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Short communication

Evaluating survival after heat challenges and investigating their correlations with field summer survival for the juvenile Pacific oyster (*Crassostrea gigas*)

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ABSTRACT

Mass mortalities of juvenile Pacific oysters (*Crassostrea gigas*) in aquaculture operations have been associated with sustained high temperatures during the summer months. Selective breeding programs to improve field survival in the *C. gigas* are expensive, labor-intensive, and often rely on prolonged field trials. Therefore, controlled challenge methods for improving selection response towards increased summer mortality resistance are required. The purpose of this study was to verify whether juvenile survival after heat challenges predicts field summer survival for full-sib families of *C. gigas*. We thermally challenged juveniles from 59 families using two separate methods (chronic heat stress and acute heat shock) and monitored their survival rates. Oysters were also deployed in two major oyster farms and then survival rates were compared between the two conditions. In this study, families from different genetic backgrounds differed in heat tolerance and field survival. Correlations between heat challenges and field trials were low to moderate and positive (0.321–0.346 for acute heat shock and 0.472–0.491 for chronic heat stress). Our results suggest that although current heat challenge trials cannot completely replace field trails in selective breeding programs, selection for heat tolerance is expected to indirectly improve field survival. An important outcome of this study is that heat challenges can discriminate some families that are resistant or susceptible to summer mortality at an early age, and that selection efficiency can be improved by identifying and eliminating poor-performing families prior to planting. In a separate experiment, we found that juvenile oysters had significantly higher summer survival when they were exposed to heat challenges than oysters that were not exposed earlier. We conclude that heat challenge of oysters is a useful method for assessing the survival capacity of hatchery-produced spat used by the oyster industry.

1. Introduction

Mortality in oysters, and particularly the summer mortality syndrome of *Crassostrea gigas*, is a major problem affecting the industry in several countries. With the increasing scale of aquaculture, large-scale mortality occurs regularly during the summer months globally (Alfaro et al., 2019). Summer mortality events have occurred in coastal areas in many countries, including France (Soletchnik et al., 2005; Royer et al., 2007), Australia (Paul-Pont et al., 2013; Go et al., 2017), and the United States of America (Friedman et al., 2005; Burge et al., 2007). According to previous studies, several factors have been associated with oyster summer mortality, with pathogens, temperature, and reproductive effort being the three most important (Samain et al., 2007; Wendling and Wegner, 2013; Huvet et al., 2010). Benefitting from the oyster selective breeding program, several lines resistant to specific pathogens or high temperatures have been selected to reduce summer mortality (Beattie et al., 1980; Dégremont et al., 2015; Ding et al., 2020; Jiang et al., 2023).

In China, oysters are produced by commercial hatcheries in late winter and early spring, and mortality events typically occur during the first summer (Chi et al., 2021). Although *Vibrio* spp. may be found in moribund oyster as opportunistic pathogens, primary and infectious agents have not been observed in oyster during summer mortality events (Wang et al., 2021; Zhang et al., 2023). Mass mortality of oysters tends to occur during the summer months, suggesting that environmental factors, especially the high temperature, may play a crucial role in mass

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mortality outbreaks (Renault et al., 2014; Pernet et al., 2016; Go et al., 2017). In coastal area of northern China, water temperature can reach over 25 °C in summer, which can be challenging for oysters (Yang et al., 2021; Liu et al., 2023). Elevated temperatures promote the growth of pathogens and suppress the immune system of oysters, making them more susceptible to opportunistic pathogens (Gagnaire et al., 2006; Vezzulli et al., 2010; Li et al., 2023) and are therefore considered to be an important causal factor for summer mortality (Cheney et al., 2000; Li et al., 2007; Samain et al., 2007).

Improved survival under field conditions is crucial for oyster growers who rely on high yields for profitability. In an effort to improve the increasing valuable *C. gigas* industry, we have conducted selective breeding for summer survival and have made good genetic progress through two generations of family selection (Chi et al., 2022). However, selective breeding programs aimed at improving field survival traits in oysters are costly, labor-intensive, and often require lengthy field trials (Lang et al., 2010). Assuming that challenge trials are correlated with summer field survival, this difficulty can be circumvented and efficiency improved by placing juveniles under controlled conditions prior to field trials and selectively eliminating poor-performing families through challenge trials (Dégremont et al., 2010; Camara et al., 2017). We chose heat challenge (heat shock and chronic heat stress) as a stressor because heat stress is a significant contributor to oyster summer mortality and because it does not involve the use of pathogens or hazardous chemicals.

The purpose of this study was to assess the potential of using survival after heat challenges as a proxy for summer survival during field challenge in a family-based breeding program. This study reports the results of two experiments, in which we 1) tested the correlations between survival to heat challenge (acute heat shock and chronic heat stress) and summer survival of juvenile oyster families reared at two commercial sites, and 2) examined whether oysters that survived from heat challenge were more resistant than control oysters when exposed to field conditions.

2. Materials and method

2.1. Biological material

In March 2021, 59 full-sib families were established at the Laizhou breeding base (37°32′N 119°09′E, Shandong Province, China). Fifteen wild families were obtained using wild oysters from non-cultivated areas of Rongcheng, Shandong Province. Thirty-three "Top selection" and eleven "Down selection" families were produced from broodstocks derived from a family selection program. The base population was derived from two fast-growing stocks which mass selection for shell height was performed over ten and eight generations, respectively. We then performed two generations of family selection based on summer survival. The selection strategy and rearing conditions for the full-sib families were described in Chi et al. (2022).

2.2. Acute heat shock

To identify the tolerance of different oyster families to heat shock, we treated 90 juvenile oysters from each of 59 full-sib families with heat shock. Three replicates were set up for each family in this experiment, with 30 oysters per replicate. All *C. gigas* families were acclimatized to the experimental environment for 7 days at 20 °C in 500-L plastic tanks, followed by a heat shock treatment. We heat-shocked oysters in filtered water at 42 °C for 2 h and then immediately released the juveniles back into ambient seawater (20 °C). Tanks were cleaned daily and juveniles were monitored daily for mortality for 5 days. Cultured phytoplankton diet of *Nitzschia closterium* was fed three times a day during the observation period. Oysters were considered as dead when they were unable to close their shells after being out of water. Dead or dying juveniles were removed as discovered and survivors were counted after 5 days. Salinity was maintained at 31.5 ± 0.5 psu throughout the experiment.

2.3. Chronic heat stress

In May 2021, chronic heat stress experiment was conducted on different oysters from the same set of 59 families. Three replicates were set up for each family in this experiment, with 30 oysters per replicate. We heated three replicate groups of 30 juveniles from each of the families. To mimic the thermal stress caused by elevated temperatures in the natural environment, we conducted a chronic increase in temperature. These oysters were acclimatized to a 20 °C environment for one week before being subjected to chronic heat stress. Temperature was gradually increased by 1 °C (1 °C per day until 32 °C) and then maintained at 32 °C until the end of the experiment. The challenge experiment was continued for 32 days to ensure that mortality trends were adequately captured (a level of 50% mortality rate) and oysters in the experimental tank were monitored daily for mortality. The choice of expected temperatures was based on previous trends in mortality at high temperatures, as well as on pilot studies with families, which showed a mortality rate of close to 50% over a one-month period at a temperature of 32 °C. During the acclimatization and challenge experiment, cultured phytoplankton diet of *Nitzschia closterium* were fed three times a day. Salinity was maintained at 31.5 ± 0.5 psu throughout the experiment.

2.4. Field test

On June 6 and June 10, 2021, juveniles were sent from the Laizhou breeding center to two commercial culture farms (Rongcheng, 37°11′N 122°48′E; Huangdao, 35°86′N 120°08′E). Families were placed in lantern nets and each replicate lantern net containing 40 oysters from each family. Three lantern nets were set up for each family in this experiment. After 104 and 107 days of deployment in Rongcheng (September 18, 2021) and Huangdao (September 25, 2021), respectively, oyster survival was assessed by counting live oysters in each lantern.

At the end of the heat challenge tests, survivors from each family were pooled together and the group was designated chronic heat-selected (CHS) and acute heat-selected (AHS). Oysters from the same family that did not experience heat challenges were named non-selected (NS). These three groups were reared in same hatchery conditions at the Laizhou breeding center. Water quality, food ration and temperature were controlled under these conditions and oysters were monitored for mortality on a daily basis. In August 2021, a field challenge experiment was conducted using the sibs (challenged oysters and unchallenged oysters) from same oyster families to determine whether prior exposure to heat challenge conferred protection during a subsequent exposure to field conditions. Twenty same families from each group (CHS, AHS and NS) were transported to Rongcheng for the field experiment. Field survival of each family was recorded in September 2021.

2.5. Statistical analyses

Statistical analyses were performed using SPSS 22.0 software. One-way ANOVA followed by multiple comparisons Tukey test was used to analyze the differences in survival rates among the families. Homogeneity of variances among means was assessed using Levene's test for equality of variance errors. Survival rate was arcsine transformed to stabilize the variances of errors. Differences were considered statistically significant if $P < 0.05$.

Spearman correlations of survival rate between different experiments were calculated using SPSS 22.0. In addition, the 15 best-performing and 15 worst-performing families in one experiment were identified on the basis of survival rate, and the proportion of these families that was also present in the 15 best-performing and 15 worst-performing families in the other experiments were determined.

3. Results

3.1. Field survival

Survival rates of different oyster families varied considerably between the two culture sites, ranging from 15.00% to 93.33% for Rongcheng and from 5.00% to 83.33% for Rushan (Fig. 1). In general, the highest survival rate was observed in the "Top selection" families (74.95% in Rongcheng and 61.39% in Huangdao). The survival rates of the wild families were 45.44% and 33.61% in Rongcheng and Huangdao, respectively, while the survival rates of the "Down selection" families were 34.17% and 23.18% in Rongcheng and Huangdao, respectively (Table 1).

Table 1
Survival rate of *C. gigas* families of different genetic backgrounds under experimental challenges. Different superscript letters indicate significant difference ($P < 0.05$).

Background	Survival rate/%			
	Field: Rongcheng	Field: Huangdao	Acute Heat shock	Chronic heat stress
Top selection	74.95^{a}	61.39^{a}	56.13^{a}	58.59^{a}
Down selection	34.17^{c}	23.18^{c}	48.69^{b}	38.69^{b}
wild	45.44^{b}	33.61^{b}	49.34^{b}	49.33^{ab}

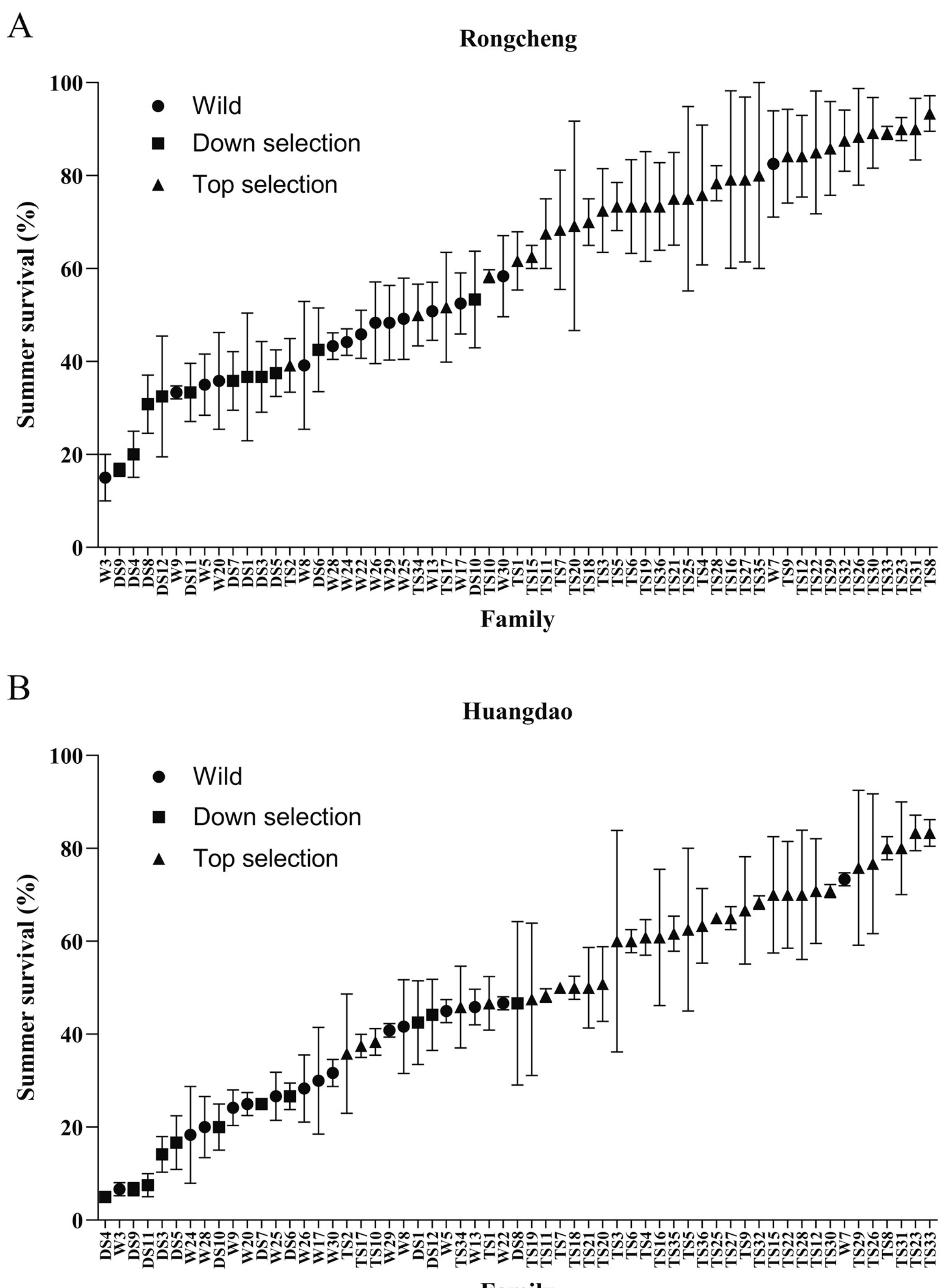

Fig. 1. Mean survival (± SD) of 59 full-sib *C. gigas* families produced in 2021 at two major oyster culture sites (Rongcheng and Huangdao). Mean survival for each family is plotted in increasing order for each oyster site.

3.2. Acute heat shock and chronic heat stress

Varying mortality rates were observed in 59 full-sib families during acute heat shock and chronic heat stress challenges. Survival rate ranged from 20.00% to 90.00% in full-sib families, with an average survival rate of 52.52% in chronic heat stress challenge (Fig. 2). In acute heat shock challenge, survival rates ranged from 35.56% to 72.22%, with a mean survival rate of 52.52% (Fig. 3).

In the chronic heat challenge, the highest survival rate was observed in the "Top selection" families (58.59%, ranging from 35.56% to 90.00%), and conversely, the lowest survival rate was observed in the "Down selection" families (38.69%, ranging from 20.00% to 50.00%). Survival rate of wild families was 49.33% (26.67%–80.00%) (Table 1). In the heat shock challenge, the survival rate of the "Top selection" families (56.13%) was significantly higher ($P < 0.05$) than that of the "Down selection" families (48.69%) and the wild families (49.34%). However, there was no significant difference in the survival rate between the wild and "Down selection" families (Table 1).

3.3. Phenotypic correlations between different challenge experiments

The correlations of the family mean survival rate between field survival and the heat shock challenge were low but positive (0.321–0.346, $P < 0.05$) (Table 2). Family mean survival rate between field survival and chronic heat stress was moderately correlated (0.472–0.491, $P < 0.01$). The correlation between acute heat shock and chronic heat stress was lower but positive (0.318, $P < 0.05$). Notably, the correlation between survival of oyster families at the two oyster farm test sites was very high (0.89, $P < 0.01$) (Table 2).

The percentages of the best-performing and worst-performing families that were shared in different experiments were shown in Table 3. The percentage of the 15 best-performing families that were shared between the two experiments ranged from 4/15 (heat shock and field: Rongcheng) to 13/15 (field: Rongcheng and field: Huangdao). The percentage of the 15 worst-performing families that were shared between the two experiments ranged from 5/15 (heat shock and field: Rongcheng; Chronic heat stress and field: Huangdao) to 9/15 (field:

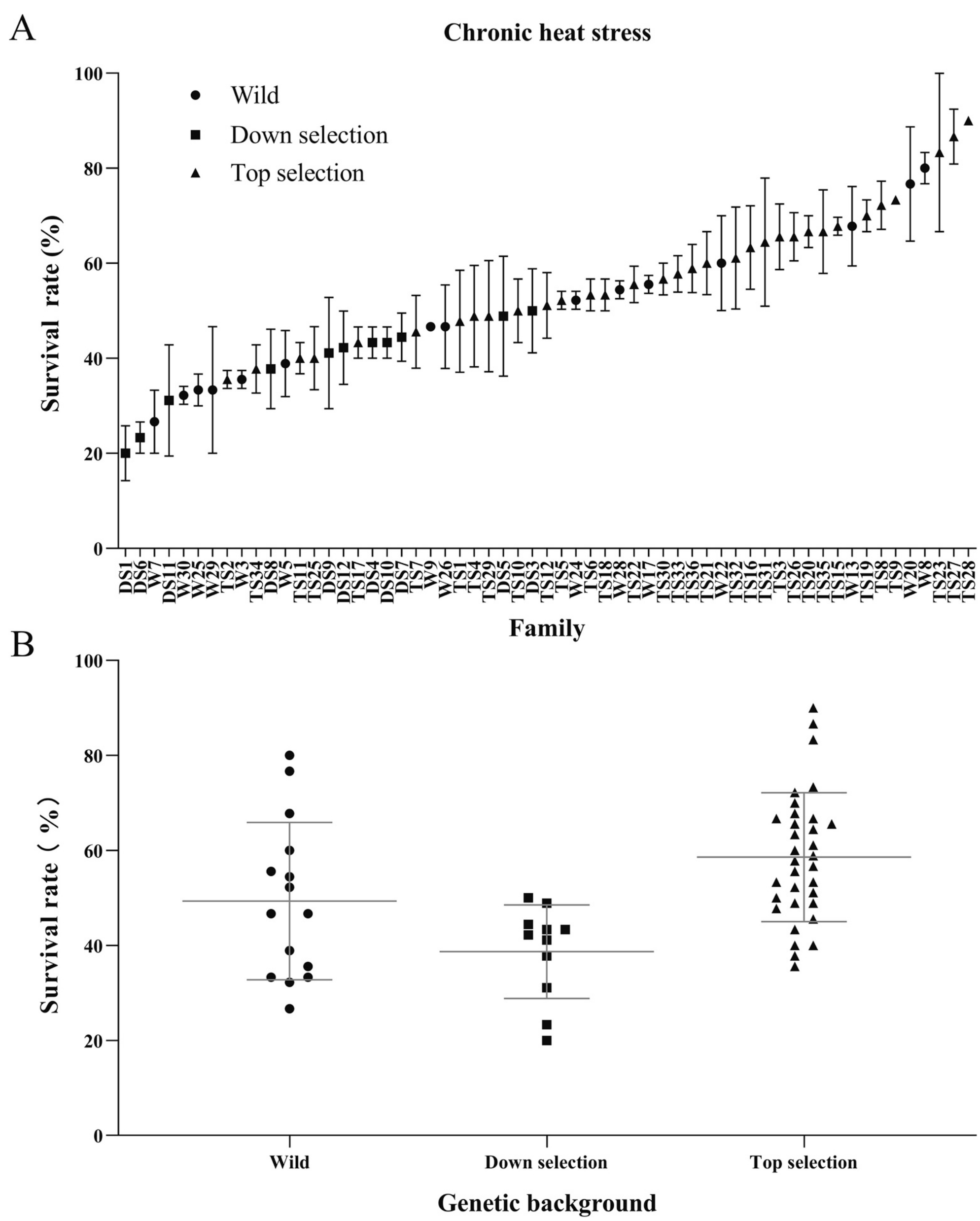

Fig. 2. Mean survival (± SD) of 59 full-sib families of *C. gigas* produced in 2021 based on number of live and dead oysters 32 days after chronic heat stress (20 °C–32 °C) experiment. Mean survival for each family is plotted in increasing order.

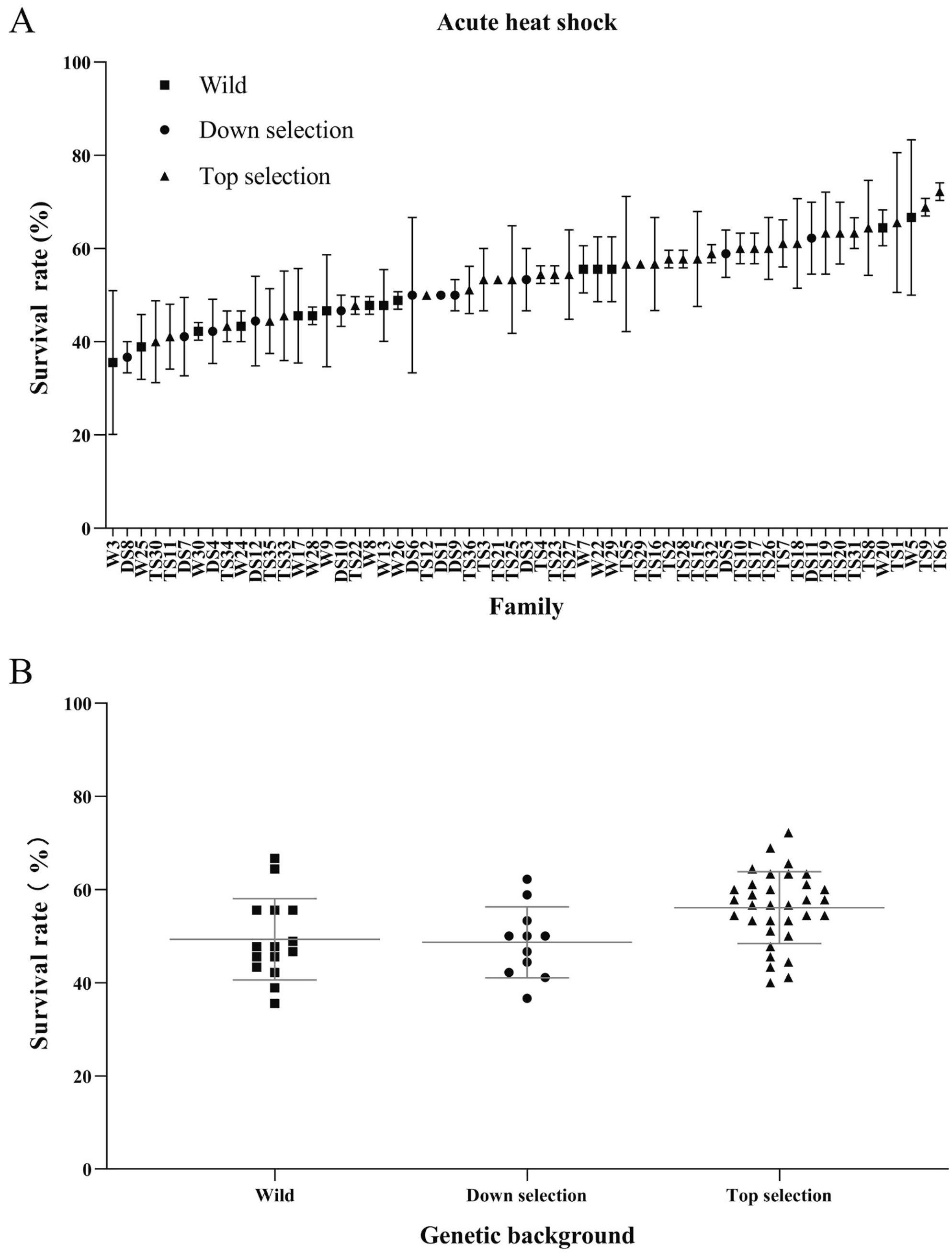

Fig. 3. Mean survival (± SD) of 59 full-sib families of *C. gigas* produced in 2021 based on number of live and dead oysters 120 h after treatment at 42 °C for 2 h in the heat shock challenge experiment. Mean survival for each family is plotted in increasing order.

Table 2
Correlation between survival of heat challenges (acute heat shock and chronic heat stress) and average survival (%) in 59 oyster families reared at a two field sites (Rongcheng and Huangdao). Correlation was significant when $P < 0.05$.

Test1	Test2	Pearson correlation coefficients	P-value
Acute heat shock	Field: Rongcheng	0.346	< 0.01
Acute heat shock	Field: Huangdao	0.321	0.013
Chronic heat stress	Field: Rongcheng	0.491	< 0.01
Chronic heat stress	Field: Huangdao	0.472	< 0.01
Acute heat shock	Chronic heat stress	0.318	0.014
Field: Rongcheng	Field: Huangdao	0.894	< 0.01

Table 3
The percentages of best-performing and worst-performing families shared in different experiments.

Challenge 1	Challenge 2	Best 15	Worst 15
Acute heat shock	Field: Rongcheng	4/15	5/15
	Field: Huangdao	8/15	6/15
Chronic heat stress	Field: Rongcheng	7/15	7/15
	Field: Huangdao	8/15	5/15
Chronic heat stress	Acute heat shock	7/15	6/15
Field: Rongcheng	Field: Huangdao	13/15	9/15

Rongcheng and field: Huangdao).

3.4. *Response of C. gigas to summer mortality after heat challenge exposure*

There was no abnormal mortality of oysters in the AHS, CHS and NS groups under breeding center conditions. After the field trial, the summer survival rate of the NS group was lower than the other two groups in the order of NS $<$ AHS $<$ CHS (Fig. 4). The survival of the CHS was significantly higher than that of NS ($P < 0.05$), but there was no significant difference between the field survival rates of AHS and NS ($P > 0.05$).

4. Discussion

Summer mortality is a major problem for *C. gigas* production worldwide. Although the causes of summer mortality are complex, rising temperature is unanimously recognized to kick off the window of oyster mortality (Solomieu et al., 2015). Multiple studies have shown that water temperature above 19 °C is a major contributor to *C. gigas* summer mortalities (Soletchnik et al., 2005; Samain, 2011; Pernet et al., 2012). Therefore, we utilized survival after acute heat shock and chronic heat stress to assess the heat tolerance of oysters. In fact, testing oyster under heat stress is considered a valid method for estimating the oyster survival capacity during oyster summer mortality outbreaks (Beattie et al., 1980; Hershberger et al., 1984; Lang et al., 2010; Dégremont et al., 2010). In the present study, *C. gigas* produced from different parent stock responded differently to heat challenges in these experiments, suggesting that heat tolerance may be a heritable trait. This is consistent with the findings of Shamseldin et al. (1997), who reported differences in heat tolerance among different populations of *C. gigas*. Notably, "Top selection" families showed greater heat tolerance and field survival than the "Down selection" and wild families, confirming the existence of genetic variation in resistance to summer mortality in *C. gigas*. Similarly, selected *C. gigas* families have been reported to have greatly improved survival in the field and in elevated temperature laboratory trials

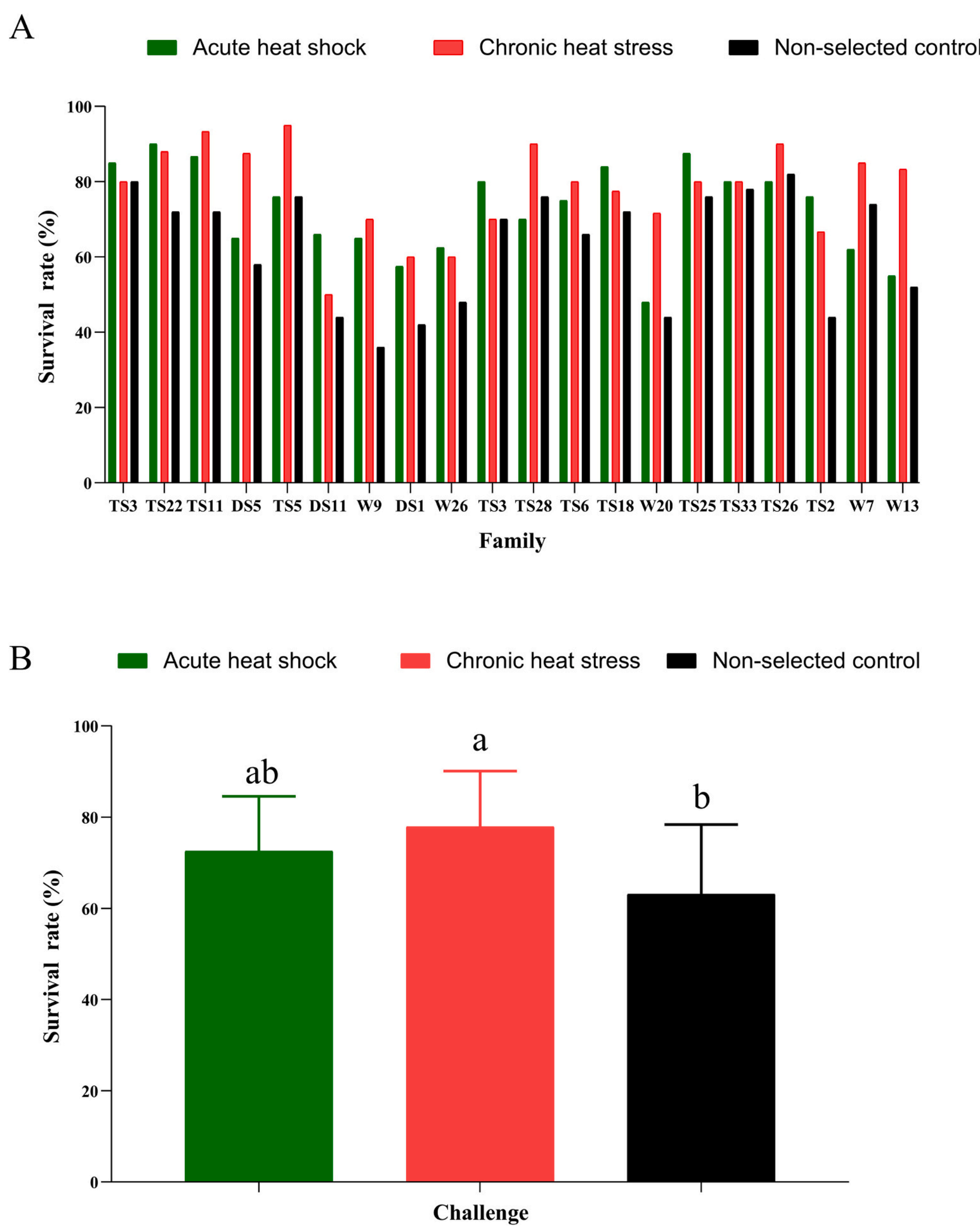

Fig. 4. Survival rate of 20*C. gigas* families after field test at Rongcheng (A). Mean survival (± SD) of three groups (CHS, AHS and NS) after field test at Rongcheng (B). Different superscript letters indicate significant difference ($P < 0.05$).

(Beattie et al., 1980). Their breeding program, which has been in place for three generations, initially challenged adult *C. gigas* in the laboratory to high temperature, which resulted in high mortality of adult *C. gigas* (Lipovsky and Chew, 1972). Most of the selected families had higher survival rates than the control families, suggesting that selection is effective in increasing resistance to heat stress, which is one of the factors that may contribute to summer mortality.

In this study, there was a low to moderate correlation (0.321–0.491, $P < 0.05$) in survival between field trials and heat challenge trails. Similarly, Boudry et al. (2007) found a significant positive phenotypic correlation between survival of summer mortality syndrome and juvenile survival to heat shock (40 °C, 2 h) in a three-month study. In contrast, Camara et al. (2017) found that the estimated heritability of heat shock survival in *C. gigas* was low, with minimal additive genetic correlation with either farm survival (in the presence of oyster herpesvirus 1) or oyster survival when exposed to laboratory virus challenge. Summer survival of *C. gigas* are influenced not only by temperature, but also by salinity and food availability (Soletchnik et al., 2007; Southworth et al., 2017; Ashton et al., 2020). In addition, oysters are exposed to many microorganisms in the field, and field results reflect the combined effects of multiple factors, not just temperature (Alfaro et al., 2019). Thus, the complex etiology of mortality in the field environment may produce survival phenotypes that are not highly correlated with the survival phenotypes observed in the heat challenges. In this study, positive correlations between survival after heat challenges and field survival suggests that selection for heat tolerance is expected to improve field summer survival. However, it would be unwise to completely replace field summer survival with survival after heat challenges in a family selective breeding program. Because field trials rely on more regular and predictable mortality outbreaks, efforts should be made to develop challenging trials that are more similar to that the oysters experience in field trials. Due to the complexity of oyster summer mortality, future challenge trials may need to consider both heat tolerance and disease resistance.

There was a strong correlation between the average survival of oyster families from the two culture sites, and no evidence of strong genotype-environment interactions that would otherwise complicate selective breeding. Moreover, the putative causal agents causing mortality may be common in different sites. Therefore, as demonstrated by resistance to summer mortality along the French Atlantic and English Channel coasts (Dégremont et al., 2007), a single selective breeding program to improve survival of *C. gigas* at sites affected by summer mortality should be effective. The correlation between chronic heat stress and field survival was higher than that of acute heat shock, suggesting that chronic heat stress challenge may be a better method of estimating oyster survival ability, which may be related to the persistent high temperatures in oyster farms during the summer months. In this study, oysters did not experience low tide exposure and heat wave at the experimental sites. Sites temperature variations throughout the experimental period were approximately 16 °C to 26 °C. In some aquaculture areas, oysters in the intertidal zone become exposed when the heat wave recedes, and oyster temperatures can rise above 40 °C for a few hours and then drop to seawater temperatures (Cheney et al., 2000). Notably, the thermal range over which oysters live or perish after heat shock was only 2° to 3 °C (Shamseldin et al., 1997; Hamdoun et al., 2003). Thus, there are practical problems with developing heat shock assays for oyster performance in the field.

An important result in this study is that heat challenges can discriminate some families that are resistant or susceptible to summer mortality at an early age. Therefore, a management strategy can be proposed that involves testing hatchery-produced spat for heat stress under hatchery conditions and selectively culling poorly performing families prior to conducting field trials (Camara et al., 2017). These methods allow for the early elimination of unfavorable (low survival) families, thus reducing the workload in the cultivation process and hopefully saving oyster farming costs. In general, most aquaculture species are highly fecund, which means that it is possible to achieve high selection intensities that result in genetic gains with minimal inbreeding (Bentsen and Olesen, 2002; Sonesson, 2005). Thus, if heat challenges can be made during the early stages of development, this can unlock the capacity of hatcheries to produce larger cohorts consisting of more families, thus increasing selection pressure and improving resistance to summer mortality more quickly.

We found that when juvenile *C. gigas* were exposed to heat challenges, the summer survival of juvenile survivors was significantly higher than oysters that were not exposed earlier. Thus, selection of survivors from heat challenges was more effective than family-based selection among unchallenged sibs. As suggested by Beattie et al. (1980), Hershberger et al. (1984), and more recently by Ding et al. (2020) and Juárez et al. (2021), heat tolerance may be a selective trait that can be used to increase summer mortality resistance. For field survival traits, we can select core families based on field survival rate, but a large number of selection candidates from a core family have the same estimated breeding value, which limits the use of within-family genetic variation to achieve genetic gain (Houston et al., 2022). Therefore, if the increase in summer mortality resistance displayed by CHS and AHS oysters in the current study has a genetic basis, within family selection for heat tolerance (sibs heat challenge) based on field survivor is expected to improve the field summer survival. The *C. gigas* has strong potential to benefit oyster breeders eager to build diversity and resiliency into their selective breeding programs. We anticipate that the vulnerability of this species to heat stress will be one of the biggest constraints to further development of *C. gigas* aquaculture in northern China. Selective breeding for greater heat tolerance might be a viable strategy for improving survival of cultured *C. gigas*.

5. Conclusion

This study investigated the heat tolerance of juvenile oysters and compared these results with those obtained in the field. Survival between field trials and heat challenges showed low to moderate correlations (0.321–0.491, $P < 0.05$), and worst families could be culled from the hatchery based on the survival rates observed here. In addition, the juvenile survivors from heat challenges had significantly higher summer survival than oysters that did not experience an earlier exposure. Thus, heat challenge can also be used in selective breeding programs to increase resistance to summer mortality and to characterize the quality of hatchery-produced spat used by oyster growers.

CRediT authorship contribution statement

Yong Chi: Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **Chengxun Xu:** Investigation, Methodology. **Qi Li:** Conceptualization, Funding acquisition, Project administration, Supervision.

Declaration of competing interest

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

Data availability

Data will be made available on request.

Acknowledgments

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