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ORIGINAL ARTICLE

Effects of salinity on early activities of artificial hybridization between *Crassostrea ariakensis* and *c. gigas*

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Abstract

The hybridization experiments have been made between *Crassostrea ariakensis* and *C. gigas* several times. However, it is difficult to obtain a large number of hybrid offspring, which becomes the bottleneck of subsequent cross-breeding. To explore whether the production of hybrid larvae is affected by salinity, we investigated the effects of salinity (16, 20, 24, 28 and 32 psu) on the early activities of artificial hybridization between *C. ariakensis* and *C. gigas at* 23–24°C. In this study, the results showed that during artificial insemination, the appropriate salinity of high-quality gametes in *C. ariakensis* and *C. gigas* was 20–32 psu and 24–32 psu respectively. Besides, the fertilization rate of AG (*C. ariakensis*♀ × *C. gigas*♂) and GA (*C. gigas*♀ × *C. ariakensis*♂) at 24–28 psu was significantly ($p < .05$) higher than that at 16–20 psu. For incubation, the optimal salinity of embryo incubation was 28 psu, under which all embryos of AG and GA can develop rapidly to the D-larvae and yielded a high hatching rate. During larval rearing, the larval shell height and survival rate of AG were of no significant difference ($p > .05$) at all salinities, and the optimum salinity for larval growth and survival of GA was 24–28 psu. These findings can contribute to the increase in the hybrid progeny yield, which can serve as the new resource for genetic improvement of oyster germplasm.

KEYWORDS
C. gigas, *Crassostrea ariakensis*, early activities, hybridization, salinity

1 | INTRODUCTION

Interspecific hybridization is an important tool to achieve the genetic improvement of aquatic economic animals, and it has been widely used in the genetic improvement of bivalve shellfish such as the oyster, abalone and scallop (Cruz & Gallardo-Escárate, 2011; Rah et al., 2013). The artificial interspecific hybridization of oysters began as early as 1882, which was also the earliest study on the hybridization of shellfish (Gaffney & Allen, 1993). Cross-breeding has aroused extensive concerns as a pretty useful tool for oyster breeding. In China, there are five common economically valuable *Crassostrea* oyster species, including *C. gigas*, *C. hongkongensis*, *C. ariakensis*, *C. angulata* and *C. sikamea*, some of which have been carried out cross-breeding experiments several times (Guo, 2009; Wang et al., 2006; Zhang et al., 2014).

C. gigas is an economic species of worldwide mariculture due to its fast growth, strong disease resistance and high environmental adaptability (Wang et al., 2012; Zhang, Fang, et al., 2012). Such excellent traits make it an important material for genetic improvement. *C. ariakensis* mainly inhabits in the estuaries and intertidal zones in the western Pacific, and it has once been considered as a new breeding object to development in several countries (Calvo et al., 2001; Yoon et al., 2008; Zhang et al., 2005). In China, *C. ariakensis*, with a natural distribution in the entire coastal areas of China, is not widely cultivated due to its slow growth and low meat weight. Thus, we launched a hybridization experiment between the two, hoping that *C. ariakensis* can exhibit the same

characteristics of fast growth and excellent meat quality as *C. gigas*, so as to realize the genetic improvement of the *C. ariakensis* and develop new germplasm resources for China's oyster aquaculture.

Salinity, as one of the basic indicators of water environment, usually varies with climate changes or human activities, thus affecting the survival of aquatic animals (Kinne, 1964; Lettieri et al., 2019; McNeil & Sasse, 2016). As an important member of aquatic animals, bivalves are affected by salinity in many aspects (Hosoi et al., 2010; Lettieri, Maione, et al., 2019; Madrones-Ladja, 2002; Taylor et al., 2004; Verween et al., 2007). Oyster is a typical euryhaline bivalve (Xu et al., 2011), and the researches on the effects of salinity on oysters are attracting attention, which mainly focus on gamete quality (Qin et al., 2018), embryonic development (Dos Santos & Nascimento, 1985), fertilization and hatching (Han & Li, 2018), larval growth and survival (Kinne, 1964; Nell & Holliday, 1988), and growth and survival of juvenile (Rybovich, Peyre, Hall, & Peyre, 2016). Interspecific hybridization has been attempted between *C. ariakensis* and *C. gigas*, but poor gamete compatibility and low larval survival made it difficult to produce more crossing progenies (Allen & Gaffney, 1993; Que & Allen, 2002; Yao, 2014), which cannot meet the demand for subsequent genetic improvement. We hypothesized that the hybridization can be influenced by salinity, since the habitats of two oysters differ greatly in salinity. In this study, we investigated the impacts of salinity on the artificial insemination, incubation and larvae rearing of hybridization between *C. ariakensis* and *C. gigas*. The data we obtained will help to improve the yield of hybrid larvae, which can serve as new genetic improvement resources.

2 | MATERIALS AND METHODS

2.1 | Broodstock cultivation

The *C. ariakensis* (SH 21.59 cm ±4.95 cm) was harvested from the wild population in the Yellow River Delta of Dongying, Shandong Province, China (38.15°N, 118.5°E). The *C. gigas* (SH 9.99 cm ±1.55 cm) was collected from the artificially cultured population in Rushan City, Shandong Province (36.4°N, 121.3°E). Both *C. ariakensis and C. gigas* were transferred to the hatchery of Dawn Fisheries Co. LTD, Yantai, Shandong Province, on 15 January 2020. Both two oyster species were cultured in a 20-m^3 concrete tank containing filtered seawater with a salinity of 28 and fed with *Nitzschia closterium* at a quantity of 250,000 – 30,0000 cells·ml^{-1}·d^{-1}. The water temperature gradually raised from 6°C to 20°C and finally kept at 18–20°C for one month to ensure that the two oyster species mature synchronously (Ma et al., 2019; Wang et al., 2011; Xu et al., 2019).

2.2 | Gamete collection

Oysters were dissected to identify the sex in accordance with routine method as described by Xu et al. (2009). For egg collection, the gonads were stripped from the oysters, sifted through a 90-μm nylon screen, rinsed on a 25-μm screen and finally immersed in the seawater of the corresponding salinity. For sperm collection, sperm was collected 20 min before fertilization. The gonads were removed from the male oysters and dissolved in seawater, and then, the sperm was filtered with a 90-μm nylon screen.

2.3 | Fertilization and incubation and larval rearing

The collected gametes were fertilized with 2 × 2 factorial crosses, and then, four fertilization combinations were obtained, including AA (*C. ariakensis*♀ × *C. ariakensis*♂), AG (*C. ariakensis*♀ × *C. gigas*♂), GA (*C. gigas*♀ × *C. ariakensis*♂) and GG (*C. gigas*♀× *C. gigas*♂). Importantly, eggs must be confirmed with no uncontrolled fertilizations before artificial insemination. After 5–10 min of adaptation, sperm was gradually added into the egg suspension and observed under the light microscope to control the presence of 10–15 sperm around each egg. After insemination, incubation was carried out at 23–24°C with a density of 30 eggs /ml. After 24 hours, D-larvae of AA, AG, GA and GG were obtained and then cultured in seawater at 23–24°C. Larval rearing was carried out according to the method described by Xu et al. (2019) which meant that 50% of water was changed once a day, and the larvae were fed with a mixture of *Isochrysis galbana* and *Chaetoceros calcitrans* at a quantity of 30,000–80,000 cells·ml^{-1}·d^{-1}.

2.4 | Experimental design

In this study, the salinity range was set according to the range of salinity changes in the coastal aquaculture areas in China (Chu et al., 2005; Liu et al., 2008). Specifically, there were five salinity levels (16, 20, 24, 28 and 32 psu) in all the experiments. And high-salt seawater was obtained by dissolving aquarium salt in natural seawater, while low-salt seawater was obtained by diluting natural seawater with filtered freshwater, and the salinity value was measured with a portable refractometer (ATAGO). Each experiment was repeated three times.

To explore the effects of salinity on gametes, the gametes were treated with different salinities, and germinal vesicle breakdown (GVBD) of stripping eggs and sperm movement was observed. The germinal vesicle breakdown (GVBD) is an important sign of stripped oocyte maturation, so we directly measure the maturity of egg by the GVBD ratio. These gametes were collected in the following ways. The gonad from the individual oyster was divided into five equal parts, and each part was treated by the method of step 2.2 to collect the gametes. The collected gametes were then immersed in seawater with the salinity of 16, 20, 24, 28 and 32 psu respectively. What called for special attention was that each gamete was treated with seawater at a corresponding salinity during gamete collection. One male or one female was used at each replication.

To explore the effects of salinity on gamete fertilization and incubation, fertilization rate, embryo development and hatching rate of different fertilization combinations were observed in different

salinities. The collected eggs were soaked in normal seawater (23–24°C, 28 psu) for 60 min. Afterwards, the egg suspension of *C. ariakensis* (*C. gigas*) was divided into 10 equal parts, five parts for self-cross and five parts for cross, and then added into five types of seawater with different salinities respectively. The collected sperm was added to the prepared egg suspension in the way of 2 × 2 factorial crosses as mentioned in 2.3, and one male was paired with one female at each replication.

To explore the effects of salinity on larval rearing, the shell height and survival rate of larvae at different salinities were compared. The gamete collection, insemination and incubation were performed in natural seawater. The D-larvae of AA, AG, GA and GG were obtained after incubation for 24 hours; then, each group was divided into five equal parts. Each part was first placed in the seawater with a salinity of 28 and then acclimated for 1 day by adjusting at a rate of 2 psu/hour (Han & Li, 2018; Wang et al., 2018). After salinity acclimatization, the larval density was adjusted to 1 ind. / ml. Three females and three males were paired in each replication.

2.5 | Sampling and measure

We observed the sperm movement and egg maturity under the light microscope every 10 min. The sperm adaptation time, fast movement time and total movement time were recorded by a stopwatch as described by Du et al. (2018). Specifically, adaptation time (AT) refers to the period in which sperms turn their basic immobility into rapid movement of about 50% of them after mixing with seawater. Fast movement time (FT) refers to the time from when the adaptation time ends to when around 70% of fast-moving sperms stop moving or move slowly. The total movement time (TT) means the time from when sperms are mixed with gradient seawater to when over 95% of sperms stop moving. The sperm vitality refers to the ratio of FT to TT. The egg maturity was measured by the GVBD ratio as described by Qin et al. (2018), and the GVBD ratio refers to the percentage of the number of oocytes without visible germinal vesicles to the total of normal oocytes.

In insemination and incubation experiments, samples were taken and fixed in 2% seawater formaldehyde at 0.5 h, 1.5 h, 12 h and 20 h respectively. The frequency distribution of embryos at various developmental stages (Figure 1) was recorded under the light microscope. Also, samples were taken at 2 h and 24 h, respectively, for the determination of fertilization rate and hatching rate. The fertilization rate, hatching rate, shell height and survival rate of larvae were calculated as previously reported (Zhang, Wang, et al., 2012). Fertilization rate means the percentage of fertilized eggs to the total number of eggs, and the hatching rate means the percentage of the number of D-larvae to the number of fertilized eggs at 24 h.

2.6 | Statistical analysis

Heterosis was calculated to evaluate the aquaculture traits, and the heterosis of the larvae of hybrid was calculated by following formula (Falconer, 1981):

$$H\ (\%) = [2F_1 - (P_1 + P_2)] / (P_1 + P_2) \times 100$$

where F_1 indicates the phenotypic value (shell height, survival rate) of the AG or GA progeny, and P_1 and P_2 indicate the mean phenotypic value (shell height, survival rate) of AA and GG respectively.

One-way ANOVA and Tukey comparisons were applied to analyse the effects of salinity on sperm movement, fertilization rate, hatching rate, larval shell height and larval survival rate. Besides, the larval shell height and survival rate of group AG and group GG at 16 psu were not counted. The reason was that in the three replicates of

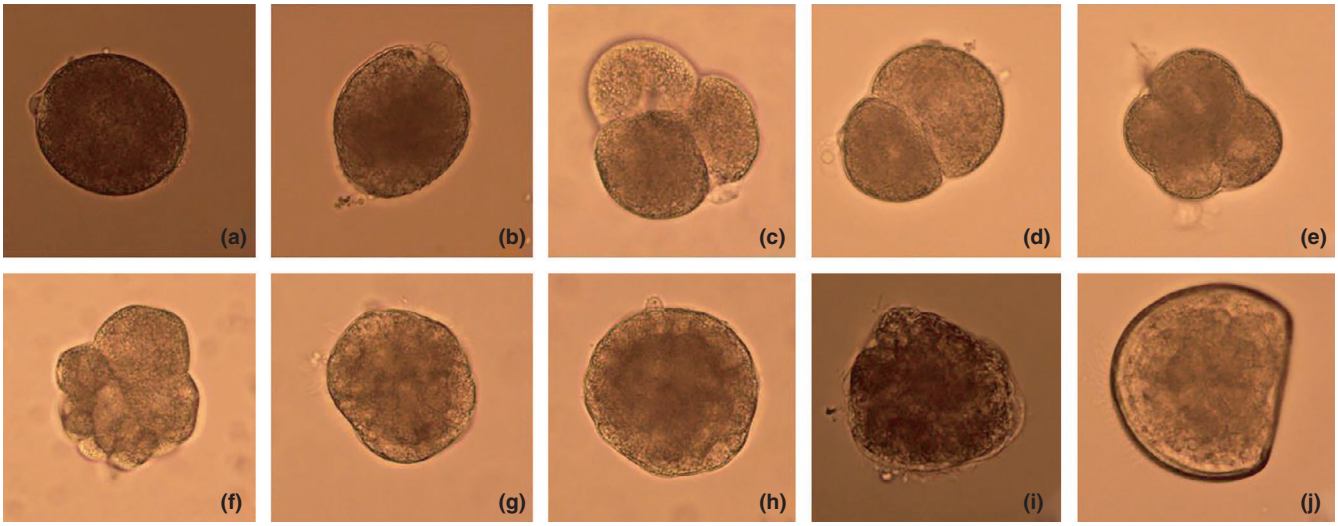

FIGURE 1 Embryonic development of *Crassostrea gigas*. (a) First polar body; (b) second polar body; (c) first cleavage; (d) two-cell stage; (e) four-cell stage; (f) sixteen-cell stage; (g) blastula stage; (h) primal period; (i) trochophore; (j) D-shaped larvae. [Colour figure can be viewed at wileyonlinelibrary.com]

AG, none of the larvae survive to 20d, and in the three replicates of GG, handful of larvae survive to 20d and they are all in one replicate.

3 | RESULTS

3.1 | Artificial insemination

At 20–32 psu, *C. ariakensis* had a faster GVBD rate, and the GVBD ratio of stripping eggs exceeded 50% and 80%, respectively, at 30 min and 60 min. Relatively, the highest GVBD ratio was at 24 psu, reaching 95.56%±3.85% at 60 min (Figure 2a). For *C. gigas*, the GVBD ratio of stripping eggs exceeded 90% at 28 −32 psu, and it also can reach 90% eventually at 24 psu even though the speed of the GVBD was relatively slow. The final GVBD ratio was less than 50% at 16-20psu (Figure 2b).

For *C. ariakensis*, the sperm adaptation time at 20–32 psu was significantly shorter than that at 16 psu. The fast movement time, total movement time and sperm vitality at 20–32 psu were significantly ($p < .05$) higher than those at 16 psu. For *C. gigas*, the sperm adaptation time was relatively shorter at 24–32 psu, all less than 10 seconds. Besides, the fast movement time, total movement time and sperm vitality at 24–32 psu were significantly ($p < .05$) higher than those at 16–20 psu (Table 1).

The fertilization rate of AA at 24–28 psu was significantly ($p < .05$) higher than that at 16-20psu, and the highest fertilization rate was 80.26%±9.08% at 28 psu. The fertilization rate of GG at 24–32 psu was significantly ($p < .05$) higher than that at other salinities, and the highest fertilization rate was 88.49%±5.57% at 28 psu. The fertilization rate of hybrid combinations (AG and GA) was lower than that of self-cross combinations (AA and GG) at all salinities. The fertilization rate of AG was higher at 24–28 psu, which was significantly ($P <. 05$) higher than that of two low salinity groups (16 and 20 psu). The fertilization rate of GA was the highest at the salinity of 24 and 28, significantly ($P <. 05$) higher than that of other salinities (Figure 3a).

3.2 | Incubation

For AA, at 20–32 psu, the embryonic development was normal and more than 50% of the embryos were at the stage δ at 20 h. The embryo developed most rapidly at 28 psu, and the frequency of the embryo at the stage δ was as high as 75.22% at the 20th hour (Figure 4AA). For AG, the embryonic development rate was faster at 24–32 psu. However, at 16–20 psu, the embryonic development was slow, the frequency of the embryo at the stage α and β was less than 20% at 0.5 h, and the frequency at the stage β was less than 20% at 1.5 h (Figure 4AG). For GA, when shorter than 1.5 h, the embryos developed most rapidly at 24–28 psu, but to 20 h, the embryo development at 32 psu gradually accelerated, and more than 15% of the embryos reached the stage **δ** (Figure 4GA). For GG, the embryonic development at 24–32 psu was normal, but the embryo development was the best at 28–32 psu, and more than 60% of the embryos reached the stage δ at 20 h (Figure 4GG).

The hatching rate of AA at 24–32 psu was significantly ($p < .05$) higher than that at 16–20 psu. For GG, the hatching rate at 28–32 psu showed no significant differences ($p > .05$) and was significantly higher ($p < .05$) than that at other salinities, and the hatching rate decreased distinctly with the decline of salinity. The highest hatching rate of AG was 59.64%±5.61% at 28 psu, and the hatching rate at other salinities was less than 50% and with no significant difference ($p > .05$). The highest hatching rate of GA reached 41.12%±5.69% at 28 psu, which was significantly ($p < .05$) higher than that of other groups (Figure 3b).

3.3 | Larval rearing

For AA, the larval shell height was of no significant difference ($p > .05$) at 24–32 psu and the shortest one is at 16 psu. The larval shell of GG at 24–32 psu was significantly ($p < .05$) higher than that at 20 psu. There were no significant differences ($p > .05$) in the larval shell height of AG at different salinities. For GA, the shell height was

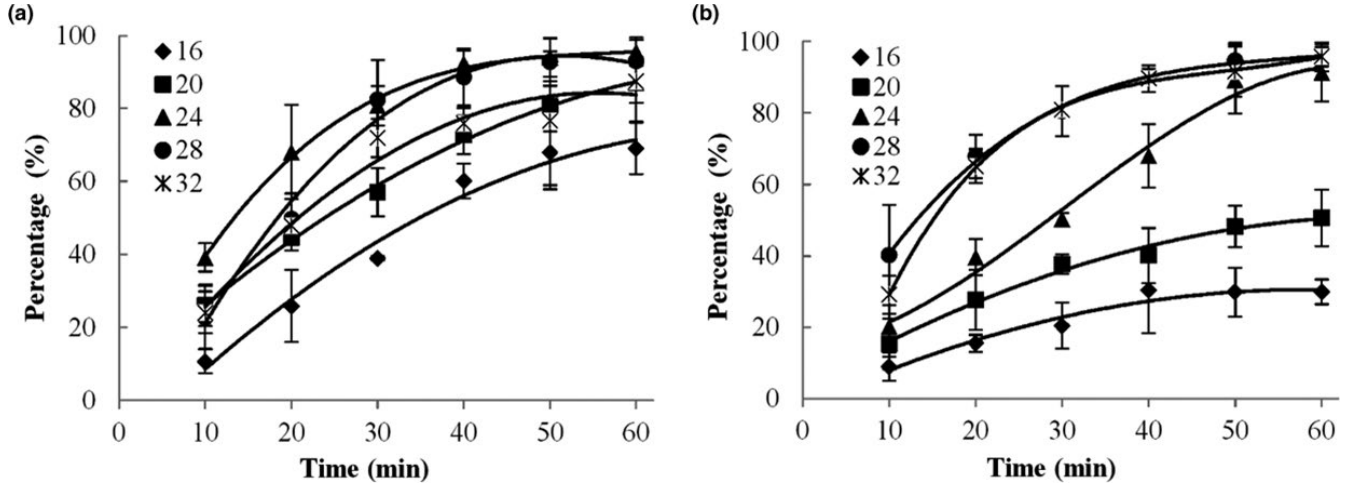

FIGURE 2 The germinal vesicle breakdown (GVBD) ratio (mean ±SD, n = 3 replicates) of stripping eggs in *C. ariakensis* (a) and *C. gigas* (b) at different salinities.

TABLE 1 The comparison of sperm movement between *C. ariakensis* and *C. gigas* (mean ±SD, n = 3 replicates).

Item	Salinity	*C. ariakensis*	*C. gigas*
AT (min)	16	21.78 ± 5.34^{b}	23.79 ± 8.04^{c}
	20	9.10 ± 3.27^{a}	18.21 ± 9.46^{bc}
	24	6.31 ± 4.59^{a}	8.64 ± 2.34^{ab}
	28	3.06 ± 1.78^{a}	4.71 ± 4.69^{a}
	32	6.23 ± 4.33^{a}	5.71 ± 3.14^{a}
FT (min)	16	13.91 ± 7.08^{a}	5.44 ± 2.67^{a}
	20	54.32 ± 7.96^{b}	21.61 ± 9.41^{b}
	24	64.04 ± 11.03^{b}	65.33 ± 4.93^{c}
	28	75.81 ± 7.72^{b}	69.33 ± 7.89^{c}
	32	50.96 ± 13.23^{b}	62.17 ± 6.36^{c}
TT (min)	16	58.73 ± 17.45^{a}	41.34 ± 7.72^{a}
	20	101.30 ± 15.75^{b}	59.39 ± 6.35^{b}
	24	128.25 ± 11.32^{b}	110.20 ± 13.88^{c}
	28	124.32 ± 15.40^{b}	128.14 ± 2.51^{d}
	32	105.43 ± 13.08^{b}	122.21 ± 9.75 ^{cd}
V (%)	16	22.75 ± 5.27^{a}	13.08 ± 6.24^{a}
	20	50.21 ± 1.30^{b}	35.66 ± 12.79^{b}
	24	49.75 ± 4.87^{b}	55.53 ± 5.36^{c}
	28	53.03 ± 3.65^{b}	54.20 ± 7.23^{c}
	32	47.88 ± 6.72^{b}	50.91 ± 4.02^{c}

Different superscript letters in each indicator indicate significant differences (P <. 05). AT, FT, TT and V, respectively, represent the sperm adaptation time, sperm fast movement time, sperm total movement time and sperm vitality.

higher at 28–32 psu, which was significantly higher (p < .05) than that of low salinity groups (16 and 20 psu), and the larvae reached to maximum shell height of 300.30 μm±6.29 μm at 28 psu (Figure 5a).

There were no significant differences (p > .05) in the survival rate of AA larvae at 20–32 psu, and the lowest survival rate was 25.28%±14.71% at 16 psu. For AG, the larvae failed to survive to 20d at 16 psu, and the larvae of three replicates died on the 2nd, 4th and 5th day respectively. Besides, the larval survival rate was lower at 20–32 psu with no more than 20% and no significant differences (p > .05). The larval survival rate of GA was higher at 24–28 psu, and the highest one was 33.37% ±4.36% at 28 psu. For GG, the larval survival rate was over 50% at 24–32 psu, which was significantly (p < .05) higher than that at 20 psu (Figure 5b).

To further compare the growth and survival advantages of the hybrid larvae at different salinities, the heterosis of the hybrid larvae was calculated. The heterosis value of the larval survival rate of the hybrid larvae was negative at all salinities. Surprisingly, the heterosis of the shell height in the GA group was positive at the salinity of 20 and 28, up to 0.35% and 6.21% respectively (Table 2).

4 | DISCUSSION

The fertilization of different oyster species was mainly achieved through artificial insemination rather than natural fertilization, and the quality of gametes was the key to the success of artificial insemination (Allen et al., 1993; Xu et al., 2019; Yan et al., 2018; Zhang et al., 2016, 2017). Compared to the naturally spawned oocytes, some of the dissected oocytes are immature (not reaching the Meiotic Metaphase I) and entail a series of treatments like being soaked in seawater to initiate maturation (Guo et al., 1992). The treatment applied in this experiment to initiate maturation of dissected oocytes was soaking them in the seawater. If most of the dissected eggs are mature before fertilization, the synchronization of fertilization will be greatly improved and the rate of polyspermy will be reduced dramatically (Qin et al., 2018). Therefore, it is necessary to obtain the seawater with suitable salinity to promote the maturation of eggs before fertilization. From the appearance, the germinal vesicle breakdown (GVBD) is an important sign of stripped oocyte maturation, so the maturation of oocytes can be directly measured by GVBD ratio.

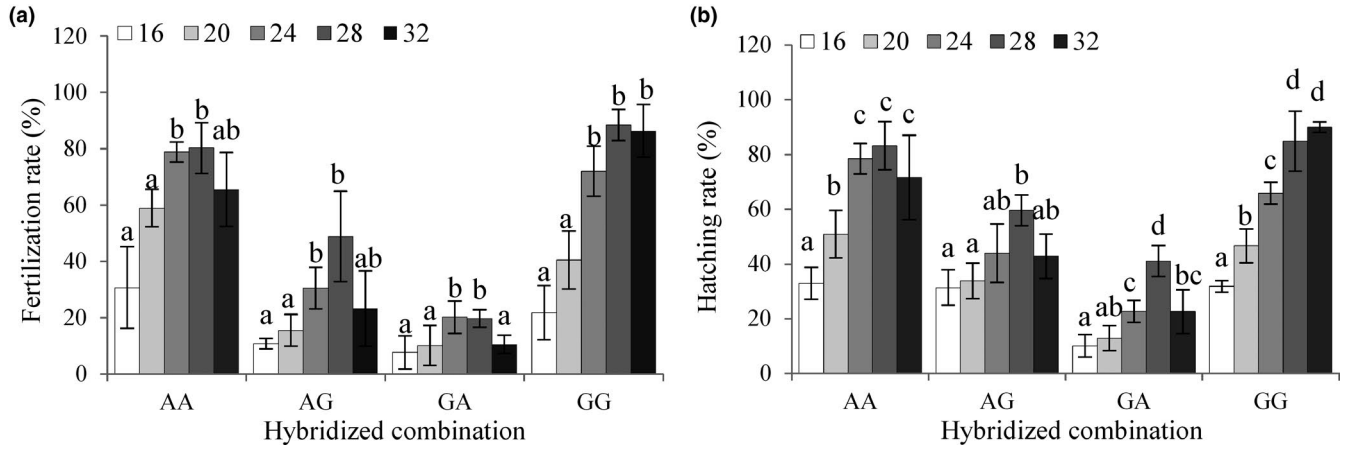

FIGURE 3 Fertilization rate (a) (mean ±SD, n = 3 replicates) and hatching rate (b) (mean ±SD, n = 3 replicates) of each combination at different salinities. Different superscript letters indicate significant difference (p < .05) among salinities at each combination. AA, AG, GA and GG represent four insemination combinations, which refer to *C. ariakensis*♀× *C. ariakensis*♂, *C. ariakensis*♀ × *C. gigas*♂, *C. gigas*♀ × *C. ariakensis*♂ and *C. gigas*♀ × *C. gigas*♂ respectively.

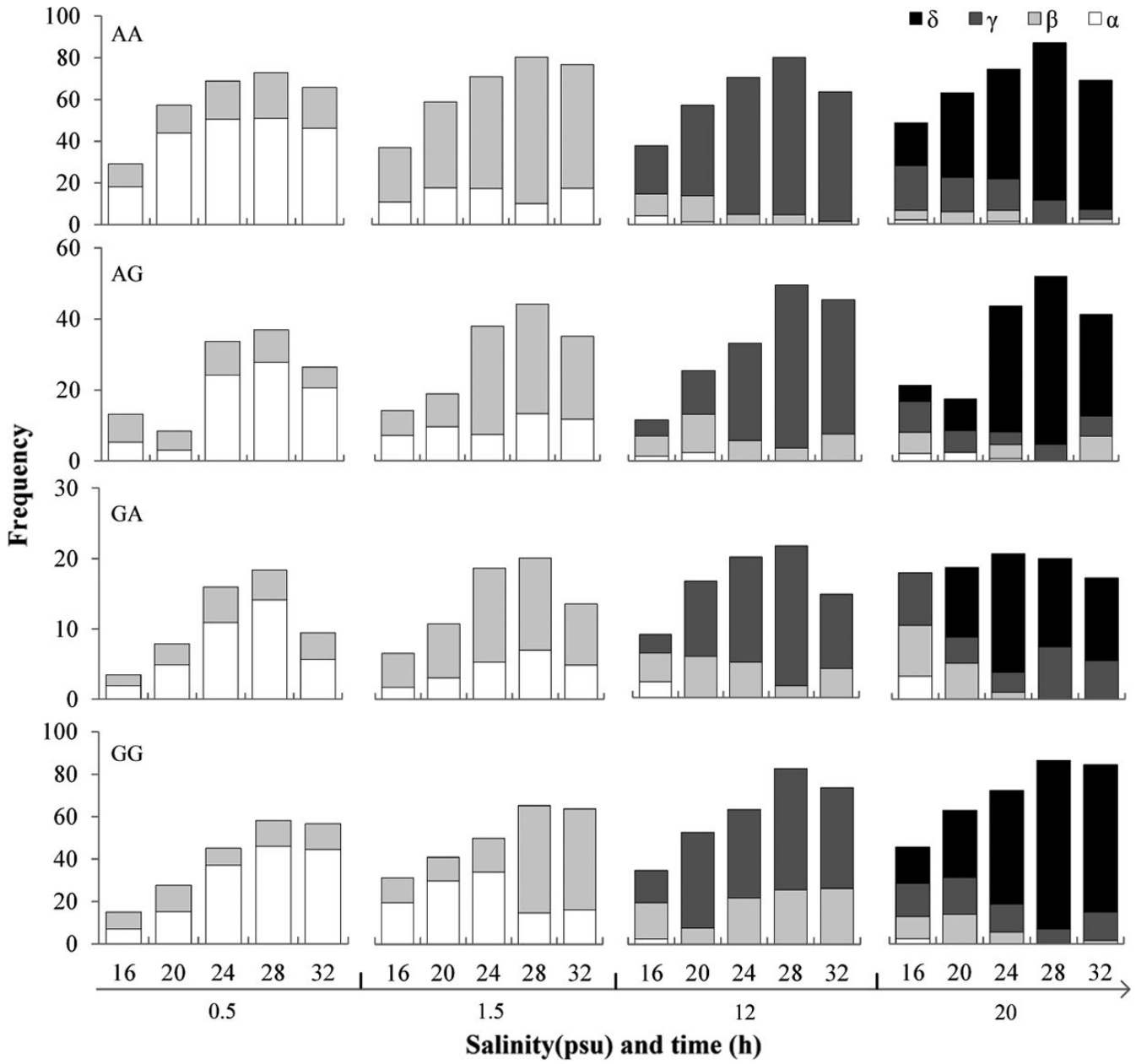

FIGURE 4 Frequency (mean, n = 3 replicates) of embryonic development stages at different salinities (16, 20, 24, 28 and 32 psu) in different times (0.5, 1, 12 and 20 h). The embryonic development stage is divided into four parts, stage "α" represents the embryo with clearly visible polar body but with no division; stage "β" represented the stage which is from egg division to the embryo before trochophore; stage "γ" represents the trochophore; stage "δ" represents D-larvae. AA, AG, GA and GG represent four insemination combinations, which refer to *C. ariakensis*♀× *C. ariakensis*♂, *C. ariakensis*♀ × *C. gigas*♂, *C. gigas*♀ × *C. ariakensis*♂ and *C. gigas*♀ × *C. gigas*♂ respectively.

For *C. ariakensis*, the GVBD of stripped eggs was normal at 20–32 psu, indicating that its eggs can mature rapidly in the seawater with the salinity of 20–32 psu. For *C. gigas*, when the eggs were soaked in the high salinity seawater (28 and 32 psu), its GVBD ratio can quickly reach more than 80%. However, it was difficult to stimulate the GVBD of stripped eggs when the salinity of seawater was lower than 24 psu. Therefore, it can be considered that the seawater with a salinity of 24–32 psu can well initiate the GVBD of stripped eggs for *C. gigas*. Oysters are in vitro fertilized organisms, and their sperm are susceptible to environmental factors in the seawater, including salinity, temperature, pH and heavy metal (Du et al., 2018; Lettieri, Maione, et al., 2019). Previous studies have shown that the sperm of marine economic animal generally preferred the environment with high osmotic pressure (Lucu & Devescovi, 1999; Morisawa & Suzuki, 1980). Similarly, the sperm of the two oyster species in this experiment showed strong vitality at relatively high salinity. The sperm of *C. ariakensis* at 20–32 psu and *C. gigas* at 24–32 psu presented superior performance, which manifested as fast sperm adaptation, long sperm movement time and strong sperm vitality. It is worth noting that, under the high salinity range (24–32 psu), the gametes of *C.*

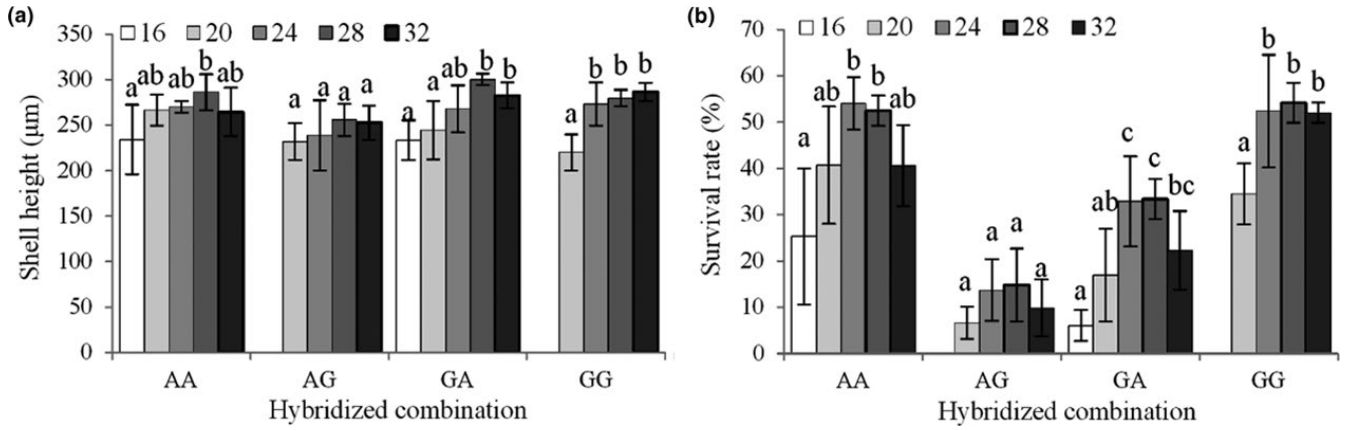

FIGURE 5 Shell height (a) (mean ±SD, n = 3 replicates) and survival rate (b) (mean ±SD, n = 3 replicates) of each combination at different salinities. Different superscript letters indicate significant difference ($p < .05$) among salinities at each combination. AA, AG, GA and GG represent four insemination combinations, which refer to *C. ariakensis*♀× *C. ariakensis*♂, *C. ariakensis*♀ × *C. gigas*♂, *C. gigas*♀ × *C. ariakensis*♂ and *C. gigas*♀ × *C. gigas*♂ respectively.

TABLE 2 Heterosis of heterozygous larvae at different salinity (mean, n = 3 replicates).

	Shell height					Survival rate				
Salinity	**16**	**20**	**24**	**28**	**32**	**16**	**20**	**24**	**28**	**32**
H_{AG} (%)		−4.69	−12.10	−9.62	−8.18		−82.35	−74.27	−72.16	−78.71
H_{GA} (%)		0.35	−5.03	6.21	−2.66		−54.90	−38.24	−37.38	−51.95

H_{AG} (%), heterosis of AG (*C. ariakensis*♀ × *C. gigas*♂); H_{GA} (%), heterosis of GA (*C. gigas*♀ × *C. ariakensis*♂)

ariakensis and *C. gigas* responded to the different salinities normally. However, at 20 psu, gametes of *C. ariakensis* were still relatively normal while the gametes of *C. gigas* were negatively affected, presenting decreased sperm motility and the inability of eggs to mature quickly. This indicated that the gametes of *C. ariakensis* can better tolerate the low-salt environment than *C. gigas*, of which the reason may be that the oysters live in the estuary area of the Yellow River Delta for a long time where the salinity changed greatly during the breeding season. Specifically, the mechanism of gamete tolerance to low salt is complex, which may require further research at the molecular level. For example, previous studies have shown that the expression of hsp70 and protein-like protein genes in sperm is closely related to its response to hyposaline conditions (Lettieri, Maione, et al., 2019). In addition, external fertilizers like oysters can form their own gamete plasticity to adapt to the specific environmental stress of their habitat (Jensen et al., 2014; Lettieri, Maione, et al., 2019). It can be concluded that *C. ariakensis* is living in the estuary with low salinity for a long time, and their gametes may have formed gamete plasticity to adapt to low-salt stress, while the gametes of *C. gigas* have no such low-salt adaptation characteristics.

The fertilization rate of self-cross combinations (AA and GG) was distinctly higher than that of the cross combinations (AG and GA) under all salinities. In other oyster hybridization studies, it was also found that the fertilization rate of the cross combination was inferior to that of the self-cross combination at various salinities (Huo et al., 2014; Xu et al., 2011). Such a low fertilization rate may be mainly on account of the internal factors like the variation of binding and lysin gene in the gametes of the two species, which were accumulated over a long period of evolution (Palumbi, 1994; Palumbi & Metz, 1991). In addition, other environmental factors may also have some effects on the fertilization rate. For example, heavy metals such as copper and cadmium can affect the sperm motility and function of bivalve shellfish (Lettieri, Mollo, et al., 2019; Piscopo, 2019; Zhao et al., 2017), resulting in a low fertilization rate. However, in fact, this impact can be neglected in this experiment. On the one hand, our experimental water is in compliance with the provisions of agricultural industry standards on the heavy metal content; on the other hand, the experimental water is treated by EDTA before use, so the water will not contain superfluous heavy metals. In interspecific hybridization, the phenomenon that the characters of hybrids are similar to female parent is maternal inheritance, while the one that they are similar to male parent is paternal inheritance. AA and GA had the fastest embryonic development at salinity 24–28, while GG and AG had the fastest embryonic development at 28–32, which indicated that the salinity adaptability of heterozygous embryo development was the same as that of their male parents, showing paternal inheritance. The cross combinations (AG and GA) only showed a higher hatching rate at salinity of 28, especially GA, whose hatching rate at salinity of 28 was significantly ($p < .05$) higher than that of other salinity groups, indicating that hybrid combinations may be able to hatch well only at salinity of 28. Obviously, the suitable salinity range for fertilization and incubation of hybrid combination is relatively narrow, which means that the incubation

of heterozygotes is more sensitive to salinity. Therefore, obtaining the suitable incubation salinity is necessary to improve the hatching rate of heterozygotes.

As for larvae growth, the growth H (%) value of GA was positive at 20 and 28 psu respectively. In fact, the growth H (%) value of GA at 20 psu was tiny. The previous study found that GA larvae had a slight growth advantage under the salinity of 22–23 psu (Que & Allen, 2002) and 20 psu (Allen & Gaffney, 1993), respectively, which was similar to our data. What's different was that in this study, the larvae of GA had an obvious growth advantage at 28 psu, and the shell height was even higher than that of their parents. For the oyster, many heterozygotes showed slower growth than their parents in the larvae stage (Banks et al., 1994; Beaumont et al., 2004; Camara et al., 2008; Xu et al., 2009; Yao, 2014; Zhang, Wang, et al., 2012). However, from the present or previous experiment results, the growth can be improved by adjusting the salinity (Huo et al., 2014; Xu et al., 2011). Noticeably, the previous studies have proved that *C. ariakensis* preferred a low-salt environment in the early stage (Cai et al., 1992; Guo et al., 1999), but the current study showed that the hatching and larval growth were the optimal under the medium-salt environment (24 and 28 psu), while they are unsatisfactory under the low-salt environment (16 psu). The difference may result from the diversity of the geographical populations of *C. ariakensis*. Certain genetic variations have been accumulated among different geographical populations during long-term geographical isolation (Kim et al., 2014; Ren et al., 2016), which is bound to be accompanied by changes in environmental adaptability. The differences in environmental adaptability among geographical groups may explain the differences in salinity adaptability of *C. ariakensis* between the current experiment and previous experiments.

Genetic incompatibility means that the heterozygous larvae of the two species are nonviable (Banks et al., 1994; Bushek et al., 2008). In this study, although the survival rate is low, a small number of heterozygous larvae still survive, which means that the two oysters have not yet developed complete genetic incompatibility. Hereby, we have a new insight about their evolution that may help to explain our results. *C. ariakensis* and *C. gigas* have accumulated certain variation in the long-term evolution, but they have not yet formed complete reproductive isolation, and a small number of hybrid larvae may be produced in their common natural distribution. However, the hybrid progenies have formed a new environmental adaptation mechanism and no longer adapt to the living environment of their parents. Therefore, the hybrid was gradually eliminated and unable to form a population. Hence, to explore the optimal environmental mechanism is the prerequisite to obtain a large number of hybrid offspring. Only salinity was explored in this study, and it may be of great significance to further study the optimization of other rearing environmental factors. The overall survival rate of AG was lower, less than 20% with no significant differences at 20-32psu, and the larvae did not even survive to 25d at 16 psu. The low survival rate of AG larvae at all salinities indicates that the larvae were insensitive to various salinities, and the improvement of the low survival rate may require the optimization of other environmental factors such as temperature, density and feed (Dove & O'Connor, 2007; Gamain et al., 2016; O'Connor & Lawler, 2004). For GA, the survival rate at 24 and 28 psu was significantly higher than that at 16–20 psu and it was 10.60% and 11.11% higher than that at 32 psu, respectively, indicating that the suitable salinity of survival rate was 24–28 psu. In summary, the fertilization, incubation, larval growth and survival of cross combinations are significantly inferior to those of their parents, so other rearing conditions need to be further optimized to obtain more hybrid offspring for research. In addition, the characteristics of adult oysters need to be further studied. If the desirable traits have not been exhibited in adult oysters, the genetic improvement of *C. ariakensis* may need to be achieved through hybridization with other species.

Overall, salinity has a distinct influence on the early activities of the artificial hybridization between *C. ariakensis* and *C. gigas*, including artificial insemination, incubation and larval rearing. Any link of early activities on the artificial hybridization has an optimum salinity range, under which the hybridization can be smoothly carried out and relatively more hybrid larvae can be obtained, thus providing a possibility for achieving genetic improvement of *C. ariakensis* through cross-breeding.

ETHICS STATEMENT

The present study was performed according to the standard operation procedures (SOPs) of the Guide for the Use of Experimental Animals of the Ocean University of China. All animal care and use procedures were approved by the Institutional Animal Care and Use Committee of Ocean University of China.

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CONFLICT OF INTEREST

None declared.

AUTHORS' CONTRIBUTION

Ruihai Yu designed the study. Haikun Li, Chunhua Li and Peizhen Ma performed the study. Haikun Li analysed the data and wrote the paper. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the first author upon request.

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REFERENCES

Allen, S. K., & Gaffney, P. M. (1993). Genetic confirmation of hybridization between *Crassostrea gigas* (Thunberg) and *Crassostrea rivularis* (Gould). *Aquaculture*, *113*, 291–300. https://doi.org/10.1016/0044-8486(93)90400-S

Allen, S. K., Gaffney, P. M., Scarpa, J., & Bushek, D. (1993). Inviable hybrids of *Crassostrea virginica* (Gmelin) with *C. rivularis* (Gould) and *C. gigas* (Thunberg). *Aquaculture*, *113*, 269–289. https://doi.org/10.1016/0044-8486(93)90399-J

Banks, M. A., Mcgoldrick, D. J., Borgeson, W., & Hedgecock, D. (1994). Gametic incompatibility and genetic divergence of Pacific and Kumamoto oysters, *Crassostrea gigas* and *C. sikamea*. *Marine Biology*, *121*, 127–135. https://doi.org/10.1007/BF00349481

Beaumont, A. R., Turner, G., Wood, A. R., & Skibinski, D. O. F. (2004). Hybridisations between Mytilus edulis and Mytilus galloprovincialis and performance of pure species and hybrid veliger larvae at different temperatures. *Journal of Experimental Marine Biology and Ecology*, *302*, 177–188. https://doi.org/10.1016/j.jembe.2003.10.009

Bushek, D., Kornbluh, A., Wang, H., Guo, X., Debrosse, G., & Quinlan, J. (2008). Fertilization interference between *Crassostrea ariakensis* and *Crassostrea virginica*: a gamete sink. *Journal of Shellfish Research*, *27*, 593–600. https://doi.org/10.2983/0730-8000(2008)27

Cai, Y., Deng, Z., & Liu, C. (1992). Studies on the ecology of *crossostrea rivularis* (Gould) in Zhanjiang Bay. *Tropic Oceanology*, *11*, 37–44.

Calvo, G. W., Luckenbach, M. W., Allen, S. K., & Burreson, E. M. (2001). A comparative field study of *Crassostrea ariakensis* (Fujita 1913) and *Crassostrea virginica* (Gmelin 1791) in relation to salinity in Virginia. *Journal of Shellfish Research*, *20*, 221. https://doi.org/10.1016/S1385-1101(01)00059-4

Camara, M. D., Davis, J. P., Sekino, M., Hedgecock, D., Li, G., Langdon, C. J., & Evans, S. (2008). The Kumamoto oyster *Crassostrea sikamea* is neither rare nor threatened by hybridization in the Northern Ariake sea, Japan. *Journal of Shellfish Research*, *27*, 313–322. https://doi.org/10.2983/0730-8000(2008)27

Chu, P. C., Chen, Y., & Kuninaka, A. (2005). Seasonal variability of the yellow sea/East China sea surface fluxes and thermohaline structure. *Advances in Atmospheric Ences*, *22*, 1–20. https://doi.org/10.1007/BF02930865

Cruz, F. L. d. l. & Gallardo-Escárate, C. (2011). Intraspecies and interspecies hybrids in Haliotis: Natural and experimental evidence and its impact on abalone aquaculture. *Reviews in Aquaculture*, *3*, 74–99. https://doi.org/10.1111/j.1753-5131.2011.01045.x

Dos Santos, A. E., & Nascimento, I. A. (1985). Influence of gamete density, salinity and temperature on the normal embryonic development of the mangrove oyster *Crassostrea rhizophorae Guilding*, 1828. *Aquaculture*, *47*, 335–352. https://doi.org/10.1016/0044-8486(85)90219-4

Dove, M. C., & O'Connor, W. A. (2007). Salinity and temperature tolerance of Sydney rock oysters Saccostrea glomerata during early ontogeny. *Journal of Shellfish Research*, *26*, 939–947. https://doi.org/10.2983/0730-8000(2007)26

Du, J., Wang, Z., Cui, Y., Li, Y., Yang, Y., Li, P., & Liu, Y. (2018). Effects of salinity on *Crassostrea angulata* sperm quality and fertilization using PI/Rh123 dual fluorescent staining and flow cytometry. *Journal of Fisheries of China*, *42*, 1737–1746. https://doi.org/10.11964/jfc.20180211171

Falconer, D. S. (1981). *Introduction to quantitative genetics* (2nd ed.). Longman.

Gaffney, P. M., & Allen, S. K. (1993). Hybridization among *Crassostrea* species: A review. *Aquaculture*, *116*, 1–13. https://doi.org/10.1016/0044-8486(93)90217-M

Gamain, P., Gonzalez, P., Cachot, J., Pardon, P., Tapie, N., Gourves, P. Y., Budzinski, H., & Morin, B. (2016). Combined effects of pollutants and salinity on embryo-larval development of the Pacific oyster, *Crassostrea gigas*. *Marine Environmental Research*, *113*, 31–38. https://doi.org/10.1016/j.marenvres.2015.11.002

Guo, X. (2009). Use and exchange of genetic resources in molluscan aquaculture. *Reviews in Aquaculture*, *1*, 251–259. https://doi.org/10.1111/j.1753-5131.2009.01014.x

Guo, X., Cooper, K., Hershberger, W. K., & Chew, K. K. (1992). Genetic consequences of blocking polar body I with cytochalasin b in fertilized eggs of the Pacific Oyster, *Crassostrea gigas*: I. ploidy of resultant embryos. *Biological Bulletin*, *183*, 381–386. https://doi.org/10.2307/1542013

Guo, X., Ford, S., Zhang, F., & Mblwhoi Library, W. H. (1999). Molluscan aquaculture in China. *Journal of Shellfish Research*, *18*, 19–31. https://doi.org/10.1016/S1385-1101(99)00006-4

Han, Z., & Li, Q. (2018). Different responses between orange variant and cultured population of the Pacific oyster *Crassostrea gigas* at early life stage to temperature-salinity combinations. *Aquaculture Research*, *00*, 1–7. https://doi.org/10.1111/are.13680

Hosoi, M., Kubota, S., Toyohara, M., Toyohara, H., & Hayashi, I. (2010). Effect of salinity change on free amino acid content in Pacific oyster. *Fisheries Science*, *69*, 395–400. https://doi.org/10.1046/j.1444-2906.2003.00634.x

Huo, Z., Wang, Z., Yan, X., & Yu, R. (2014). Hybridization between *Crassostrea hongkongensis* and *Crassostrea ariakensis* at different salinities. *Journal of the World Aquaculture Society*, *45*, 226–232. https://doi.org/10.1111/jwas.12098

Jensen, N., Allen, R. M., & Marshall, D. J. (2014). Adaptive maternal and paternal effects: Gamete plasticity in response to parental stress. *Functional Ecology*, *28*, https://doi.org/10.1111/13652435.12195

Kim, W. J., Dammannagoda, S. T., Jung, H., Baek, I. S., Yoon, H. S., & Choi, S. D. (2014). Mitochondrial DNA sequence analysis from multiple gene fragments reveals genetic heterogeneity of *Crassostrea ariakensis* in East Asia. *Genes and Genomics*, *36*, 611–624. https://doi.org/10.1007/s13258-014-0198-5

Kinne, O. (1964). The effects of temperature and salinity on marine and brackish water animals: 2. Salinity and temperature-salinity relations. *Oceanography and Marine Biology an Annual Review*, *2*, 597–602. https://doi.org/10.1590/S0085-56262003000400010

Lettieri, G., Maione, M., Ranauda, M. A., Mele, E., & Piscopo, M. (2019). Molecular effects on spermatozoa of *Mytilus galloprovincialis* exposed to hyposaline conditions. *Molecular Reproduction Development*, *86*, 650–660. https://doi.org/10.1002/mrd.23141

Lettieri, G., Mollo, V., Ambrosino, A., Caccavale, F., Troisi, J., Febbraio, F., & Piscopo, M. (2019). Molecular effects of copper on the reproductive system of *Mytilus galloprovincialis*. *Molecular Reproduction Development*, *86*, 1357–1368. https://doi.org/10.1002/mrd.23114.

Liu, Z., Hu, D., & Tang, X. (2008). Tidal current observation in the southern Yellow Sea in the summers of 2001 and 2003. *Journal of Oceanology and Limnology*, *026*, 121–129. https://doi.org/10.1007/s00343-008-0121-5

Lucu, Č., & Devescovi, M. (1999). Osmoregulation and branchial $Na^{+},^{K}$+-ATPase in the lobster Homarus gammarus acclimated to dilute seawater. *Journal of Experimental Marine Biology and Ecology*, *234*, 291–304. https://doi.org/10.1016/S0022-0981(98)00152-X

Ma, P., Wang, Z., & Yu, R. (2019). Triploid induction by hyperosmotic shock in the Yesso scallop, *Patinopecten yessoensis*. *Journal of the World Aquaculture Society*, *50*, 922–933. https://doi.org/10.1111/jwas.12583

Madrones-Ladja, J. A. (2002). Salinity effect on the embryonic development, larval growth and survival at metamorphosis of Placuna placenta Linnaeus (1758). *Aquaculture*, *214*, 411–418. https://doi.org/10.1016/S0044-8486(02)00401-5

McNeil, B. I., & Sasse, T. P. (2016). Future ocean hypercapnia driven by anthropogenic amplification of the natural CO_2 cycle. *Nature*, *529*, 383–386. https://doi.org/10.1038/nature16156

Morisawa, M., & Suzuki, K. (1980). Osmolality and potassium ion: their roles in initiation of sperm motility in teleosts. *Science*, *210*, 1145–1147. https://doi.org/10.1126/science.7444445

Nell, J. A., & Holliday, J. E. (1988). Effects of salinity on the growth and survival of Sydney rock oyster and Pacific oyster larvae and spat. *Aquaculture*, *68*, 39–44. https://doi.org/10.1016/0044-8486(88)90289-X

O'Connor, W. A., & Lawler, N. F. (2004). Salinity and temperature tolerance of embryos and juveniles of the pearl oyster, Pinctada imbricata Röding. *Aquaculture*, *229*, 493–506. https://doi.org/10.1016/S0044-8486(03)00400-9

Palumbi, S. R. (1994). Genetic divergence, reproductive isolation, and marine speciation. *Annual Review of Ecology and Systematics*, *25*, 547–572. https://doi.org/10.1146/annurev.es.25.110194.002555

Palumbi, S. R., & Metz, E. C. (1991). Strong reproductive isolation between closely related tropical sea urchins (genus Echinometra). *Molecular Biology and Evolution*, *8*, 227. https://doi.org/10.1007/BF02342749

Piscopo, M. (2019). Seasonal dependence of cadmium molecular effects on *Mytilus galloprovincialis* (Lamarck, 1819) protamine-like protein properties. *Molecular Reproduction Development*, *86*, 1418–1429. https://doi.org/10.1002/mrd.23240

Qin, Y., Xiao, S., Ma, H., Mo, R., Zhou, Z., Wu, X., Zhang, Y., & Yu, Z. (2018). Effects of salinity and temperature on the timing of germinal vesicle breakdown and polar body release in diploid and triploid Hong Kong oysters, *Crassostrea hongkongensis*, in relation to tetraploid induction. *Aquaculture Research*, *49*, 3647–3657. https://doi.org/10.1111/are.13833

Que, H., & Allen, S. K. (2002). Hybridization of tetraploid and diploid *Crassostrea gigas* (Thunberg) with diploid *C. ariakensis* (Fujita). *Journal of Shellfish Research*, *21*, 137–143.

Rah, M. A., Arshad, A., Marimuthu, K., Ara, R., & Amin, S. M. N. (2013). Inter-specific hybridization and its potential for aquaculture of Fin fishes. *Asian Journal of Animal and Veterinary Advances*, *8*, 139–153. https://doi.org/10.3923/ajava.2013.139.153

Ren, J., Hou, Z., Wang, H., Sun, M. A., Liu, X., Liu, B., & Guo, X. (2016). Intraspecific variation in mitogenomes of five *Crassostrea* species provides insight into oyster diversification and speciation. *Marine Biotechnology*, *18*, 242–254. https://doi.org/10.1007/s10126-016-9686-8

Rybovich, M., Peyre, M. K. L., Hall, S. G., & Peyre, J. F. L. (2016). Increased temperatures combined with lowered salinities differentially impact oyster size class growth and mortality. *Journal of Shellfish Research*, *35*, 101–113. https://doi.org/10.2983/035.035.0112

Taylor, J. J., Southgate, P. C., & Rose, R. A. (2004). Effects of salinity on growth and survival of silver-lip pearl oyster, *Pinctada maxima*, spat. *Journal of Shellfish Research*, *23*, 375–377. https://doi.org/10.1016/j.seares.2003.11.002

Verween, A., Vincx, M., & Degraer, S. (2007). The effect of temperature and salinity on the survival of *Mytilopsis leucophaeata* larvae (Mollusca, Bivalvia): The search for environmental limits. *Journal of Experimental Marine Biology and Ecology*, *348*, 111–120. https://doi.org/10.1016/j.jembe.2007.04.011

Wang, C., Liu, B., Li, J., Liu, S., Li, J., Hu, L., Fan, X., Du, H., & Fang, H. (2011). Introduction of the Peruvian scallop and its hybridization with the bay scallop in China. *Aquaculture*, *310*, 380–387. https://doi.org/10.1016/j.aquaculture.2010.11.014

Wang, H., Qian, L., Zhang, G., Liu, X., Wang, A., Shi, Y., Jiao, N., & Guo, X. (2006). Distribution of *Crassostrea ariakensis* in China. *Journal of Shellfish Research*, *25*, 789–790.

Wang, Q., Li, Q., Kong, L., & Yu, R. (2012). Response to selection for fast growth in the second generation of Pacific oyster (*Crassostrea gigas*). *Journal of Ocean University of China*, *11*, 413–418. https://doi.org/10.1007/s11802-012-1909-7

Wang, T., Li, Q., Zhang, J., & Yu, R. (2018). Effects of salinity, stocking density, and algal density on growth and survival of Iwagaki oyster *Crassostrea nippona* larvae. *Aquaculture International*, *26*, 947–958. https://doi.org/10.1007/s10499-018-0261-3

Xu, F., Guo, X., Li, L., & Zhang, G. (2011). Effects of salinity on larvae of the oysters *Crassostrea ariakensis*, *C. sikamea* and the hybrid cross. *Marine Biology Research*, *7*, 796–803. https://doi.org/10.1080/17451000.2011.569555

Xu, F., Zhang, G., Liu, X., Zhang, S., Shi, B., & Guo, X. (2009). Laboratory hybridization between *Crassostrea ariakensis* and *C.* Sikamea. *Journal of Shellfish Research*, *28*, 453–458. https://doi.org/10.2983/035.028.0305

Xu, H., Li, Q., Han, Z., Liu, S., Yu, H., & Kong, L. (2019). Fertilization, survival and growth of reciprocal crosses between two oysters, *Crassostrea gigas* and *Crassostrea nippona*. *Aquaculture*, *507*, 91–96. https://doi.org/10.1016/j.aquaculture.2019.04.012

Yan, L., Su, J., Wang, Z., Zhang, Y., Yan, X., & Yu, R. (2018). Growth performance and biochemical composition of the oysters *Crassostrea sikamea*, *Crassostrea angulata* and their hybrids in southern China. *Aquaculture Research*, *49*, 1020–1028. https://doi.org/10.1111/are.13549

Yao, T. (2014). Hybridization between *Crassostrea ariakensis* and *C. gigas*, *C. angulata*. Doctor thesis, Ocean university of china.

Yoon, H. S., Jung, H. T., & Choi, S. D. (2008). Suminoe oyster (*Crassostrea ariakensis*) culture in Korea. *Journal of Shellfish Research*, *27*, 505–508. https://doi.org/10.2983/0730-8000(2008)27

Zhang, G., Fang, X., Guo, X., Li, L. I., Luo, R., Xu, F., Yang, P., Zhang, L., Wang, X., Qi, H., Xiong, Z., Que, H., Xie, Y., Holland, P. W. H., Paps, J., Zhu, Y., Wu, F., Chen, Y., Wang, J., … Wang, J. (2012). The oyster genome reveals stress adaptation and complexity of shell formation. *Nature*, *490*, 49–54. https://doi.org/10.1038/nature11413

Zhang, Q., Allen, S. K., & Reece, K. S. (2005). Genetic variation in wild and hatchery stocks of Suminoe oyster (*Crassostrea ariakensis*) assessed by PCR-RFLP and microsatellite markers. *Marine Biotechnology*, *7*, 588. https://doi.org/10.1007/s10126-004-5105-7

Zhang, Y., Wang, Z., Yan, X., Yu, R., Kong, J., Liu, J., Li, X., Liu, Y., & Guo, X. (2012). Laboratory hybridization between two oysters: *Crassostrea gigas* and *Crassostrea hongkongensis*. *Journal of Shellfish Research*, *31*, 619–625. https://doi.org/10.2983/035.031.0304

Zhang, Y., Wang, Z., & Yu, Z. (2014). A recent review of interspecific hybridization among cultivated oysters. *Journal of Fisheries of China*, *4*, 612–623. https://doi.org/10.3724/SP.J.1231.2012.27999

Zhang, Y., Zhang, Y., Jun, L., Wang, Z., Yan, X., & Yu, Z. (2016). Phenotypic trait of *Crassostrea hongkongensis*♀×*C. angulata*♂ hybrids in southern China. *Aquaculture Research*, *47*, 3399–3409. https://doi.org/10.1111/are.12787

Zhang, Y., Zhang, Y., Li, J., Wang, Z., Yan, X., & Yu, Z. (2017). Incomplete sterility of hybrids produced by *Crassostrea hongkongensis* female× *Crassostrea gigas* male crosses. *Aquaculture Research*, *48*, 1351–1358. https://doi.org/10.1111/are.12941

Zhao, L., Ru, Y., Liu, M., Tang, J., & Shi, H. (2017). Reproductive effects of cadmium on sperm function and early embryonic development in vitro. *PLoS One*, *12*, e0186727. https://doi.org/10.1371/journal.pone.0186727

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