Fisheries Science (2019) 85:821–828
https://doi.org/10.1007/s12562-019-01330-2

ORIGINAL ARTICLE

Aquaculture

Fertilization, survival and growth of hybrids between *Crassostrea gigas* and *Crassostrea sikamea*

Hongqiang Xu[1] · Qi Li[1,2] · Lingfeng Kong[1] · Hong Yu[1] · Shikai Liu[1]

Received: 15 March 2019 / Accepted: 15 May 2019 / Published online: 18 June 2019

Abstract
Crossbreeding is a powerful tool for animal breeding and genetic improvement in aquaculture. In this work, artificial interspecific hybridization was carried out and three crosses were successfully produced, namely *Crassostrea gigas* (GG), *C. sikamea* (SS) and *C. sikamea* ♀ × *C. gigas* ♂ (SG), although *C. gigas* ♀ × *C. sikamea* ♂ formed nonviable hybrid offspring. The fertilization and hatching success of the SG cross was at an acceptable level compared with that of the intraspecific crosses. At the larval stage, the growth rate of the SG cross corresponded to that of the SS cross, but was significantly lower than the GG cross, and the survival rate of the SG cross did not differ from that of either parental cross. During the spat stage, the single-parent heterosis value of the growth rate for the SG cross ranged from 8.85 to 24.43%. Considering survival rates, notable mid-parent heterosis (27.16–76.00%) and clear single-parent heterosis (30.53–73.28%) were observed, though no significant differences were observed among the three crosses. Our results clearly demonstrate that the production performance of the hybrid SG cross is comparable to that of the maternal parental SS cross. The high survival advantage and single-parent heterosis for the growth trait observed in the SG cross provides a promising method for the genetic improvement of oysters.

Keywords *Crassostrea gigas* · *Crassostrea sikamea* · Heterosis · Hybridization · Phenotypic trait

Introduction

Interspecific hybridization, which refers to mating between individuals of two different species (FAO 2007), has made a great contribution to finfish aquaculture and stocking programs (Bartley et al. 2001). In marine mollusks, several cases of interspecific hybridization between close species involving commercial utility have been reported. In scallops, the 'Bohai Red', a superior strain from the hybrid between the bay scallop *Argopecten irradians irradians* and the Peruvian scallop *A. purpuratus*, has demonstrated higher growth performance and enhanced temperature tolerance in northern China (Wang et al. 2017), and a new strain *Patinopecten yessoensis* × *P. canrinus* with high resistance to *Perkinsus qugwadi* was reported in Canada (Heath 1995). In abalones, *Haliotis discus hannai* × *H. discus discus* known as 'black abalone', *H. rubra* × *H. laevigata* known as 'tiger abalone', *H. discus discus* × *H. diversicolor* and *H. laevigara* × *H. conicopora* are being grown commercially (Lafarga de la Cruz and Gallardo-Escárate 2011). However, in oysters, only a new oyster strain, 'Huanan No. 1', has been reported. Huanan No. 1 was selected from the backcross progeny between *C. hongkongensis* ♀ and *C. gigas* ♂ fertile hybrids and their parental species, with higher growth performance and enhanced salinity tolerance (Zhang et al. 2016c). As growth divergence of hybrids was observed in several other oyster interspecific hybridizations (Zhang et al. 2017), fertilization barriers, contamination of cultures and the different cultured environmental conditions may contribute to the negative performance of hybrids (Allen et al. 1993; Dégremont et al. 2005).

Generally speaking, hybridization can enhance performance in yield or survival compared to the parental lines—a phenomenon known as heterosis (Hedgecock and Davis 2007). However, interspecific hybridization can sometimes

✉ Qi Li
qili66@ouc.edu.cn

[1] Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China

[2] Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China

result in poor growth and lower survival compared with pure crosses in oyster aquaculture. In the previous century, almost all oyster interspecific hybrids with genetic confirmation were nonviable, with little growth (Allen et al. 1993; Allen and Gaffney 1993). In recent decades, the genetic materials for hybridization have been focused on newly described or classified species such as *C. hongkongensis* and *C. sikamea* (Allen and Gaffney 1993; Wang et al. 2004). Similarly, breeding depression or hybrid breakdown has been documented because of gamete incompatibility in most interspecific hybridization cases (Xu et al. 2009; Zhang et al. 2012, 2017). However, positive growth performance was produced in the following crosses: *C. hongkongensis* × *C. angulate* (Zhang et al. 2016b), *C. hongkongensis* × *C. sikamea* (Zhang et al. 2017) and *C. hongkongensis* × *C. ariakensis* (Huo et al. 2013). Moreover, the growth and survival superiority of backcross progeny was observed between *C. hongkongensis* × *C. gigas* fertile hybrids and their parental species mentioned above (Zhang et al. 2016c), which indicates that interspecific hybridization also has a potential use in genetic improvement in oyster aquaculture.

The Kumamoto oyster *C. sikamea* is widely distributed in China, Korea and Japan (Hamaguchi et al. 2013), and it is well-known that *C. sikamea* is a sympatric species of *C. gigas* in a few regions (Hedgecock et al. 1999; Hong et al. 2012). The distinguishing morphological characteristics between the two species are the more deeply cupped left valve and a highly wrinkled or ridged shell in *C. sikamea* (Wang et al. 2013). However, shell morphology is irregular and highly variable; thus the use of morphological characteristics often leads to errors in oyster identification and classification (Wang et al. 2004). To improve oyster resource management, genetic markers have been used to solve this problem, and subsequently a small number of hybrid progenies were detected in the wild by genetic analysis (Banks et al. 1994; Camara et al. 2008; Hong et al. 2012). The presence of naturally occurring hybrids between *C. gigas* and *C. sikamea* is interesting and raises the question of whether hybridization between the two species is improvable for aquaculture. *C. sikamea*, known for its smooth texture and sweet fruity flavor despite its small size and slow growth, has been cultivated and bred artificially on a large scale in America. On the other hand, *C. gigas*, characterized by rapid growth, large size and wide distribution around the world, especially the new Pacific oyster strain selected by Li et al. (2011), has been developed as a principal cultivated oyster species in north China. It is apparent that the commercially important traits of each species would be a useful trait for the other species. Consequently, hybridization between *C. sikamea* and *C. gigas* may be useful for the genetic improvement of the two species.

In this study, a two-by-two factorial cross between *C. gigas* and *C. sikamea* was carried out under common hatchery and nursery conditions, and a detailed comparison of the fertilization, survival and growth performance of the progeny were carried out among experimental groups to determine whether heterosis exists in growth and survival traits at different stages. The purpose of the study was to obtain a new potential oyster stock combining the desirable commercially important traits of each species.

Material and methods

Brood stocks and rearing conditions

C. sikamea were collected from cultured stock in the area of Rushan, Shandng Province, China. Two-year-old *C. gigas* (successively selected since 2007) with rapid growth performance were collected from Rongcheng, Shandong Province, China. In the summer of 2017, both brood stocks were initially identified by shell morphology and separately conditioned with a mixed algal diet in the hatchery as described by Li et al. (2011). To achieve the synchronization of spermiation and ovulation of brood stocks, sexually mature *C. gigas* were reared at low temperatures ranging from 16.9 to 18 °C, with temperature maintained by a chiller vessel circulation system, while the *C. sikamea* were conditioned in a 1000-L polyethylene bucket with the water temperature kept at 27.2–30.8 °C and salinity from 28 to 30 psu.

Fertilization and embryo hatching

After *C. sikamea* reached the partially spawned stage in July 2017, four males and four females from each species were selected for the experiment. Gametes from the two species were obtained by dissecting mature gonads, and eggs of each female were divided equally into two 5-L buckets. Before fertilization, gametes were examined under a microscope to ensure no sperm contamination or self-fertilization. After gamete collection, the adductor muscle of each animal was fixed in 95% ethanol for subsequent genetic identification.

Fertilization was carried out 30 min later after egg collection; eggs of each individual were fertilized with sperm from *C. gigas* (G) and *C. sikamea* (S) (Table 1). For interspecific crosses, approximately 30–50 sperm surrounded an egg to enhance the fertilization success, according to Zhang et al. (2017). Four different combinations were produced: *C. gigas* ♀ × *C. gigas* ♂(GG), *C. gigas* ♀ × *C. sikamea* ♂(GS), *C. sikamea* ♀ × *C. gigas* ♂(SG), and *C. sikamea* ♀ × *C. sikamea* ♂(SS). The experiment was carried out four times using different sets of parents. After most of the fertilized eggs developed into eight-cell stages, the fertilized eggs of each combination were placed into a 70-L bucket with a density of 30–40 eggs mL^{-1} for incubation. The temperature

Table 1 Experimental design for the hybridization between *C. gigas* and *C. sikamea*

Parents	G1♀	S1♀	G2♀	S2♀	G3♀	S3♀
G1♂	GG1	SG1	–		–	–
S1♂	GS1	SS1	–		–	–
G2♂	–	–	GG2	SG2	–	–
S2♂	–	–	GS2	SS2	–	–
G3♂	–	–	–	–	GG3	SG3
S3♂	–	–	–	–	GS3	SS3

GG and SS indicate the intraspecific crosses *C. gigas* ♀×*C. gigas* ♂ and *C. sikamea* ♀×*C. sikamea* ♂, respectively; GS and SG indicate the interspecific crosses *C. gigas* ♀×*C. sikamea* ♂ and *C. sikamea* ♀×*C. gigas* ♂, respectively. The subscript numbers 1, 2, 3 denote 4 replicates; each replicate was carried out by one female mating with one male

of rearing seawater was maintained at 29–30 °C and the salinity was 30 psu.

Rearing, nursery and grow-out

After 24 h incubation, the D-larvae from each combination were collected on a 48-μm sieve and stocked into a larval rearing bucket (70-L). The larval density of each culture vessel was maintained at four larvae mL^{-1} by adjusting water volume. The rearing of larvae and spat followed routine culture procedures, as described by Wang et al. (2012). In brief, larvae were maintained on *Isochrysis galbana* for the first 8 days, and added on *Platymonas* sp. in the later stage. Thirty percent of the seawater was exchanged once a day and 100% every 5 days. Water temperature was kept at 27–30 °C and the salinity was 28–30 psu. When 50% of the progeny reached eyed stage, string of scallop shells were placed in the buckets. About 10 days after metamorphosis, all spat were transported to an outdoor nursery pond for 1 month to adapt to the ocean environment. Subsequently, 60-day-old juveniles were cultivated, carefully employing a lantern net hanging culture system to avoid contamination from other spat in Sanggou Bay, China. During the grow-out period, the density was randomly adjusted monthly and similar levels were maintained among various groups; the seawater temperature varied from 1.9 to 29 °C.

Genetic confirmation

The parental species and their hybrids were identified using different molecular techniques. The parental species were confirmed using the multiplex polymerase chain reaction (PCR) assay of mitochondrial cytochrome oxidase I (COI) marker as described by Wang and Guo (2008a) (photo not shown here). For every experimental group, 120 individuals (4 replicates × 30) were examined during larval and spat periods. Samples were collected and fixed with ethanol for genetic confirmation, and genomic DNA from larvae samples was extracted employing Chelex 100. The hybrid status of individuals in the experimental hybrid families was confirmed by PCR–restriction fragment length polymorphism (RFLP), using the internal transcribed spacer 1 (ITS1) marker as described by Wang and Guo (2008b). The primer sequences for ITS1 were 5′-GTTTCCGTAGGTGAACCT GC (28S forward) and 5′-ACACGAGCCGAGT GATCCA C (5.8S forward). Each reaction contained 1 μL PCR buffer (Mg^{2+}), 0.2 μM dNTP, 1 μM of each primer, 0.25 U *Taq* DNA polymerase (TaKaRa) and 15–30 ng template DNA. Thirty cycles were completed, each consisting of 95 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min, with a final elongation at 72 °C for 5 min to complete the run. The ITS-1 PCR product was digested with *Hin*dIII to display species-specific RFLP patterns. Available sequence data for *C. gigas* (GenBank AJ543743) predicted *Hin*dIII digestion fragments of 175 bp and 281 bp, whereas no *Hin*dIII cut site was predicted for the *C. sikamea* amplicon (partial sequence data, GenBank AB735523). Digestion was carried out with 1.5 U of the enzyme for 10 h using the conditions recommended by the manufacturer. PCR and digestion products were analyzed by 1.5% agarose gel electrophoresis and visualized on a UV transilluminator to confirm amplification and digestion of the target amplicon.

Sample collection

The egg diameters of the two species were calculated before fertilization. Sixty minutes after fertilization, a 2-mL sample was collected from each bucket, and fertilization success was measured as the percentage of fertilized eggs (cells divided) against the total number of eggs. The hatching rate was calculated as the percentage of D-larvae among fertilized eggs according to the same procedure (the data for the cross GS were collected in an additional study), considering differences in fertilization success, and the percentage of eggs that developed to D-stage was calculated (Table 2).

For the larval stage, the larvae of each group were sampled every 3 days after the D-stage, and larva survival was calculated based on the total number of live larvae on different days post-fertilization. Subsequently, larvae were photographed using an Olympus BX53 microscope, the

Table 2 Hatching index, cumulative survival rate of *C. gigas* (GG), *C. sikamea* (SS), and their hybrids (GS and SG), heterosis (H_t) and single-parent heterosis (I_{SG})

Hatching index					Cumulative survival rate (%)				
Items	Egg diameter (μm)	Fertilization rate (%)	Hatching rate (%)	Eggs to D-larvae (%)	Day 12	Day 21	Day 120	Day 210	Day 320
GG1	50.98	84.13	72.73	61.18	26.67	5.67	4.99	2.81	1.02
GG2	50.9	92.36	85.71	79.17	24	11.33	8.16	6.35	1.59
GG3	51.19	90	86.96	78.26	16	3.67	3.58	3.23	0.95
GG4	51.62	93.15	87.88	81.86	18	4.33	3.15	3.02	0.61
Mean	51.17 ± 0.32^a	89.91 ± 4.08^a	83.32 ± 7.12^a	75.12 ± 9.41^a	21.17 ± 5^a	6.25 ± 3.49^a	4.97 ± 2.27^a	3.85 ± 1.67^a	1.04 ± 0.41^a
SG1	–	94.29	90.57	85.39	31.5	7	5.32	4.03	2
SG2	–	91	85	77.35	26	10	8.47	5.3	2.33
SG3	–	80	73.14	58.51	30	7.5	5.05	3.4	2.05
SG4	–	83.33	76.36	63.64	35	6.5	6.33	5.17	1.07
Mean	–	87.15 ± 6.62^a	81.27 ± 7.97^a	71.22 ± 12.35^a	30.63 ± 3.73^a	7.75 ± 1.55^a	6.29 ± 1.55^a	4.47 ± 0.92^a	1.86 ± 0.55^a
GS1	–	1.32	0.73	–	–	–	–	–	–
GS2	–	0.2	–	–	–	–	–	–	–
GS3	–	0.08	–	–	–	–	–	–	–
GS4	–	0.43	0.072	–	–	–	–	–	–
SS1	43.68	96	91.67	88	35	7.75	3.97	3.5	1.77
SS2	43.47	96.51	95.56	92.22	20	7.75	5.52	4.15	0.78
SS3	44.02	81.54	73.48	59.91	15	4.5	2.16	1.24	0.41
SS4	43	94.44	87.5	82.64	40	12	7.63	3.84	1.34
Mean	43.54 ± 0.43^b	92.12 ± 7.11^a	87.05 ± 9.63^a	80.69 ± 14.4^a	27.5 ± 11.9^a	8 ± 3.08^a	4.82 ± 2.32^a	3.18 ± 1.32^a	1.08 ± 0.6^a
H_t (%)	–	−4.24	−4.6	−8.6	25.86	8.77	28.52	27.16	76
I_{SG} (%)	–	−5.39	−6.64	−11.36	11.36	−3.13	30.53	40.45	73.28

For egg diameter, $n = 120$ (4 replicates × 30 individuals); for fertilization, hatching and cumulative survival rates, $n = 4$ (replicates) in each combination. As the GS hybrids wee nonviable, the cumulative survival data for GS are absent. Different superscript letters in each column indicate significant differences ($p < 0.05$)

shell height of 30 larvae were measured by Image-Pro Plus 6.0 image analysis software. During nursery and grow-out, 30 spat were randomly selected and the shell height at spat stage was measured to the nearest 0.01 mm with an electronic Vernier caliper.

Statistical analysis

As hybrids of the GS group were nonviable and died at the D-stage, data for the GS cross are absent here. Differences in hatching index, growth and survival data between groups and replicates were analyzed by multiple comparisons using a two-way analysis of variance (ANOVA). The differences in growth and survival among the three experimental groups were analyzed with one-way ANOVA. The growth parameters were transformed to a natural logarithm to obtain normality and homoscedasticity, and the hatching and survival rates were arcsine-transformed prior to analysis. Statistical analyses were conducted using SPSS 19.0 software, and the significance level for all analyses was set to $p < 0.05$.

Heterosis was calculated to evaluate the production traits. The equation to determine mid-parent heterosis (H_t) was taken from Cruz and Ibarra (1997):

$$H_t(\%) = (2SG - GG - SS)/(GG + SS) \times 100,$$

where GG and SS are the average phenotypic value of the two purebred offspring, and SG indicates the mean value of hybrid offspring. To estimate the increase in survival and growth of the hybrids compared with that of the *C. sikamea*, the increase in production (I_{SG}) was calculated using the following equation:

$$I_{SG}(\%) = (X_{F1} - X_{Ai}) \times 100/X_{Ai},$$

where X_{F1} is the mean phenotypic value for the hybrid progeny, and X_{Ai} is the mean phenotypic value for the *C. sikamea*.

Results

Fertilization

A remarkable distinction between *C. gigas* and *C. sikamea* is the size of their eggs, with average egg size of 51.17 µm for *C. gigas* and 43.54 µm for *C. sikamea* (Table 2). With respect to fertilization rates, the GS cross had the poorest rate of fertilization and hatching, despite the presence of additional sperm. The average fertilization rate was 0.51%, and the fertilized eggs had a significant delay to the cleavage stage compared with the other three crosses. The mean rates of fertilization success were 89.91%, 87.15% and 92.12% for the GG, SG and SS crosses, respectively (Table 2); embryonic development in the SG group was good and comparable to that of the pure crosses, with no obvious delays or abnormality.

Survival

The hybrids of the GS cross died during the D-stage because of poor gamete compatibility, so data from the other three crosses are presented here. Similar to fertilization success, the proportion of fertilized eggs developing into normal larvae in the SG cross was not significantly different from that of intraspecific crosses (Table 2). The survival of fertilized eggs to the D-stage was 83.32% for the GG cross, 81.27% for the SG cross, and 87.05% for the SS cross (Table 2). Considering differences in fertilization, the percentage of eggs that were fertilized and developed to the D-stage was 71.13% for the GG cross, 70.83% for the SG cross, and 80.19% for the SS cross (Table 2). The variation in survival was attributable to genetic differences among groups, replicates and their interaction in the larval stage (Table 3).

The survival rates for the SG cross and inbred crosses did not differ significantly during the larval stage, but followed the order SG > SS > GG (Table 2). For example, at days 12 and 21, the mid-parental heterosis values for the SG cohort were 25.86% and 8.77%, respectively. During the grow-out

Table 3 Two-way analyses of variance for the effect of genetic group and replicate set on performance traits

Item	Source	Survival				Growth			
		d.f	MS	*F* value	Sig	d.f	MS	*F* value	Sig
Fertilization rate	G	2	0.004	8.008	0.002**	–	–	–	–
	R	3	0.006	11.905	<0.001***	–	–	–	–
	G×R	6	0.003	7.193	<0.001***	–	–	–	–
Hatching rate	G	2	0.003	10.682	<0.001***	–	–	–	–
	R	3	0.005	20.879	<0.001***	–	–	–	–
	G×R	6	0.006	25.089	<0.001***	–	–	–	–
Day 9	G	2	0.148	159.811	<0.001***	2	0.206	17.180	<0.001***
	R	3	0.063	68.060	<0.001***	3	0.006	0.538	0.657
	G×R	6	0.103	111.861	<0.001***	6	0.005	0.403	0.877
Day 12	G	2	0.084	124.767	<0.001***	2	0.569	66.659	<0.001***
	R	3	0.080	120.071	<0.001***	3	0.006	0.753	0.521
	G×R	6	0.041	61.583	<0.001***	6	0.009	1.078	0.375
Day 21	G	2	0.067	45.671	<0.001***	2	0.549	63.669	<0.001***
	R	3	0.122	83.309	<0.001***	3	0.008	0.987	0.403
	G×R	6	0.066	44.846	<0.001***	6	0.003	0.387	0.887
Day 120	G	2	0.072	8.401	0.002**	2	2.755	148.482	<0.001***
	R	3	0.173	20.221	<0.001***	3	0.085	4.584	0.004**
	G×R	6	0.057	6.615	<0.001***	6	0.13	7.027	<0.001***
Day 210	G	2	0.107	4.308	0.025*	2	2.149	105.925	<0.001***
	R	3	0.190	7.639	0.001***	3	0.055	2.713	0.045*
	G×R	6	0.067	2.702	0.038*	6	0.059	2.893	0.009**
Day 320	G	2	0.417	4.240	0.026*	2	0.298	34.186	<0.001***
	R	3	0.087	0.888	0.461	3	0.007	0.795	0.497
	G×R	6	0.212	2.153	0.084	6	0.017	2.004	0.064

For fertilization, hatching and survival rates, $n = 36$ (3 samples × 12 groups); for growth data, $n = 3600$ (30 larvae × 12 groups)

*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$

stage, the SG cohort survived better than the SS cohort; the heterosis value of I_{SG} was 30.53% at day 120 and 40.45% at day 210. However, there was no significant difference among the three crosses ($p > 0.05$) (Table 2); the variation in survival was attributable to genetic differences among groups during the entire spat stage (Table 3).

Growth

The data for shell height during the larval and spat stages are shown in Table 4. The mean shell height of the GG cross was significantly greater than that of the SG and SS crosses ($p < 0.05$; Tables 3, 4). The shell height of the hybrid larvae was less than that of the SS cross at the larval stage, although the difference was not statistically significant ($p > 0.05$). However, the shell height of the SG cross was significantly greater than that of the SS cross at the spat stage (Table 4), while the I_{SG} value was 23.32% at day 120 and 24.43% at day 210. ANOVA demonstrated that the group (experimental combinations) had a significant impact on shell size during the entire grow-out stage ($p < 0.001$) (Table 3).

Genetic confirmation

The amplified bands of ITS1 produced by *C. gigas* and *C. sikamea* were similar in size, approximately 550 bp (Fig. 1a), which made it difficult to distinguish the hybrid from the parental species by standard agarose gels. However, *Hin*dIII-digested PCR-amplified ITS1 products of *C. gigas* obtained two fragments (200 and 300 bp), while no fragments were obtained from *C. sikamea*, enabling identification of both the parental species and the F1 hybrids (Fig. 1b).

Table 4 Shell height of *C. gigas* (GG), *C. sikamea* (SS), and their hybrids (SG) at different days, as well as heterosis (H_t) and single-parent heterosis (I_{SG})

Items	Day 9 (μm)	Day 12 (μm)	Day 21 (μm)	Day 120 (mm)	Day 210 (mm)	Day 320 (mm)
GG1	122.40	141.56	291.51	12.63	14.43	31.17
GG2	139.42	133.28	298.63	12.96	15.34	32.01
GG3	121.13	142.33	286.94	14.44	15.22	32.87
GG4	125.75	140.29	285.23	14.27	15.07	32.58
Mean	127.17 ± 29.47^a	139.37 ± 37.39^a	290.58 ± 47.27^a	13.57 ± 0.9^a	15.02 ± 0.41^a	32.16 ± 6.52^a
SG1	102.39	116.39	213.53	9.99	11.01	30.20
SG2	99.03	115.90	216.37	10.32	9.99	28.01
SG3	102.72	119.73	216.26	7.71	10.91	28.14
SG4	98.23	115.33	210.58	6.84	10.42	27.75
Mean	100.59 ± 19.77^b	116.84 ± 29.89^b	214.19 ± 42.84^c	8.72 ± 1.7^b	10.59 ± 0.47^b	28.52 ± 1.13^b
SS1	103.91	125.31	258.80	8.25	8.84	28.13
SS2	104.95	115.70	254.48	6.52	9.43	26.23
SS3	104.63	112.34	262.64	6.62	9.25	25.82
SS4	89.90	119.41	239.97	6.88	6.52	24.64
Mean	100.85 ± 28.5^b	118.19 ± 30.95^b	253.97 ± 59.81^b	7.07 ± 0.8^c	8.51 ± 1.35^c	26.2 ± 4.78^c
H_t (%)	−11.77	−9.27	−21.34	−15.56	−10.00	−2.25
I_{SG} (%)	−0.25	−1.14	−15.67	23.32	24.43	8.85

For shell height, $n = 120$ in each cross. Different superscript letters in each column indicate significant differences ($p < 0.05$). The GS hybrids were nonviable after the D-stage, so there are no GS data in this table

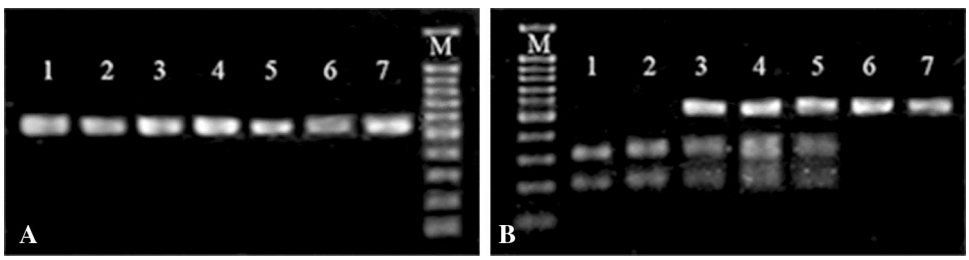

Fig. 1 Agarose gel images of *C. gigas*, *C. sikamea* and their hybrids. a ITS1 amplicons. b ITS1 amplicons after *Hin*dIII digestion. M, size standard (100 bp); lanes 1–2, *C. gigas* parent; lanes 3–5, hybrid spats from *C. sikamea* female × *C. gigas* male cross; lanes 6–7, *C. sikamea* parent

Discussion

In this study, we introduced *C. sikamea* into northern China and hybridized them successfully with *C. gigas*. The aquaculture performance traits of hybrid crosses were firstly compared with two pure crosses under laboratory conditions. High asymmetry in fertilization was revealed: *C. sikamea* eggs were readily fertilized by *C. gigas* sperm, while eggs from *C. gigas* were hardly fertilized with *C. sikamea* sperm. In fact, asymmetric gamete compatibility is commonly found in oyster interspecific hybridization; the lack of gamete recognition proteins between *C. gigas* eggs and *C. sikamea* sperm may account for the asymmetry (Zhang et al. 2012).

Successful embryo development is critical for reliable spat production (Le et al. 2018). In our research, the mean fertilization success of the SG cross was 87.15%, which was lower than that of the GG and SS crosses. Furthermore, no abnormal fertilized eggs were documented in the early embryonic development of zygotes. Generally, fertilization success of interspecific hybrids is lower than parent species among the *Crassostrea* oyster species (Allen et al. 1993; Xu et al. 2009; Yurchenko and Kalachev 2016). Gamete recognition barriers were considered to be the strongest predictor of fertilization success in previous reports (Rawson et al. 2003; Slaughter et al. 2008). However, it is hard to make accurate analysis as fertilization rate can be influenced by water temperature, salinity, and gamete longevity (Banks et al. 1994; Bushek et al. 2008; Xu et al. 2009). In our study, high fertilization success and hatching level were observed among four SG crosses, suggesting that there was no sperm–egg recognition barrier between the *C. gigas* males and *C. sikamea* females. On the contrary, *C. gigas* eggs are hardly fertilized by *C. sikamea* sperm, indicating that bindin divergence might develop with the combination (Moy and Vacquier 2008; Wu et al. 2011). Overall, since fertilization is a key parameter to assess commercial production of interspecies hybrids (You et al. 2015), the incubation index of the SG cross was acceptable for large-scale production in aquaculture.

Survival weakness has been observed in most interspecific hybrids among the *Crassostrea* genus (Allen et al. 1993; Allen and Gaffney 1993; Soletchnik et al. 2002). However, in the present study, there were no significant differences in the survival of the SG cross throughout the entire life cycle, and the hybrids showed positive survival advantages in larval and spat stages. This suggests that the adaptability of the SG hybrid might be stronger than that of intraspecific progeny under certain environmental conditions. Indeed, the viability of an aquaculture animal is known to be affected by the environment (Dégremont et al. 2010; Rawson and Feindel 2012). Similar results were observed for the hybrids of *C. hongkongensis* × *C. angulate* (Zhang et al. 2016a) and *C. hongkongensis* × *C. sikamea* (Zhang et al. 2017), for which survival advantages were maintained throughout the entire lifetime.

The interspecific hybrids exhibited hybrid inferiority in growth performance at the larval stage, though there was no significant difference in average shell size between the SG and SS cross larvae. Similar results were observed in the crosses of *C. ariakensis* × *C. sikamea*, *C. virginica* × *C. gigas* and *C. gigas* × *C. angulata* (Allen et al. 1993; Soletchnik et al. 2002; Xu et al. 2009). Interspecific mating may hinder genetic exchange between the normally developed parents and result in genetic incompatibility in the F1 generation, ultimately causing a set of weakness symptoms (Sun et al. 2017). Interestingly, notable heterosis (in terms of *C. sikamea*) was also found in the spat stage compared to the SS cross, and the growth rate increased by 23.32% for the SG cross at day 120 and 24.43% at day 210. The results demonstrated that genome compatibility between the two species was acceptable. Zhang et al. (2017) attributed a portion of this growth heterosis as likely the different adaptability under particular environmental conditions. Indeed, the growth performance of oysters is sensitive to apparently small environmental differences (Sheridan 1997); the SG hybrid progeny might be more suitable for culturing under the nursery conditions in north China, as its growth was better than that of *C. sikamea* in the spat stage.

In conclusion, an interspecific hybridization experiment between *C. gigas* and *C. sikamea* was successfully carried out in north China. The excellent fertilization rate and comparatively high survival and growth rates compared with *C. sikamea* indicate that the hybrids could be a potential aquaculture oyster stock in north China. Though the production performance of hybrids is still below the commercially desired *C. gigas*, it may be increased to desired levels through selection or backcross over a few generations. Clearly, further study is needed to obtain additional information abou hybrid fertility and to develop strategies to maintain or develop the hybrid advantage.

Acknowledgements This work was supported by grants from the National Natural Science Foundation of China (31772843), Fundamental Research Funds for the Central Universities (201762014), Taishan Scholars Seed Project of Shandong, and Industrial Development Project of Qingdao City (17-3-3-64-nsh).

References

Allen SK, Gaffney PM (1993) Genetic confirmation of hybridization between *Crassostrea gigas* (Thunberg) and *Crassostrea rivularis* (Gould). Aquaculture 113:291–300

Allen SK, Gaffney PM, Scarpa J, Bushek D (1993) Inviable hybrids of *Crassostrea virginica* (Gmelin) with *C. rivularis* (Gould) and *C. gigas* (Thunberg). Aquaculture 113:269–289

Banks MA, McGoldrick DJ, Borgeson W, Hedgecock D (1994) Gametic incompatibility and genetic divergence of Pacific and Kumamoto oysters, *Crassostrea gigas* and *C. sikamea*. Mar Biol 121:127–135

Bartley DM, Rana K, Immink AJ (2001) The use of inter-specific hybrid in aquaculture and fisheries. Rev Fish Biol Fisher 10:325–337

Bushek D, Kornbluh A, Wang H, Guo X, Debrosse G, Quinlan J (2008) Fertilization interference between *Crassostrea ariakensis* and *Crassostrea virginica*: a gamete sink? J Shellfish Res 27:593–600

Camara MD, Davis JP, Sekino M, Hedgecock D, Li G, Langdon CJ, Evans S (2008) The Kumamoto oyster *Crassostrea sikamea* is neither rare nor threatened by hybridization in the Northern Ariake Sea, Japan. J Shellfish Res 27:313–322

Cruz P, Ibarra AM (1997) Larval growth and survival of two catarina scallop (*Argopecten circularis*, Sowerby, 1835) populations and their reciprocal crosses. J Exp Mar Biol Ecol 212:95–110

Dégremont L, Bédier E, Soletchnik P, Ropert M, Huvet A, Moal J, Samain JF, Boudry P (2005) Relative importance of family, site, and field placement timing on survival, growth, and yield of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). Aquaculture 249:213–229

Dégremont L, Bédier E, Boudry P (2010) Summer mortality of hatchery-produced Pacific oyster spat (*Crassostrea gigas*). II. Response to selection for survival and its influence on growth and yield. Aquaculture 299:21–29

FAO (2007) Assessment of freshwater fish seed resources for sustainable aquaculture. FAO Fisheries Technical Paper, Rome

Hamaguchi M, Shimabukuro H, Kawane M, Hamaguchi T (2013) A new record of the Kumamoto oyster *Crassostrea sikamea* in the Seto Inland Sea, Japan. Mar Bio Record 6:6–11

Heath WA (1995) Developments in shellfish culture in British Columbia. J. Shellfish Res 14:228

Hedgecock D, Davis JP (2007) Heterosis for yield and crossbreeding of the Pacific oyster *Crassostrea gigas*. Aquaculture 272:17–29

Hedgecock D, Li G, Banks MA, Kain Z (1999) Occurrence of the Kumamoto oyster *Crassostrea sikamea* in the Ariake Sea, Japan. Mar Biol 133:65–68

Hong JS, Sekino M, Sato S (2012) Molecular species diagnosis confirmed the occurrence of Kumamoto oyster *Crassostrea sikamea* in Korean waters. Fish Sci 78:259–267

Huo Z, Wang Z, Yan X, Gaffney PM (2013) Fertilization, survival, and growth of *Crassostrea hongkongensis* ♀ × *Crassostrea ariakensis* ♂ hybrids in northern China. J Shellfish Res 32:377–385

Lafarga de la Cruz F, Gallardo-Escárate C (2011) Intraspecies and interspecies hybrids in *Haliotis*: natural and experimental evidence and its impact on abalone aquaculture. Rev Aquac 3:74–99

Le DV, Young T, Alfaro AC, Ragg NLC, Hilton Z, Watts E, King N (2018) Practical fertilization procedure and embryonic development of the New Zealand geoduck clam (*Panopea zelandica*). J Mar Biol Assoc UK. https://doi.org/10.1017/S0025315416001685

Li Q, Wang Q, Liu S, Kong L (2011) Selection response and realized heritability for growth in three stocks of the Pacific oyster *Crassostrea gigas*. Fish Sci 77:643–648

Moy GW, Vacquier VD (2008) Bindin genes of the Pacific oyster *Crassostrea gigas*. Gene 423:215–220

Rawson P, Feindel S (2012) Growth and survival for genetically improved lines of Eastern oysters (*Crassostrea virginica*) and interline hybrids in Maine, USA. Aquaculture 326:61–67

Rawson PD, Slaughter C, Yund PO (2003) Patterns of gamete incompatibility between the blue mussels *Mytilus edulis* and *M. trossulus*. Mar Biol 143:317–325

Sheridan AK (1997) Genetic improvement of oyster production—a critique. Aquaculture 153:165–179

Slaughter C, Mccartney MA, Yund PO (2008) Comparison of gamete compatibility between two blue mussel species in sympatry and in allopatry. Biol Bull 214:57–66

Soletchnik P, Huvet A, Le Moine O, Razet D, Geairon P, Faury N, Philippe G, Boudry P (2002) A comparative field study of growth, survival and reproduction of *Crassostrea gigas*, *C. angulata* and their hybrids. Aquat Living Resour 15:243–250

Sun S, Wu Y, Lin X, Wang J, Yu J, Sun Y, Miao Y, Li Q, Sanguinet KA, Liu B (2017) Hybrid weakness in a rice interspecific hybrid is nitrogen-dependent, and accompanied by changes in gene expression at both total transcript level and parental allele partitioning. PLoS One 12:e0172919

Wang H, Guo X (2008a) Identification of *Crassostrea ariakensis* and related oysters by multiplex species-specific PCR. J Shellfish Res 27:481–487

Wang Y, Guo X (2008b) ITS length polymorphism in oysters and its use in species identification. J Shellfish Res 27:489–493

Wang H, Guo X, Zhang G, Zhang F (2004) Classification of jinjiang oysters *Crassostrea rivularis* (Gould, 1861) from China, based on morphology and phylogenetic analysis. Aquaculture 242:137–155

Wang Q, Li Q, Kong L, Yu R (2012) Response to selection for fast growth in the second generation of Pacific oyster (*Crassostrea gigas*). J Ocean Univ China 11:413–418

Wang H, Qian L, Wang A, Guo X (2013) Occurrence and distribution of *Crassostrea sikamea* (Amemiya 1928) in China. J Shellfish Res 32:439–446

Wang C, Liu B, Liu X, Ma B, Zhao Y, Zhao X, Liu F, Liu G (2017) Selection of a new scallop strain, the Bohai Red, from the hybrid between the bay scallop and the Peruvian scallop. Aquaculture 479:250–255

Wu Q, Xu F, Bao Y, Li L, Zhang G (2011) Bindin gene from the Kumamoto oyster *Crassostrea sikamea*, and divergence of the fucose lectin repeats of bindin among three species of *Crassostrea*. J Shellfish Res 30:55–64

Xu F, Zhang G, Lio X, Zhang S, Shi B, Guo X (2009) Laboratory hybridization between *Crassostrea ariakensis* and *C.* Sikamea. J Shellfish Res 28:453–458

You W, Guo Q, Fan F, Ren P, Luo X, Ke C (2015) Experimental hybridization and genetic identification of Pacific abalone *Haliotis discus hannai* and green abalone *H. fulgens*. Aquaculture 448:243–249

Yurchenko OV, Kalachev AV (2016) Is gamete morphology involved in hybridization in oysters? Mar Biol 163:1–11

Zhang Y, Wang Z, Yan X, Yu R, Kong J, Liu J, Li X, Li Y, Guo X (2012) Laboratory hybridization between two oysters: crassostrea gigas and Crassostrea hongkongensis. J Shellfish Res 31:619–625

Zhang Y, Su J, Li J, Zhang Y, Xiao S, Yu Z (2016a) Survival and growth of reciprocal crosses between two stocks of the Hong Kong oyster *Crassostrea hongkongensis* (Lam & Morton, 2003) in southern China. Aquac Res 48:1–11

Zhang Y, Zhang Y, Jun L, Wang Z, Yan X, Yu Z (2016b) Phenotypic trait of *Crassostrea hongkongensis* ♀ × *C. angulata* ♂ hybrids in southern China. Aquac Res 47:3399–3409

Zhang Y, Zhang Y, Li J, Xiao S, Xiang Z, Wang Z, Yan X, Yu Z (2016c) Artificial interspecific backcrosses between the hybrid of female *Crassostrea hongkongensis* × male *C. gigas* and the two parental species. Aquaculture 450:95–101

Zhang Y, Li J, Zhang Y, Ma H, Xiao S, Xiang Z, Yu Z (2017) Performance evaluation of reciprocal hybrids derived from the two brackish oysters, *Crassostrea hongkongensis* and *Crassostrea sikamea* in southern China. Aquaculture 473:310–316