Aquaculture potential of Bohai Red and its hybrid with *Agropecten irradians concentricus* in southern China

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Abstract

Argopecten irradians concentricus (Say), one of four geographic subspecies of the bay scallop, has become the major cultured species in southern China since its introduction in 1995. However, its population has been significantly reduced due to high mortality. Also, poor growth rate has been observed following decades of culture. Therefore, the introduction or creation of new varieties is an urgent need. This study describes the first introduction of the new strain, Bohai Red, from the north to southern China. Hybridization trials were conducted between the recently introduced new strain and the local specie, *A. irradians concentricus* (Say). The success of hybridization was confirmed by SSR marker. The adult and juvenile Bohai Red cannot tolerate high temperatures compared to *A. irradians concentricus* (Say), whether in natural waters or under laboratory conditions. Fertilization rate for *A. irradians concentricus* (Say) $\times$ Bohai Red exceeded 80%, and hatching rate was 70%. The hybrids exhibit heterosis in survival rate, growth rate, and high-temperature tolerance, demonstrating substantial potential to replace *A. irradians concentricus* (Say) in southern China.

Key Words: *Argopecten irradians concentricus*; Bohai Red; Hybridization; Temperature tolerance; Heterosis;

Running Head: Introduction and hybridization of a new strain of scallop

1. Introduction

As an economically important marine bivalve, the bay scallop *Argopecten irradians* (A.i.) has four geographic subspecies: *A.i. irradians* (Lamarck), *A.i. amplicostatus* (Dall), *A.i. concentricus* (Say), and *A.i. taylorae* (Petuch), which are naturally distributed along the coast of the Atlantic Ocean and the Gulf of Mexico (Wilbur, Gaffney, 1997). The original stocks of *A. irradians concentricus* (Say) were first introduced to China from North Carolina in 1995 (Zhang et al, 2000). Due to the fast growth rate, and large adductor muscle, the production of the bay scallop reached about 600,000 tons in 2007 (Guo, 2009; Wang et al, 2014). However, since the bay scallop is a hermaphroditic animal, significant decreases in population due to high mortality, and also poor growth rate had been noticed following decades of culture. (Wang et al, 2014; Wang et al, 2007; Zheng et al, 2008). Efforts such as intra-specific and inter-specific hybridization did result in improved growth rate, but its tolerance to environmental conditions such as *Argopecten purpuratus* × *A. irradians concentricus*, *Argopecten irradians* × *A. irradians concentricus* and *Mimachlamys nobilis* × *A. irradians concentricus* were minimal. New varieties to replace bay scallops in southern China have not been identified and the problem of germplasm degradation in the southern bay scallop has not been effectively solved.

Bohai Red is a new scallop strain formed from the hybridization of the *Argopecten purpuratus* and bay scallop *Argopecten irradians* (Lamarck) through six generations of selection which exhibits excellent heterosis in growth. Compared to bay scallops, this strain is 125.9–138.9% larger by whole body weight and has a 145.4–156.2% larger adductor muscle. So the *A. i. irradians* (Lamarck) was quickly being replaced by the Bohai Red after the new strain passed the certification of Fisheries Law of People's Republic of China. By 2016, in the north of China, Bohai Red production reached 556470 tons, replacing more than 50% of the traditional culture areas of the *A. i. irradians* (Lamarck) (Wang et al, 2017; Wang et al, 2011). But as a northern cultured scallop variety, it is only been cultured in Bohai Bay of north china but not in the south where the culture area is 2-3 times higher than that of the northern culture area. Considering the degradation of *Argopecten irradians*

100 *concentricus* in south China, Bohai Red may provide an opportunity for genetic
101 improvement of *A. irradians concentricus*. However, it is unknown if Bohai Red can
102 be successfully introduced in the south due to the significant temperature differences
103 between the north and south.

104 Although the mechanism of heterosis still remains enigmatic (Wang et al, 2017;
105 Wang et al, 2011), hybridization is considered as one of the most effective methods for
106 genetic improvement in livestock and plant production. In aquaculture, hybridization
107 has been extensively applied to enhance growth rate, survival rate, and environmental
108 tolerance in the oyster (Hedgecock et al, 1995; Kong et al, 2017), abalone (You et al,
109 2009; You et al, 2015) and scallop (Cruz, Ibarra, 1997; Cruz et al, 1998; Wang et al,
110 2011). For example, due to high-temperature tolerance and accelerated growth rate,
111 the Penglai red hybrid between *Chlamys farreri* and *Patinopecten yessoensis* was
112 commercially introduced in China in 2003 (Sun et al, 2004; Yang et al, 2004). Since
113 Bohai Red is the hybrid between the *Argopecten purpuratus* and *Argopecten irradians*
114 (Lamarck), it is likely that Bohai Red and *A. irradians concentricus* (Say) can be
115 successfully crossed to produce offspring with heterosis, furthermore, we are
116 interested in the ability of hybrids to tolerate the high temperatures as this is an
117 obligatory adaptation for southern China.

118 In order to improve the sustainability of bay scallops in China, the Bohai Red,
119 which was first introduced from the north of China, was evaluated for growth and
120 survival rate in southern China to determine if it will be able to adapt to the
121 environment of southern China. In addition, crossbreeding between the Bohai Red
122 and *Argopecten irradians concentricus* was conducted and heterosis in growth and
123 survival traits were assessed by comparing the hybrid crosses with purebred offspring.

124

125 **2. Material and methods**

126

127 *2.1. Comparisons of survival in two broodstocks*

128 One thousand and one hundred adult Bohai Red scallops, which were 40
129 mm–50 mm in shell length, were collected in Qindao, Shangdong, China

(35.7°N,120.0°E) by professor Chunde Wang, who created the new strain. Five hundred adult *Argopecten irradians concentricus* were collected from culture stocks in Beibu bay (20.4°N, 109.9°E) with shell length ranging from 40 to 50 mm. The two stocks were cultured in Beibu bay, Guangdong, China (20.8°N, 109.7°E) from December to April. The water temperature was measured at 16:00 each day to calculate the monthly average water temperature. Dead scallops were collected to calculate the survival rate.

2.2. Gamete collection and hybridization

When the broodstocks of Bohai Red (designated as Z) and *Argopecten irradians concentricus* (designated as M) were sexually mature, the two broodstocks were hybridized. Ten sexually mature individuals were chosen from each stock to spawn by flowing water stimulation for two hours followed by injection of 0.1 mL of 0.05 mmol/L serotonin (5-hydroxytryptamine, Sigma, USA) into the adductor muscle (Cruz, Ibarra, 1997). All scallops begin to release eggs after 30 minutes of releasing of the sperm. At this time, all scallops were separately placed in a 2-L box filled with seawater of the same temperature to collect eggs or sperm. The eggs were discarded once the first polar bodies were identified under the microscope following 15 minutes of egg collection. Eggs and sperm of the two stocks were collected separately. The gametes from different broodstock were mixed with eggs from another stock to create hybrids groups ($M_{\text{♀}} \times Z_{\text{♂}}$, $M_{\text{♂}} \times Z_{\text{♀}}$). Those from the same stock were used to create parental groups ($M_{\text{♀}} \times M_{\text{♂}}$, $Z_{\text{♀}} \times Z_{\text{♂}}$). Three parallel parental and hybrid groups were created, and a limited number of fertilized eggs were collected to evaluate fertilization success and survival to D-stage larvae.

2.3 Larvae and juvenile rearing

The larvae were kept in 500-L aerated plastic tanks at 22–25°C. The *Isochrysis zhanjiangensis* and *Chlorella vulgaris* were mixed in equal amounts and fed. The density of larvae was maintained at 20 larvae/ml. The collectors were placed in the nursery tanks when 30% of larvae had eyespots. Three 2-ml samples were taken from each group and the numbers of larvae were used to calculate the density. The survival rate was recorded based on the live larvae on

different days. The larvae were removed from the collector when the shell length of larvae reach 450 μm and was placed in a 0.15mm mesh screen to transfer them to the Beibu Bay, China,(20.4°N,109.9°E) for the nursery stage. The spats were sorted at a day intervals, first into larger mesh bags and finally into adult cages. Forty progenies per replicate were randomly sampled and their growth index (shell length/mm, shell height/mm and weight/g) was measured with vernier calipers (0.02 mm accuracy) and an electronic balance (0.01 g accuracy) every month(from seeding to harvesting for about 200d).

2.4 Genetic confirmation

DNA was extracted from samples of the hybrids and parents progeny using the marine animal DNA extraction kit (transgene, China). The SSR primer information reference literature (Jie et al, 2018).The procedure was as follows:

(1) Primer M13 (containing HEX HEX or 6-FAMFAM fluorescent material, sequence 5' -CACGACGTTGTAAAACGACCGACGTTGTAAAACGAC) was added to the 5' end of the forward primer (completed by Sangon Biotech, China), and the reverse primer was unchanged. (2) PCR reactions involving genomic DNA templates from the parents and hybrids. The PCR was conducted as follow: 3 min at 95°C (pre-denaturation); 10 cycles of denaturing for 30 s at 95°C, annealing for 30 s at 60°C and extending for 30 s at 72°C;20 cycles of denaturing for 30 s at 95°C,annealing for 30 s at 55°C and extending for 30 s at 72°C;followed by a final extension of 6 min at 72°C(3) 50 pg of PCR product and with 10 μl of Buffer (HiDi formamide and ROX500 in a ratio of 99:1) were mixed, then rapidly cool on ice-water mixture immediately after 5 min at 98°C. The capillary electrophoresis was performed on the 3730XL (Applied Biosystems) and the results were analyzed using Genemapper software.

2.5 Temperature tolerance test

To determine the temperature tolerance, hybrid cohorts and inbred cohorts weighing 0.22–0.24 g, with shell lengths of 10.8–10.9 mm, were first acclimated to the laboratory conditions for 24 h prior to the test. The temperature groups were set at

18°C and 30°C in 10-L plastic containers equipped with temperature control units. Sixty individuals were randomly selected from each cohort, and three replicates were assigned. The scallops were fed with *Chlorella vulgaris* twice a day. The seawater was changed and the dead scallops were picked out to calculate the survival rate every 24 h. The final shell length was measured to calculate the growth rate after 15 d.

2.6. Heterosis estimate and data analysis

The data are expressed as mean±SD. Statistical analyses were performed using IBM SPSS, Version 19 (IBM SPSS Inc, Chicago, IL, USA). The differences in survival and growth among the experimental lines and heterosis were analyzed using one-way ANOVA, and significance for all analyses was set at $P < 0.05$. Mid-parent heterosis was calculated using the formula (Falconer, 1996).

$$H(\%) = [(MZ + ZM) - (MM + ZZ)] \times 100 / (MM + ZZ)$$

where MZ and ZM are the mean survival rate (or weight, size) of the hybrid F1 cohort, and MM, ZZ is the mean survival rate (or weight, size) of the *A. irradians concentricus* Say and Bohai Red, respectively.

Single-parent heterosis was calculated using the formula (Cruz, Ibarra, 1997):

$$H_H = \frac{F - H_H}{H_H} \times 100$$

where H_X is the single-parent heterosis of stock X, P_X is the mean size (or weight, survival rate) of the cohort parental stock X (ZZ, MM), and F is the mean size (or weight survival rate) of the hybrid cohort (MZ, ZM).

3. Result

3.1 Comparisons the survival of two broodstocks in the southern waters

The water temperature in the culturing area was measured continuously over five months. The lowest average temperature, 18°C, was observed in February, while the highest average temperature was observed in April at 26.8°C. The two broodstocks exhibited different survival rates at the end of the 5 months of growth and the survival rate for Bohai Red was closely related to temperature (Fig. 1). Survival rate exceeded 90% for two broodstocks from December to March, but the survival rate dropped to

219 70.2% for Bohai Red when the average water temperature reached 26.8°C in April
220 while the survival rate of *Argopecten irradians concentricus* remained high survival
221 rate(92%) during this period.

222 3.2 Fertilization and hatching rates

223 The hatching and fertilization rates are displayed in Table 2. The highest
224 hatching rate was recorded in MM (90.63%) with the highest fertilization in MZ
225 (78.80%), While ZM had the lowest hatching (88.31%) and fertilization rate
226 (74.6%). There was no significant difference between all groups ($P > 0.05$).

227 3.3 Growth and survival during the larval stage

228 During the larval stage, there were significant differences between the four
229 groups in shell length (Table 3). The hybrid group (MZ, ZM) exhibited substantial
230 heterosis (4.50%–18.57%) during the larval stage which gradually increased over time.
231 The single parent heterosis of MZ was significantly higher than that of the ZM group
232 at 5, 10, 20 and 30 d. The growth rates of the two-hybrid groups were greater than the
233 parent groups (MM ZZ), with middle-parental heterosis of 21.59%. Shell length of
234 MZ was significantly greater than all other groups, while no significant difference
235 was found between the pure groups ($P > 0.05$). In term of survival rate, it ranged from
236 41.83–49.09% with no significant difference between all groups at the larval stage.

237 3.3 Growth and survival during growth-out stage

238 The survival and growth rates of the inbred and hybrid cohorts are shown in Fig.
239 2 and Fig. 3. The growth rates for shell length of the hybrid groups (MZ, ZM) were
240 higher than those of the parental crosses (MM, ZZ), and the shell length for MZ
241 reached 53.64 mm with a growth rate 0.45 mm/d, the greatest rate among the hybrid
242 groups ($H_M = 37.20\%$, $H_Z = 27.14\%$ and $H = 32.23\%$). The shell length for the MM
243 group was significantly lower than the other groups at 47.76 mm ($P > 0.05$). The
244 middle-parental heterosis of the whole body weight was greater than shell length
245 heterosis, with an average value of 14.41%. While the whole body weight growth
246 rates for the hybrid groups (MZ, ZM) were greater than the parental crosses (MM,
247 ZZ), there was no significant difference between ZM (24.70 g) and ZZ (24.52 g). The
248 average values were 0.73% for H_Z and 29.81% for H_M , and 14.44 % for H .

249 In terms of survival rate, mortality rose rapidly for the ZZ group at high water
250 temperatures. The survival rate for the ZZ group was only 44.46% at harvest, while
251 the other groups were all higher than 80%. No significant differences in survival rates
252 were observed between hybrid cohorts ($P > 0.05$), with average values of 7.25% for
253 H_M, 92.58% for H_Z and 27.35% for H.

254

255 3.4 Parentage analyses

256 Parentage analyses with the three SSR primers (MQ1, MQ2, MQ3) are shown in
257 Fig.4 For the *Argopecten irradians concentricus* Say, only one band ~146 bp was
258 amplified by MQ1, one band ~132 bp was amplified by MQ2 and two bands, ~116
259 bp, and ~124 bp, by MQ3. For the Bohai Red only one band was amplified by
260 MQ1(~148 bp),MQ2(~134bp) and MQ3(~134bp)In all the hybrids, only two bands
261 were amplified, one was only appeared in the *Argopecten irradians concentricus* (Say)
262 and another only appeared in Bohai Red, indicating that the success of hybridization
263 can be confirmed.

264

265 3.5 Temperature tolerance test

266 Average summer temperature (30°C) and average winter temperature (18°C)
267 in southern China were used for temperature tolerance test.The growth and survival
268 rates of the hybrids (MZ, ZM) and inbreds (MM, ZZ) under laboratory conditions are
269 presented in Figs.5 and Figs.6. The results indicate that the high-temperature tolerance
270 ability of MM was greatest with growth rate (0.3084 mm/d) and survival
271 rate(78.34%),followed by ZM and MZ.The ZZ was weakest with growth rate
272 (0.1015mm/d) and survival rate(25%).There was no significant difference recorded
273 among the groups in the low-temperature(18°C). The inbred ZZ exhibited the highest
274 growth rate 0.30 mm/d ($P>0.05$) in the low-temperature environment while the worse
275 growth rate was recorded under high temperature (30°C) tolerance with survival rate
276 25% and growth rate 0.1015mm/d ($P<0.05$).Our result demonstrate that the ability to
277 tolerate the high temperature of hybrids was improved compared to Bohai Red.

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281 **4 Discussion**

282 In this study, Bohai Red was introduced into the south of China for the first
283 time. We found that the adult and juvenile Bohai Red cannot tolerate high
284 temperatures compared to *A. irradians concentricus*, whether in natural waters or
285 under laboratory conditions. The results matched the reports about different ability of
286 temperature tolerance among the two species. Preliminary results showed that the
287 critical thermal maxima were 32°C for *Argopecten irradians concentricus* (Liu, 2007),
288 compared to 29 °C for Bohai Red (Wang et al, 2017). High mortality was observed in
289 Bohai Red with the increasing temperature demonstrating that thermal stress may
290 also be the primary challenge for the introduction of Bohai Red, and it may not be
291 suitable for direct introduction to southern China where the maximum water
292 temperature reaches 32°C in summer. However, the hybrids between *A. irradians*
293 *concentricus* and Bohai Red are adaptable to south water which suggests that ability
294 of hybrids to tolerate the high temperatures was increased through hybridization. Same
295 results had appeared in *Haliotis discus hannai* × *H. fulgens* (You et al, 2015), red
296 abalone ♀ × Pacific abalone ♂ (Cruz, Gallardo-Escárate, 2011) and *Argopecten*
297 *purpuratus* × *Argopecten irradians* (Cruz, Gallardo-Escárate, 2011). The hybrids could
298 change physiological status to get more resistant to disease and thermal stresses than
299 their parents (Liang et al, 2014; Nan et al, 2016).

300 Summer mortality is multifactorial process, including
301 genetics, pathology, physiology, and immunology (Samain et al, 2008) which could be
302 caused by an interaction between abiotic environmental factors and biotic at high
303 water temperatures (Stone et al, 2014). The reasons for high mortality of Bohai Red in
304 the high temperature were complex, while the temperature is only one of the factors
305 related to mortality. Our results demonstrate that hybridization could be an effective
306 way to improve the ability of Bohai Red to tolerate high temperature conditions.

307 Bohai Red, non-native genetic material, was used for genetic improvement of
308 native species (*A. irradians concentricus*) through interspecies

309 hybridization. Compared to *A. irradians concentricus* (Say), shell length and whole
 310 body weight of the hybrid increased by 12.31%, and 29.81%, respectively.
 311 Generally, hybrids could develop heterosis in disease resistance, growth, or other
 312 traits than parental species through hybridization that promotes genomic transfer
 313 between species and produces phenotypic and genotypic changes (Stone et al,
 314 2014)(Stelkens et al, 2009; Zhang et al, 2014). Similarly, our results also have shown
 315 that the growth of the hybrids was likely to be greatly improved by the introgression
 316 gene flow between two parental species which it is likely an important plastic force in
 317 the morphological and genetic diversity of the hybrids (Bierne et al, 2006). In our
 318 study, the growth rate of MZ significantly exceeded that of ZM in hybrid
 319 groups. Differences between reciprocal crosses are also commonly observed in shellfish such
 320 as *Crassostrea hongkongensis* (Bierne et al, 2006), *Argopecten irradians* (Zhang et al, 2014),
 321 it may be due to the cytoplasmic inheritance, sex-linked genes, or parental
 322 effects (Zhang et al, 2014). This study demonstrates that the cross between Bohai Red and
 323 *A. irradians concentricus* may be a good way in increasing growth of *A. irradians*
 324 *concentricus*.

325 Bay scallop is hermaphroditic animal that spawns sperm and eggs
 326 simultaneously, So it is hard to separate sperm and eggs at a large spawning scale,
 327 as performed in one-to-one crossbreeding (Zhang et al, 2007). Furthermore, there
 328 are phenomena of gynogenesis, natural polyploid, androgenesis in shellfish (Zhang
 329 et al, 2007), we can not confirm hybrids just basing on morphologies such as
 330 number of the ribs or shell collar (Hedgecock et al, 1995). Genetic identification of
 331 interspecific hybrids among shellfish has been reported using karyological
 332 studies, SSR (Hedgecock et al, 1995), CO I, 16S (Ahmed et al, 2008; An et al, 2005)
 333 and ITS (Xu et al, 2019). In our research, primers MQ1, MQ2 and MQ3 were
 334 developed as species-specific marker to distinguish *A. irradians concentricus* and
 335 “Bohai Red” as only one band ~146 bp was amplified by MQ1, only one band
 336 ~132 bp was amplified by MQ2 in *Argopecten irradians concentricus* and only
 337 one band was amplified by MQ1 (~148 bp), MQ2 (~134 bp) and MQ3 (~134 bp) in

338 “Bohai Red” , but Both hybrids, two bands were amplified at the same time which
339 can confirm the hybrid at the genetic level.

340 Three-way crosses were defined as: the hybridization of a binary hybrid of two
341 varieties (lines) to another cultivar (line) with excellent traits(Yan et al, 2010).It can
342 combine the ideal traits of three species to make the most of heterosis and had been
343 widely used in the breeding of crops (Padhi et al, 2016) and livestock(Yamak et al,
344 2014) .For shellfish in aquaculture, three-way crosses had only been reported in
345 *Ruditapes philippinarum* (Yan et al, 2010).In this study, three-way crosses were
346 conducted between *Argopecten irradians concentricus*,*Argopecten purpuratus*, and
347 *Argopecten irradians irradians*. The results shown that compared to the heterosis
348 which only in growth but not high temperature tolerance in hybridization of
349 *Argopecten irradians concentricus*×*Argopecten irradians irradians*. *Argopecten*
350 *irradians concentricus*×*Chlamys nobilis* and *Argopecten irradians*
351 *concentricus*×*Argopecten purpuratus* (Nan et al, 2012),the ternary hybrid has shown
352 heterosis in both growth and high-temperature tolerance which demonstrating that
353 three-way crosses are also an effective method for genetic improvement in *A.*
354 *irradians concentricus*.Furthermore,Shellfish usually spawn a lot(Wang, Li, 2010),for
355 example,a bay scallop can produce between 500,000 and 600,000 gametes at a time
356 (Lautert et al, 2012)and some of shellfish species are hermaphroditic animal which
357 means that the inheritance of some ideal traits can be stabilized quickly,So we
358 believe three-way crosses could suitable for shellfish breeding and have advantages
359 overcrop and livestock in some aspects .

360 Although many experimental species of scallop hybridization have shown
361 promising results and positive heterosis in aquaculture, only a few hybrids were
362 commercially introduced until now.In the present study,we were excited to find
363 that the hybrids of*A. irradians concentricus* and Bohai Red have improved in
364 growth and tolerance to higher temperature when compared to parents. In order to
365 produce the hybrid with stable inheritance and better growth performance in the
366 future prospective research will focus on backcrossing with parental species and

selective breeding to find strategies to maintain hybrid advantage.

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378 **Declaration of Competing Interest**

379 The authors affirm that there are no conflicts of interest.

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Fig. 1. Survival rate of Bohai Red and Bay Scallop from December to April

Fig. 2. Shell length of inbred and hybrid scallops from 60 to 150 day age

Fig. 3. Body weight of inbred and hybrid scallops from 60 to 150 day age.

Fig. 4. Genotyping results of three SSR loci in “Bohai Red”, *A.i.concentricus* and their hybrid.

Note: MQ-M: Genotyping results of *A.i.concentricus*

MQ-Z: Genotyping results of “Bohai Red”

MQ-H: Genotyping results of hybrid

Fig. 5. The growth of hybrid and inbred cohorts under different water temperatures in the laboratory.

Fig. 6. Survival rate of hybrid and inbred cohorts under different water temperatures in the laboratory.

Table 1

Primers for parentage analyses

Primers	Sequence	Annealing temperatures
MQ3	Forward: 5'-ACTATCCAAATGATCCCAGGAGT	55°C
	Reverse 5'-ACCTTACCAGACACTTCAATGACA	
MQ2	Forward: 5'-AAAATAGCGAAAATGGGAGGTCC	55°C
	Reverse 5'-TCGCGAATTAAAGCTCCGTTTT	
MQ1	Forward: 5'-AATATCTTTAAGCATGCACCTGC	54°C
	Reverse 5'-ACTGCATACGGATACAATGTCCTT	

Table 2

Fertilization and hatching rates in different groups

	MM	MZ	ZZ	ZM
Fertilization rate (%)	90.63±0.69 ^a	89.41±1.20 ^a	88.73±0.87 ^a	88.31±1.77 ^a
Hatching rate (%)	77.40±5.77 ^a	78.80±3.90 ^a	76.40±5.94 ^a	74.60±4.04 ^a

Notes: The same letters indicate no significant difference ($P > 0.05$)

Table 3

Growth and survival of reciprocal and parental crosses at the larval stage

Combination	Shell length (um)				Growth rate (um/d)	Survival rate (%)
	5 d	10 d	20 d	30 d		
MM	112.63±8.93 ^b	148.30±10.50 ^b	300.57±12.49 ^b	625.13±35.28 ^c	20.50±1.16 ^c	41.83±3.41 ^a
MZ	116.33±10.14 ^a	153.13±9.66 ^a	353.30±21.16 ^a	754.60±83.23 ^a	25.53±3.06 ^a	49.09±5.73 ^a
ZZ	103.47±8.11 ^d	140.23±7.38 ^c	290.80±10.46 ^c	603.13±45.34 ^c	19.98±1.61 ^c	47.87±4.35 ^a
ZM	109.50±8.13 ^c	146.73±7.04 ^b	313.63±24.25 ^b	701.73±64.18 ^b	23.69±2.39 ^b	47.41±4.99 ^a
H _M (%)	3.28	3.26	17.54	20.70	24.54	17.36
H _Z (%)	5.83	4.64	7.80	16.35	18.57	-0.96
H (%)	4.50	3.93	12.78	18.57	21.59	7.58

Notes: The same letters indicate no significant difference ($P > 0.05$).

Table 4

Comparisons of growth and survival of the hybrid and inbred cohorts at harvest

Combination	Shell length (mm)	Growth rate (mm/d)	Weight (g)	Growth rate (g/d)	Survival rate (%)
MM	47.76±4.10 ^c	0.40±0.034 ^c	21.87±4.17 ^c	0.18±0.035 ^c	81.60±3.04 ^b
MZ	53.64±5.03 ^a	0.45±0.042 ^a	28.39±6.62 ^a	0.24±0.055 ^a	87.52±3.00 ^a
ZZ	50.93±3.23 ^b	0.42±0.027 ^b	24.52±6.87 ^b	0.20±0.057 ^b	44.46±3.33 ^c
ZM	51.47±4.95 ^b	0.43±0.041 ^b	24.70±4.90 ^b	0.21±0.041 ^b	85.62±2.37 ^{ab}
H _M (%)	12.31	-	29.81	-	7.25
H _Z (%)	1.05	-	0.73	-	92.58
H	6.51	-	14.44	-	37.35