Service Platform of China (http://mds.nmdis.org.cn/).

3. Results

3.1. Hatching index

The fertilization and hatching rates of the hybrid and inbred groups are shown in Fig. 2 and Table 1. The fertilization rates of reciprocal hybrids were slightly lower than those of the two inbred groups, with the mid-parental heterosis of −2.25%. Similarly, the hatching rates of hybrid cohorts were not different from that of the inbred ($P > 0.05$), with the mid-parental heterosis of −3.34%. The high fertilization and hatching rate of the hybrid cohorts are related to the phylogenic proximity of the *C. gigas* and *C. angulata*.

3.2. Survival, growth and metamorphosis of larvae

No significant difference in survival were observed between GA and AG throughout the larval stage ($P > 0.05$). However, the survival rate of reciprocal hybrids was significantly higher than that of the inbred groups ($P < 0.05$) (Fig. 2), with the mid-parental heterosis of 16.60% at day 5, 22.21% at day 10 and 27.84% at day 15, respectively (Table 1). The survival rate of GA was the highest among the four groups at day 10 (76.41%) and day 15 (70.66%), with a single patent heterosis $I_{(GA/GG)}$ of 21.41% and 53.52%, respectively. Notably, the heterosis of reciprocal crosses (16.60% - 27.84%) gradually increased as the larvae grew (Table 1). Furthermore, the survival of GG (46.03%) was significantly

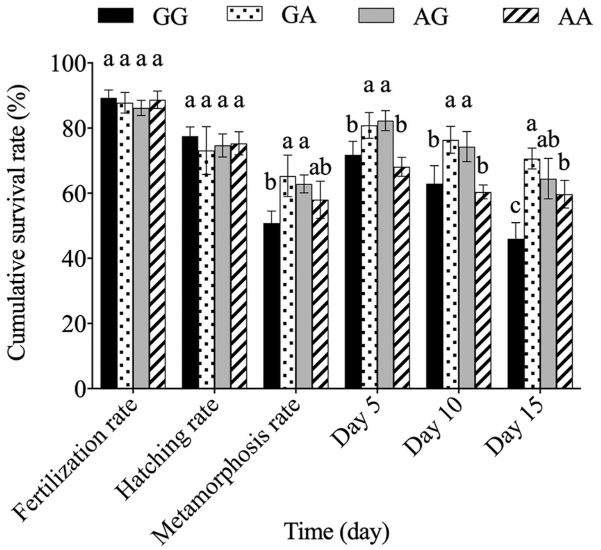

Fig. 2. Hatching index, metamorphosis rate and larval cumulative survival rate for two inbred groups and two hybrid groups. GG: *C. gigas* ♀ × *C. gigas* ♂; GA: *C. gigas* ♀ × *C. angulata* ♂; AG: *C. angulata* ♀ × *C. gigas* ♂; AA: *C. angulata* ♀ × *C. angulata* ♂. Different superscript letters at the same time indicate significant difference ($P < 0.05$).

lower than that of AA (59.69%) at day 15 ($P < 0.05$) (Fig. 2).

Shell heights at the larval stage are shown in Fig. 3. Among the four groups, the shell heights of newly hatched D-larvae were significantly different ($P < 0.05$), and GA (70.85 ± 1.06 μm) was slightly higher than AG (69.92 ± 1.30 μm) and AA (69.52 ± 1.47 μm). From day 10, the mean shell heights of GA and AG were significantly larger than those of inbred groups ($P < 0.05$), followed the order of GA > AG > GG > AA. At day 15, GA (338.60 ± 20.25 μm) was significantly larger than other three groups ($P < 0.05$), with a values of single patent heterosis $I_{(GA/AA)}$ at 23.51% (Table 1). The heterosis of reciprocal crosses (4.52% - 15.93%) gradually increased as the larvae grew (Table 1).

For the four groups, the critical stage where highest mortality occurred during the larvae development was the metamorphosis stage. There were significant differences between hybrid and inbred cohorts in metamorphosis ($P < 0.05$) (Fig. 2). Specifically, the metamorphosis rate was 65.33% in GA, 62.89% in AG, 58.00% in AA, and 50.89% in GG, with mid-parental heterosis of 17.75%; whereas the $I_{(GA/GG)}$ and $I_{(AG/GG)}$ in metamorphosis rate was 28.37% and 23.58%, respectively (Table 1).

3.3. *Survival and growth of juvenile and adult*

The highest temperature of coastal areas in Shandong Province was observed in August with an average temperature up to 25 °C (Fig. 4). High mortality was observed in Pacific oysters during the summer months. At day 90 and 450, 31.67% and 55.89% of oysters in the GG group were dead, respectively (Fig. 5A). However, the reciprocal hybrids exhibited excellent performance in survival. Except 25.19% of oysters in the AG group died at day 450, the survival rates of reciprocal hybrids were higher than 82% during both summer periods (Fig. 5A). The mid-parental heterosis and single patent heterosis $I_{(GA/GG)}$ in incremental survival rate were 38.20% and 97.41% at day 450,

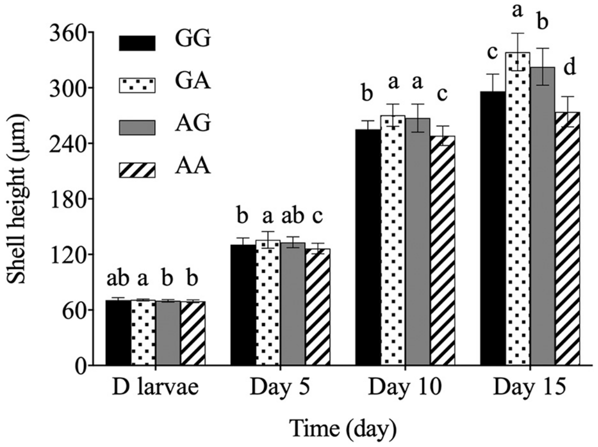

Fig. 3. Shell height for two inbred groups and two hybrid groups in the larval stage. Different superscript letters at the same time indicate significant difference ($P < 0.05$).

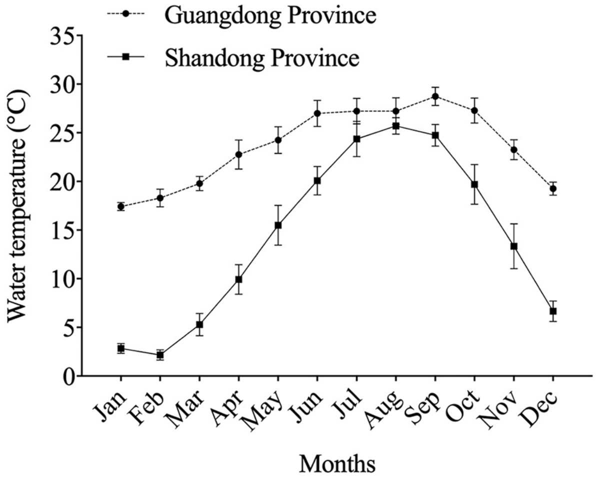

Fig. 4. Annual seawater temperature in coastal areas of Shandong Province and Guangdong Province in 2019, China (Data downloaded from http://mds.nmdis.org.cn/).

Table 1
Heterosis (H and I) for survival rate and shell height in *C. gigas* (GG), *C. angulata* (AA), and their hybrids (GA and AG) at the larval stage.

Items	Heterosis (%)	Fertilization rate	Hatching rate	Metamorphosis rate	Day 5	Day 10	Day 15
Survival rate	H	−2.25	−3.34	17.75	16.60	22.21	27.84
	$I_{(GA/GG)}$	−1.74	−5.74	28.37	12.57	21.41	53.52
	$I_{(AG/GG)}$	−3.48	−3.73	23.58	14.58	18.07	40.11
	$I_{(GA/AA)}$	−1.00	−2.95	12.64	18.73	26.52	18.38
	$I_{(AG/AA)}$	−2.76	−0.88	8.43	20.85	23.04	8.04
Shell height	H	–	–	–	4.52	6.85	15.93
	$I_{(GA/GG)}$	–	–	–	3.80	6.07	14.27
	$I_{(AG/GG)}$	–	–	–	1.80	4.84	8.92
	$I_{(GA/AA)}$	–	–	–	7.32	8.90	23.51
	$I_{(AG/AA)}$	–	–	–	5.26	7.65	17.72

H indicates mid-parent heterosis; $I_{(GA/GG)}$ and $I_{(AG/GG)}$ indicate the single patent heterosis of GA and AG relative to the *C. gigas* parental group, respectively; $I_{(GA/AA)}$ and $I_{(AG/AA)}$ indicate the single patent heterosis of GA and AG relative to the relative to the *C. angulata* parental group respectively.

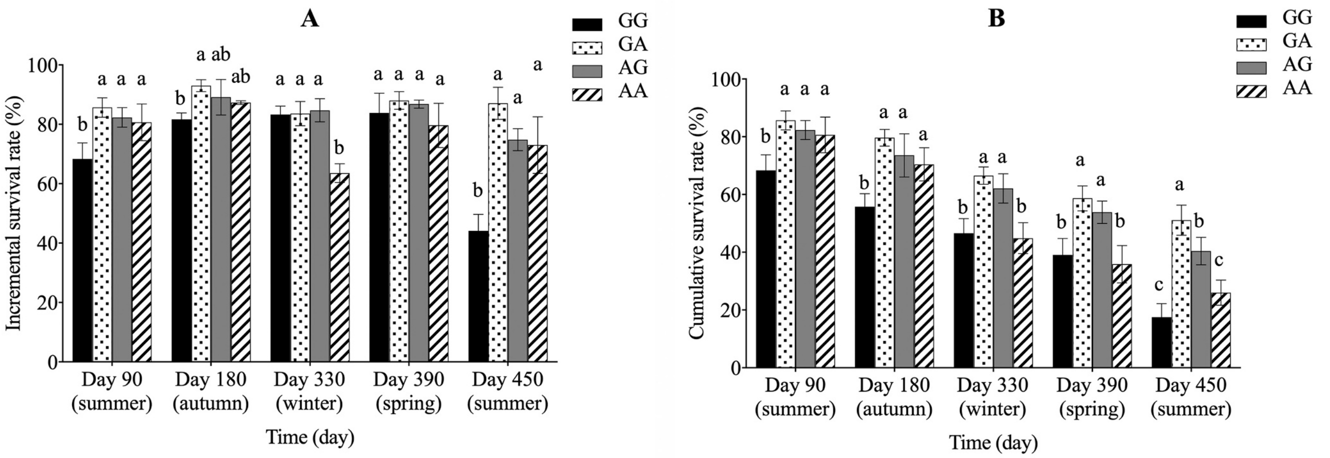

Fig. 5. Incremental survival rate (A) and cumulative survival rate (B) for two inbred groups and two hybrid groups in the juvenile and adult stage. Different superscript letters at the same time indicate significant difference ($P < 0.05$).

respectively (Table 2). After 450 days of grow-out, the cumulative survival rate of GG was only 17.56%, while the two reciprocal hybrids all had survival rates higher than 40% (Fig. 5B). Notably, the cumulative survival rate of GA increased by nearly 34% compared with GG at day 450, with a single patent heterosis of 191.14% (Table 2). The value of mid-parental heterosis in cumulative survival rate tended to increase with age, ranging from 12.75% to 110.20% (Table 2). In addition, the incremental survival rates of AA (63.56%) were significantly lower than those of the other three groups (GG: 83.31%, GA: 83.61%, AG: 84.72%) at day 330 (winter). (Fig. 5A). According to Fig. 4, the average water temperature was lower than 4 °C during winter period. Compared with AA, the two reciprocal hybrids showed higher survival advantage in winter, with a single patent heterosis $I_{(GA/AA)}$ of 31.55% and $I_{(AG/AA)}$ of 33.29% (Table 2).

The growth data at spat and adult stages are shown in Fig. 6. At all periods, reciprocal hybrids grew faster than inbred cohorts followed the order of GA > AG > GG > AA (Fig. 6A), with positive mid-parent heterosis ranging from 12.95% to 35.44% (Table 3). However, unlike survival, the growth mid-parent heterosis slowly increased from day 90 to day 390 and then decreased, with a maximum at day 390 (35.44%). From day 90 to 450, the shell heights of GA were significantly higher than those of two inbred groups ($P < 0.05$), though not always significantly higher from AG (Fig. 6A). Furthermore, the single patent heterosis $I_{(GA/AA)}$ *and* $I_{(AG/AA)}$ in shell height all fall in the range of 12.65% - 44.66%, which was larger than that of $I_{(GA/GG)}$ and $I_{(AG/GG)}$ (2.92% - 33.88%) in the same period (Table 3). The average yield differed substantially among different groups (Fig. 6B). The yield of hybrid crosses GA and AG were significantly higher than that of parental lines GG and AA at day 450 ($P < 0.05$), with the mid-parental heterosis of 182.72% (Table 3). The GA group had the highest yield (9855.64 g), with a single patent heterosis $I_{(GA/GG)}$ of 239.46% and $I_{(GA/AA)}$ of 209.35% (Table 3).

3.4. *High temperature tolerance*

Survival rate were 100% at 24 °C in all four groups and then decreased as the temperature elevated, followed the order of GA > AG > AA > GG. Specifically, at 30 and 32 °C the survival rate of GA was significantly higher than that of GG ($P < 0.05$), but no significant difference between the other three groups ($P > 0.05$). At 34 °C, the survival rate of hybrids GA and AG were significantly higher than that of GG ($P < 0.05$), while no significant differences within cohorts between GA, AG and AA and between AA and GG were observed ($P > 0.05$). At 36 °C, the survival rate of GA, AG and AA were significantly higher than that of GG ($P < 0.05$), but there was no significant difference between GA, AG and AA ($P > 0.05$). When the temperature increased to 38 °C, all four groups of oysters died after 96 h (Fig. 7).

4. Discussion

Hybridization is widely used to improve specific traits in animal and agricultural stocks, thus artificially producing offspring with phenotypic and genotypic improvements (de la Cruz and Gallardo-Escarate, 2011). In aquaculture, the desired goal is to obtain hybrid that perform better than purebred broodstock in terms of the survival rates, growth rates, meat quality, and stress resistance (Bartley et al., 2000; Zheng et al., 2019). Here, we discussed the potential of applying hybridization between Pacific oyster and Fujian oyster to breed a new hybrid variety with rapid growth and high summer survival characteristics.

4.1. *Survival*

In this study, the cumulative survival of the reciprocal hybrids was significantly higher than that of the GG during the grow-out stage, which

Table 2
Heterosis (H and I) for cumulative survival rate and incremental survival rate in *C. gigas* (GG), *C. angulata* (AA), and their hybrids (GA and AG) at the juvenile and adult stage.

Items	Heterosis (%)	Day 90 (summer)	Day 180 (autumn)	Day 330 (winter)	Day 390 (spring)	Day 450 (summer)
Cumulative survival rate	H	12.75	21.39	40.70	50.07	110.20
	$I_{(GA/GG)}$	25.37	42.83	42.96	50.00	191.14
	$I_{(AG/GG)}$	20.49	31.87	33.41	37.78	130.38
	$I_{(GA/AA)}$	6.20	13.09	48.27	63.47	96.58
	$I_{(AG/AA)}$	2.07	4.42	38.37	50.15	55.56
Incremental survival rate	H	12.75	7.82	14.61	6.95	38.20
	$I_{(GA/GG)}$	25.36	13.93	0.36	5.01	97.41
	$I_{(AG/GG)}$	20.49	9.15	1.69	3.57	69.59
	$I_{(GA/AA)}$	6.20	6.57	31.55	10.51	19.25
	$I_{(AG/AA)}$	2.07	2.10	33.29	9.00	2.44

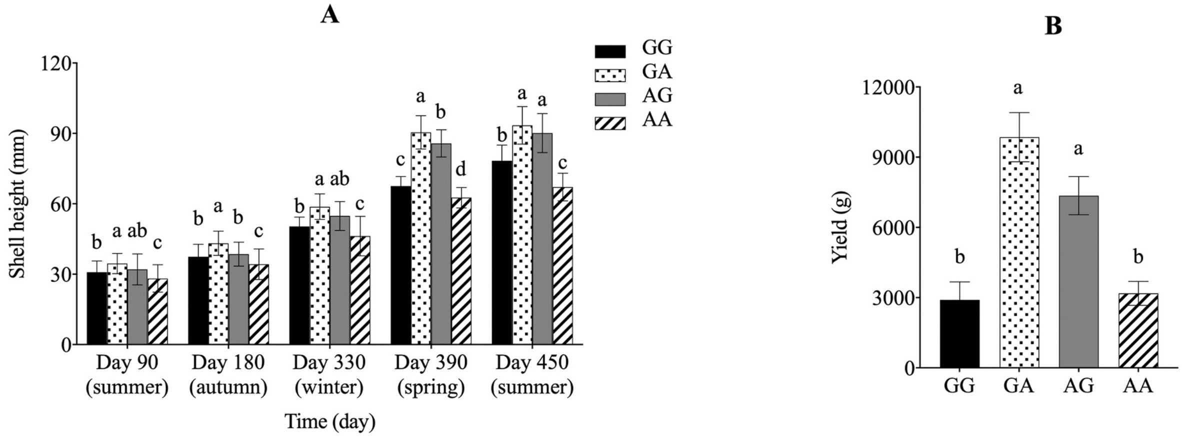

Fig. 6. Shell height (A) and yield (B) for two inbred groups and two hybrid groups in the juvenile and adult stage. Different superscript letters at the same time indicate significant difference ($P < 0.05$).

Table 3
Heterosis (H and I) for Shell height (SH) and Yield (Y) in *C. gigas* (GG), *C. angulata* (AA), and their hybrids (GA and AG) at the juvenile and adult stage.

Heterosis (%)	Day 90 SH	Day 180 SH	Day 330 SH	Day 390 SH	Day 450 SH	Day 450 Y
H	12.95	14.06	17.74	35.44	26.31	182.72
$I_{(GA/GG)}$	11.85	15.34	16.83	33.88	19.31	239.46
$I_{(AG/GG)}$	3.93	2.92	8.93	26.91	15.16	153.49
$I_{(GA/AA)}$	22.86	26.25	27.35	44.66	39.32	209.35
$I_{(AG/AA)}$	14.17	12.65	18.74	37.13	34.48	131.01

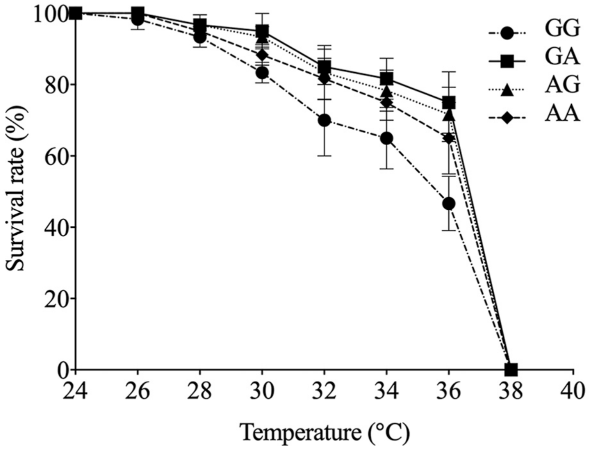

Fig. 7. The high temperature tolerance range for two inbred groups and two hybrid groups.

may result from the stronger adaptability of reciprocal hybrids than that of Pacific oysters under particular culture conditions (Zhang et al., 2015). Hybridization could increase genetic heterozygosity and weaken the influence of recessive lethal genes, thereby improving the environmental adaptability of hybrids and producing heterosis (Whitlock et al., 2000). From the day 5 to day 15 and day 90 to day 450, the mid-parent heterosis in cumulative survival rate increased continuously, reaching the maximum value of 110.20% at day 450. In general, the magnitude of heterosis is not constant throughout life history, and tended to increase with age in marine molluscs, such as scallops (Zheng et al., 2006) and abalones (You et al., 2009). Nevertheless, when hybrids inherit incompatible traits from their parents, adaptation to the environment is reduced, which would cause out breeding depression or hybrid breakdown (Stelkens and Seehausen, 2009).

Similar to previous results (Mao et al., 2005; Lian et al., 2010), the highest mortality rate for GG was observed in summer (day 90 and 450), demonstrating that the summer mass mortalities were still the primary challenge for *C. gigas* in northern China. The average seawater temperature in the coastal area of Shandong Province reached about 25 °C in summer (July to September) (Fig. 4). Therefore, summer mortality for Pacific oysters may be closely related to high water temperature (Shiel et al., 2017; Yang et al., 2021). Surprisingly, the hybrid GA outperformed the purebred GG by nearly 43% in incremental survival at day 450 (the second summer), with a single patent heterosis $I_{(GA/GG)}$ of 97.41%. This result showed that the over-summer survival rate of oysters could be improved through hybridization. You et al. (2015) also reported that hybrid abalone had higher summer survival rate than Pacific abalone. The significant improvement in survival of GA may have been contributed by partial transfer of the high temperature tolerance of Fujian oysters to the hybrid GA. Consequently, when GG reach a physiological limit at a temperature lower than that of the hybrid population, the hybrid GA could better cope with this stress. However, the hybrids between the red abalone and the green abalone could do moderately well, because none of the parent species could not tolerate high temperature in summer (You et al., 2015).

4.2. *Growth*

Studies from crossbreeding experiments between *C. gigas* and *C. angulata* suggested no heterosis of the reciprocal hybrids (Menzel, 1974; Soletchnik et al., 2002; Huvet et al., 2002; Batista et al., 2007; Batista et al., 2008). Therefore, most breeders rarely conduct crossbreeding between these two species. Conversely, this study indicated that the reciprocal hybrids were significantly larger than the two parental progenies with positive mid-patent heterosis (12.95% - 35.44%). Superior performance in growth could be partly explained by genetic component differences of the broodstocks. The Pacific oyster used in this study was from a selected line, which had been successfully bred for 12 generations with rapid growth trait. When the parent populations were continuously artificially selected, the hybrid will exhibit significant heterosis due to the accumulation of a large number of different non-additive genetic variation (Sheridan, 1997). Nevertheless, environment may alter gene expression levels and affect additive and non-additive genetic components, resulting in differences in phenotypes (Yan et al., 2018).

Yield is an important characteristic in oyster commercial production. In this research, the reciprocal hybrids outperformed its two parental species in final yield, and the mid-parent heterosis was as high as 182.72%. Compared to GG, the yield of hybrid GA increased by about 239.46%. Similarly, the high heterosis for yield was also found in intraspecific hybrids of *C. gigas*, in which the high-parent heterosis for yield can be as high as about 100% (Hedgecock and Davis, 2007). Variations in yield may be explain by the different growth and mortality patterns among the four cohorts (Rawson and Feindel, 2012). The cumulative survival rate of hybrid groups (GA: 51.11%; AG: 40.44%) were approximately two times of that of purebred groups (GG: 17.56%; AA: 26.00%) at the final stage. On the other hand, faster growth of hybrid cohorts was observed during the whole field experiment, which also contributed significantly to differences in yield. Consequently, the extraordinary high yield characteristics of the hybrids between *C. gigas* and *C. angulata* indicate great potential to oyster farming industry in northern of China.

4.3. Difference performance of reciprocal hybrids in northern and southern China

There is a substantial amount of research suggesting that the environmental factors (e. g. temperature, salinity, food) may contribute to the phenotypic variation of hybrids (Rawson and Feindel, 2012; Xu et al., 2019). In this study, the growth of the hybrid GA was significantly faster than that of AG after one year of growth in Rongcheng, which was completely opposite to the results in Shantou, Guangdong Province, China (Tan et al., 2020) (Fig. 1). Growth difference in different environments was also observed in the crosses of *C. ariakensis* × *C. sikamea* (Xu et al., 2011) and *C. hongkongensis* × *C. ariakensis* (Qin et al., 2020). The annual seawater salinity in Shantou and Rongcheng varies from 31 psu to 33 psu (data not shown), while the seawater temperature varies widely, from 16.37 °C to 28.04 °C and from 2.16 °C to 25.72 °C, respectively (Fig. 4). Considering the small salinity variation between the two sites, we believe that the temperature may be a major environmental factor contributing to the difference of hybrid growth. Meanwhile, maternal effects may be another reason for this difference (Zhang et al., 2017). Due to the different direction of hybridization, the rapid growth traits of the selected line of Pacific oyster were more transferred to GA instead of AG. In addition, hybrids at both sites showed positive heterosis in growth (Rongcheng: 12.95% - 35.44% for shell height, Shantou: 3.93% - 13.83% for shell length), which suggested that the hybridization between *C. gigas* and *C. angulata* can be applied to improve the growth of oyster in northern and southern China.

The cumulative survival rate of GA (51.11%) was significantly higher than that of AG (40.44%) in Rongcheng after one year, while the difference between the two hybrids was not significant in Shantou (GA: 55.40%, AG: 55.70%) (Tan et al., 2020). This suggested that GA might have an adaptability advantage over AG in low or moderate temperature environmental conditions. Moreover, the mid-parent heterosis for juvenile cumulative survival rate at Rongcheng (12.75% - 110.20%) was significantly larger than that at Shantou (1.30% - 25.40%). The *C. gigas* and *C. angulata* cultured in Rongcheng suffered mass death in summer and winter, respectively. In Shantou, however, the survival rate of *C. angulata* was not significantly different from other groups in winter because the average water temperature in winter was kept at about 16 °C. Meanwhile, the reciprocal hybrids remained high survival rates during the whole farming period at both sites. Consequently, a higher survival advantage of hybrids was observed in Rongcheng rather than Shantou, which suggested that the hybrid might had more application value under the nursery conditions in north China. Overall, this study demonstrated production traits variability of reciprocal hybrids across different sites, indicating the necessity for different strategies for their management and aquaculture.

4.4. Difference of heterosis between survival and growth

It is noteworthy that the heterosis in cumulative survival rate was much greater than that in shell height at both the larval and rearing stages. In particular, the mid-patent heterosis of cumulative survival reached to 110.20% at day 450, while the heterosis of shell height was 26.31%. Similar results were observed in the Bay scallop *Argopecten irradians irradians* (Wang and Li, 2010). Theoretically, two reasons may explain the difference of heterosis between survival and growth. On the one hand, since no directional selection was carried out for the survival traits of *C. gigas* and *C. angulata*, it is possible that the survival-related loci of these two oysters may be more diverse than that of growth (Han et al., 2020). The magnitude of heterosis in hybrid is determined by the square of the difference in gene frequency between the parental species, and the larger genetic difference of populations and the higher homozygosis frequency, the greater heterosis would cause (Kong et al., 2017). On the other hand, fitness-related traits such as survival are more likely to exhibit directional dominance compared with morphological-related traits (Lynch and Walsh, 1998). The expression of heterosis mainly depends on the directional dominance of genes related to the target trait and the increase of mean value on crossbreeding is a consequence of dominance at the loci concerned with the trait related to enhance performance (Falconer and Mackay, 1996; Lamkey and Edwards, 1998). As a result, the heterosis was expressed more in the increasing survival but less contribution to shell height.

4.5. High temperature tolerance

The single-parent heterosis $I_{(GA/GG)}$ and $I_{(AG/GG)}$ for incremental survival rate was positive in the field and closely related to seawater temperature, with a maximum in summer. Therefore, it is necessary to identify whether hybridization could improve the tolerance of oysters to higher temperature. After 96 h heat shock, the survival rates of hybrids and parental groups were significantly different (GA > AG > AA > GG), indicating that crossbreeding enabled the hybrids to tolerate higher temperatures compared to *C. gigas*. Similarly, the improvement for thermal tolerance was found in the hybridization between female red abalone and male pacific abalone (Lafarga de la Cruz and Gallardo-Escárate, 2011). Furthermore, we evaluated the differences in heat tolerance of *C. gigas*, *C. angulata* and their reciprocal hybrids at juvenile stage in a previous study (Jiang et al., 2021). Results showed that the semi-lethal temperature (LT50) of GA, AG, AA and GG were 42.48 °C, 41.94 °C, 41.63 °C and 41.55 °C, respectively. Therefore, we could conclude that hybrid oysters were more resistant to high temperature than *C. gigas* in both juvenile and adult stages, which also explains the high survival rate of reciprocal hybrids during the first (day 90) and second summer (day 450) in the field trials. However, this heterosis for thermotolerance of hybrids needs to be explained by further physiological studies. High temperature is known to result in chemical changes in water quality (higher ammonia levels, pH changes, lower dissolved oxygen, and nutritional factors), which impair the immune system and make species more susceptible to disease (Vandepeer, 2006). Based on the results in field trials and heat shocks, we confirmed that high temperature was the crucial reason causing summer mass mortality of *C. gigas* in northern China.

In summary, this study clearly demonstrated that that the reciprocal hybrids between *C. gigas* and *C. angulata* exhibited high heterosis in growth and survival at larval and grow-out stages in northern China. At day 450, the cumulative survival rate was increased by nearly 34% in GA compared with GG, with the single patent heterosis of 191.14%. In particular, the final yield of reciprocal hybrids was significantly higher than that of inbred lines, with a mid-parent heterosis of 182.72% and $I_{(GA/GG)}$ of 239.46%. This study also showed that the reciprocal hybrids had higher thermal tolerance than parental species. Based on the performances in growth, survival and thermotolerance, GA (*C. gigas* ♀ × *C. angulata* ♂) has a large potential to become the preferred alternative variety in oyster farming in northern China.

Author statement

Gaowei Jiang: Investigation, Conceptualization, Formal analysis, Writing - original draft. **Qi Li:** Supervision, Conceptualization, Resources, Writing - review & editing, Funding acquisition. **Chengxun Xu:** Supervision, Resources. **Shikai Liu:** Resources. **Lingfeng Kong:** Data curation. **Hong Yu:** Data curation.

Declaration of Competing Interest

The authors declare no conflict of interest.

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Appendix A. Supplementary data

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