Phenotypic evaluation of two genetically improved strains selected from the reciprocal hybrids of *Crassostrea gigas* and *C. angulata*

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ABSTRACT

The Pacific oyster (*Crassostrea gigas*) is one of the most economically important aquaculture shellfish in China, but further development of its industry is constrained by germplasm degradation and summer mass mortality episodes. To this end, a three-generation mass selection on the reciprocal hybrids, GA-*C. gigas* ♀ × *C. angulata* ♂ and AG-*C. angulata* ♀ × *C. gigas* ♂, was conducted to develop two strains (GA and AG selected strain) with fast-growing and warm-adapted characteristics. The aim of the present study was to determine whether the genetic improvement in growth, survival and thermal tolerance could be achieved by mass selection. For shell height, a genetic gain of 7.77–14.60%, a selection response of 0.67–0.91, and a realized heritability of 0.39–0.57 were observed in the selected strains after three generations of selection; for total weight, a genetic gain of 11.33–14.85%, a selection response of 0.73–1.06, and a realized heritability of 0.41–0.67 were observed in the selected strains after three generations of selection. The significant selection response of growth traits suggests the potential for genetic improvement of growth traits in both strains. With selection, the LT_{50} increased from 41.81 °C to 42.10 °C in the GA selected strain and from 41.61 °C to 42.06 °C in the AG selected strain. By the F3 generation, the summer survival rates of the GA and AG selected strain were 10.53% and 8.98% higher than that of the control strain, respectively, and 20.80% and 18.14% higher than that of the *C. gigas* strain, respectively. The consistent results of LT_{50} and summer survival suggest that thermotolerance selection may provide a viable strategy for improving summer survival in oysters. After three generations of selection, the total yield of the two strains increased by 29.63% and 19.78%, respectively, compared to *C. gigas* strain. This study provides a source of superior strains for the sound development of the oyster industry and can be used as a reference for shellfish breeding strategies.

1. Introduction

The Pacific oyster *Crassostrea gigas* is a leading species in world shellfish farming thanks to its characteristics for rapid growth and wide environmental tolerance. *C. gigas* is naturally distributed along the northwestern Pacific coast and is mainly cultured in China. In 2021, *C. gigas* contributes over 1.58 million metric tons to oyster production in China, representing approximately 27% of the oyster total production (BOF, Bureau of Fisheries, 2022). However, due to improper broodstock management and deficient parental selection, germplasm degradation of *C. gigas* has occurred in recent years, resulting in slow growth, small size and poor stress resistance (Wang et al., 2016; Zhang et al., 2018; Xu et al., 2019a). Meanwhile, episodes of massive mortalities present another significant challenge to *C. gigas* culture in China (Mao et al.,

2005; Lian et al., 2010; Bai et al., 2021). The massive mortalities were associated with physiological stress caused by high temperatures, which are the main limiting environmental factor for oyster culture in northern China (Yang et al., 2021; Li et al., 2023). Therefore, breeding a superior strain with fast growth rate and high survival rate is necessary to promote the healthy development of oyster industry.

Selection and hybridization are two classic and effective approaches for genetic improvement of aquatic animals. Selective breeding refers to the selection of individuals with a trait of interest from a stock or family, with the aim of directionally screening the superior traits, to obtain varieties with desired phenotype (Sun et al., 2022). The basis of selection is the existing genetic variation among species, families or individuals (Bosworth et al., 2003). Mass selection is a common technique used for the genetic improvement of aquatic species, such as fish
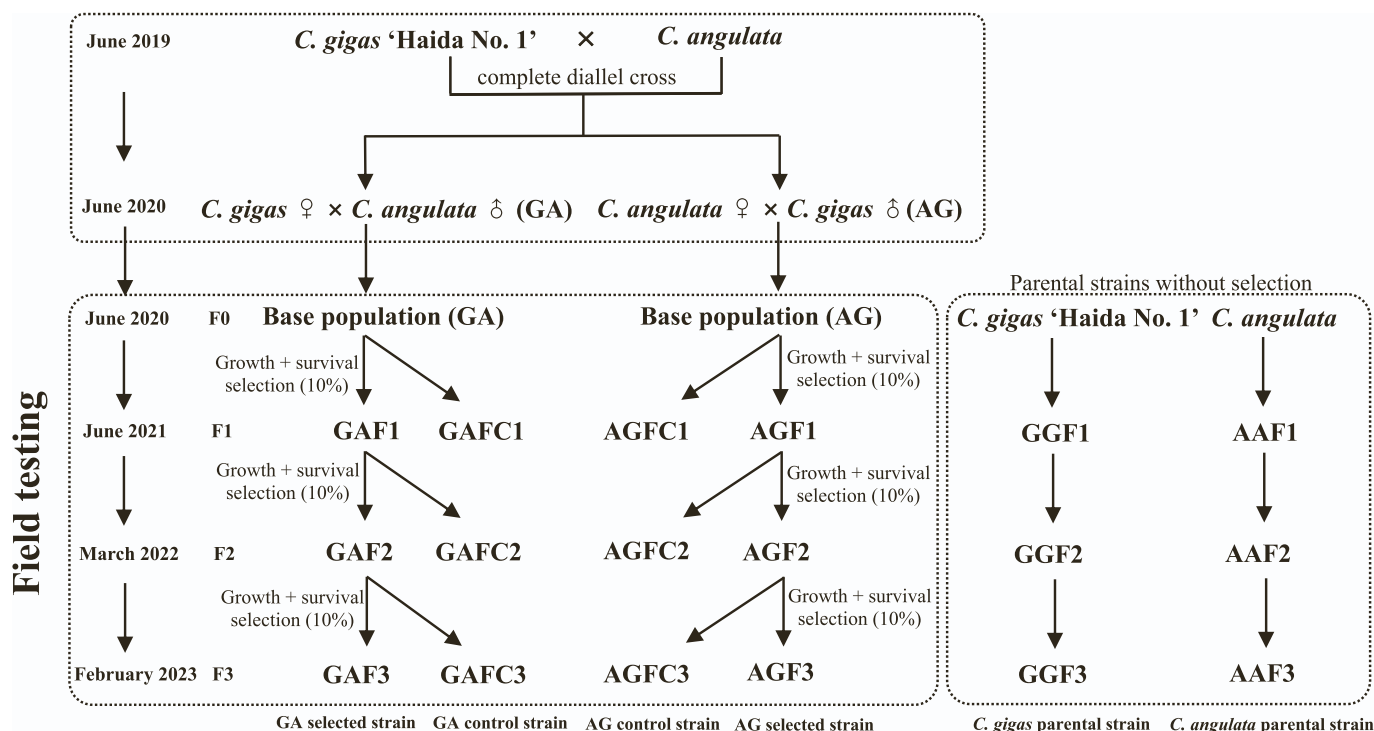

Fig. 1. Schematic overview of mass selection experiments.

Table 1
Number and Shell height (mean ± SD) of selected parents, cutoff point, and selection intensity in different selective breeding strains.

Year	Strain	Selected parents			Cut point	Selection
		N_f	N_m	Shell height (mm)	(mm)	intensity
2020	GAF1	50	50	90.83 ± 7.95^{ns}	80.12	1.56
	AGF1	50	50	90.44 ± 8.04^{ns}	75.84	1.54
2021	GAF2	40	40	90.02 ± 8.51^{ns}	81.94	1.57
	AGF2	40	40	89.18 ± 8.38^{ns}	73.10	1.62
2022	GAF3	40	40	$86.60 \pm 8.44^{**}$	75.73	1.61
	AGF3	40	40	$81.04 \pm 5.21^{**}$	73.17	1.71

N_f and N_m represented the number of females and males broodstock, respectively. The difference in shell height between the two selected parents in the same year was indicated by asterisk (** – $P < 0.01$; ns – not significant).
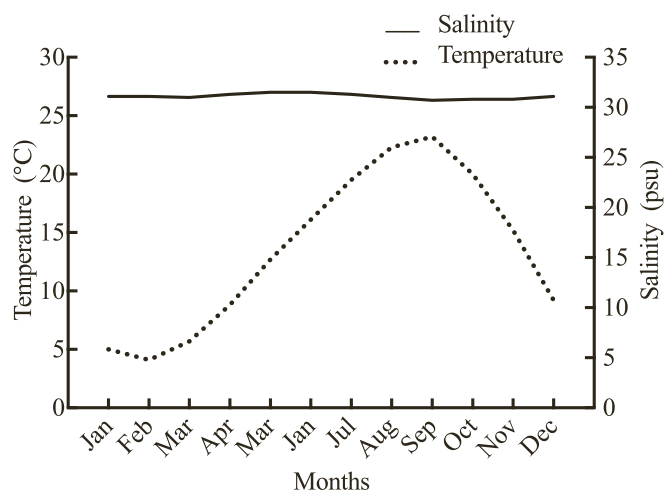
Table 2
Two-way analyses of variance testing for strain origin (SO), selection generation (SG) and SO × SE interaction effects on shell height (SH), total weight (TW), incremental survival rate in summer (IS), cumulative survival rate (CS), final yield (Y) and LT_{50}.

Traits	Effect	d.f.	MS	F-value
SH	SO	3	0.078	24.311***
	SG	2	0.098	30.260***
	SO × SG	6	0.001	0.464^{ns}
TW	SO	3	0.102	25.381***
	SG	2	0.381	95.125***
	SO × SG	6	0.002	0.609^{ns}
IS	SO	3	0.094	9.823***
	SG	2	0.564	59.042***
	SO × SG	6	0.002	0.256^{ns}
CS	SO	3	0.075	5.793**
	SG	2	0.323	24.947***
	SO × SG	6	0.004	0.290^{ns}
Y	SO	3	0.071	20.772***
	SG	2	0.265	77.258***
	SO × SG	6	0.005	1.549^{ns}
LT_{50}	SO	3	0.817	9.958***
	SG	2	0.578	7.039**
	SO × SG	6	0.013	0.161^{ns}

The P-value associated with each F-value are indicated by asterisks (* – $P < 0.05$; ** – $P < 0.01$; *** – $P < 0.001$; ns – not significant).

Fig. 2. The average monthly temperature and salinity of the breeding site (data downloaded from http://mds.nmdis. org.cn/).

(Garduño-Lugo et al., 2004; Chatchaiphan et al., 2019), shrimps (Donato et al., 2005), scallop (Zheng et al., 2006; Wang et al., 2020) and oyster (Li et al., 2011; Zhang et al., 2018). On the other hand, hybridization is frequently used as an important method of genetic improvement to combine desirable traits or eliminate undesirable trait in aquaculture (Cruz and Ibarra, 1997; Šimková et al., 2022). This method is also used to establish a composite base population with abundant genetic variation (Grant and Grant, 1994; Bartley et al., 2001), allowing the creation of a synthetic strain with superior traits by selective breeding (Bosworth et al., 2003; Wang and Côté, 2012; In et al., 2017). In aquaculture, hybridization is often used in combination with selection to produce superior strains. For example, a tilapia strain with increased growth and
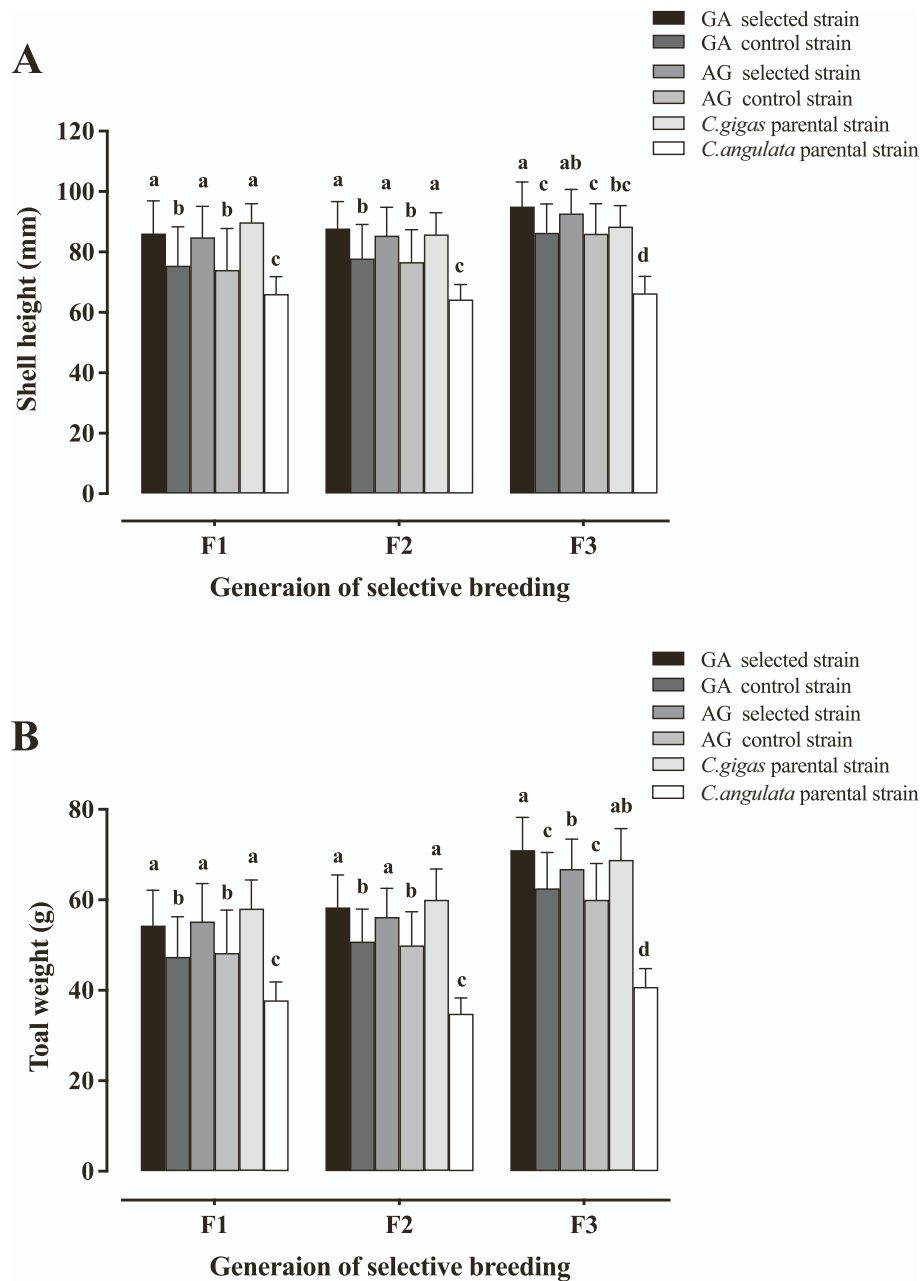
Fig. 3. Shell height and total weight of the GA selected strain, GA control strain, AG selected strain, AG control strain, *C. gigas* parental strain and *C. angulata* parental strain for all three generations. The differences were not statistically significant ($P > 0.05$) when the same superscript letters were shared for shell height and total weight in the same generation.

salinity tolerance were produced by selection from the hybrid between *Oreochromis mossambicus* and *O. niloticus* (de Verdal et al., 2014). The scallop strain of 'Bohai Red', obtained by selection from the hybrid between *Argopecten irradians irradians* and *A. purpuratus*, is characterized by fast growth and thermal tolerance (Wang et al., 2017).

The Fujian oyster *C. angulata* is a closely-related species of *C. gigas*, widely distributed in southern seas of China, with populations concentrated in Fujian province. Compared to *C. gigas*, *C. angulata* is well adapted to subtropical warm seawater but does not grow fast (Huvet et al., 2002). Previous studies have shown that the hybrids between *C. gigas* and *C. angulata* is improved for growth, survival (especially in summer), thermal tolerance, and have higher yields compared to the parental species (Tan et al., 2020; Jiang et al., 2021; Jiang et al., 2022). However, this strategy has significant drawbacks, such as the necessity to maintain and control pure parental populations and the need to

conduct artificial selection on both parents if hybrid phenotype must be improved, which is complicated and laborious (de Verdal et al., 2014; Argue et al., 2014). One possible alternative to eliminate these drawbacks is to carry out founder hybridization and subsequently select these hybrids on target traits to breed a superior intercross strain (Behrends et al., 1990; Bosworth et al., 2003).

Adopting rapid growth and thermal tolerance as the target traits, we performed a mass selection through three successive generations with same selection intensity to obtain two new oyster strains from the reciprocal hybrids of *C. gigas* and *C. angulata*. The aim of the present study was to estimate whether the genetic improvement in growth, survival and thermal tolerance could be achieved by the mass selection program.

Table 3
Production increase (*PI*), variation coefficients (CV), standardized response to selection (SR), current genetic gains (GG) and realized heritability (h_R^2) of shell height (SH) and total weight (TW) in different selective breeding strains.

Items	SH						TW					
	GA selected strain			AG selected strain			GA selected strain			AG selected strain		
	GAF1	GAF2	GAF3	AGF1	AGF2	AGF3	GAF1	GAF2	GAF3	AGF1	AGF2	AGF3
$PI_{(S/C)}$ (%)	14.12	12.74	10.13	14.6	11.36	7.77	14.65	14.85	13.45	14.5	12.65	11.33
$PI_{(S/G)}$ (%)	− 4.11	2.27	7.57	− 5.54	− 0.47	4.9	− 6.32	− 2.76	3.19	− 4.77	− 6.24	−2.86
$PI_{(S/A)}$ (%)	30.35	36.57	43.28	28.41	32.9	39.74	43.84	67.47	74.12	46.22	61.49	63.9
CV_s (%)	12.50	10.15	8.52	12.13	11.05	8.65	14.39	12.37	10.24	15.18	11.28	9.89
CV_c (%)	17.05	14.52	11.11	18.56	13.91	11.54	18.77	14.15	12.64	19.76	15.00	13.36
SR	0.83	0.88	0.91	0.79	0.82	0.67	0.78	1.05	1.06	0.73	0.84	0.85
GG (%)	14.12	12.73	10.13	14.60	11.36	7.77	14.65	14.85	13.44	14.50	12.65	11.33
h_R^2	0.53	0.56	0.57	0.51	0.50	0.39	0.67	0.63	0.57	0.58	0.59	0.41

$PI_{(S/C)}$, $PI_{(S/G)}$ and $PI_{(S/A)}$ indicate the production increase of the GA or AG selected strain relative to the corresponding control strain, *C. gigas* parental strain and *C. angulata* parental strain in the same generation of selective breeding, respectively. CV_s and CV_c indicate the variation coefficients of the selected strains and the corresponding control strain.

2. Materials and methods

2.1. Establishment of the base populations

The production of the reciprocal crosses of the *C. gigas* and *C. angulata* has been previously described by Jiang et al. (2021). In May 2019, 500 individuals of *C. angulata* were collected from a cultured stock in Zhangzhou, Fujian Province (24°28′N, 118°16′E), while 500 individuals of *C. gigas* were collected from a selected line of *C. gigas* 'Haida No. 1' in Rongcheng, Shandong Province (37°11′N, 122°35′ E). All broodstocks were conditioned to sexually mature at a hatchery in Laizhou, Shandong Province (37°31′N, 119°90′ E). In June 2019, the eggs from 40 *C. gigas* or *C. angulata* were pooled together and fertilized with pooled sperm from 40 *C. angulata* or *C. gigas* to produce reciprocal hybrids *C. gigas* ♀ × *C. angulata* ♂ (GA) and *C. angulata* ♀ × *C. gigas* ♂ (AG). In May 2020, approximately 50,000 adult oysters were obtained from each of the two hybrid populations.

2.2. Mass selection

In May 2020, approximately 3000 oysters of GA and AG were sourced from Rongcheng and selected as the base population to breed the first-generation GA selected strain (GAF1) and AG selected strain (AGF1) (Fig. 1). A sub-sample of each stock containing 200 oysters was collected to produce the non-selected first-generation GA control strain (GAFC1) and AG control strain (AGFC1). A total of 600 healthy oysters in GA and AG were respectively selected from the top end of shell-height distribution of each stock with a 20% selected rate. Subsequently, size-selected individuals of GA and AG were heat-shocked at 34 °C for approximately 96 h to induce 25–30% initial mortality. The survivors were transferred to an outdoor shallow pond (25–30 °C) for a recovery phase of 7 to 15 days, then moved to an indoor tank (21–23 °C) for conditioning. Delayed mortality occurred during the recovery and conditioning phases. The duration of each challenge phase was up to the point where the cumulative mortality of the size-selected hybrid stocks reached about 50% (approximately 300 individuals remaining in both GA and AG). In Jun 2020, all survivors from GA stock were opened to determine the sex using a microscope, and the preferred females (with ovoid or pear-shaped eggs) and males (with >95% active sperm) were used as parents for mass spawn to produce GAF1. Meanwhile, AGF1 was produced using survivors derived from the AG stock in the same process. An equal number of oysters were randomly selected from the sub-samples as parents to produce the control strains GAFC1 and AGFC1. In addition, two first-generation *C. gigas* parental strain (GGF1) and *C. angulata* parental strain (AAF1) were established as commercial controls using the equal number of parents. The second-generation selected strains (GAF2 and AGF2) and the third-generation selected

strains (GAF3 and AGF3) were established in June 2021 and March 2022, respectively, using the same approach as the selection of the previous generation, with parents from the first-generation selected strains (GAF1 and AGF1) and the second-generation selected strains (GAF2 and AGF2), respectively (Fig. 1). Details of selected parents and selection intensity for each generation are given in Table 1. In the same way, the second-and third-generation control strains and parental strains were established.

2.3. Hatchery, nursery and grow-out

The resulting larvae and spats were raised according to the conventional culture procedure as detailed by Li et al. (2011). After hatching, the D-stage larvae were reared in 30-m^3 cement tank with filtered seawater at 21–23 °C, salinity of 29–30 psu, constant aeration and daily seawater renewal of 30%. The density of larvae for each strain was maintained at 5 larvae/ml and decreased with age. The larvae were fed three times a day with a mixture of *Isochrysis galbana* and *Platymonas* sp. at an estimated concentration of 100,000 cells mL^{-1}. Scallop shells were put into the tanks for eyed larvae to set on. The spat were temporarily reared in an outdoor pond for 10 days and then transferred to Rongcheng for cultivation using suspended-longline method at a density of 15 to 25 individuals per shell. After two weeks, the spat of each strain were artificially detached from scallop shells and cultured in three replicate 10-layer cages, with 100 spat per cage 10 individuals in each layer to avoid the potential effects of overcrowding.

2.4. Field testing

Performance was monitored from July 2020 to June 2021 for F1, from July 2021 to June 2022 for F2 and from April 2022 to February 2023 for F3. For each selected generation, shell height data were collected at the sampling endpoint on 30 individuals taken at random from each strain using an electronic vernier caliper (0.01 mm). The total weight (TW) of 30 randomly picked oysters from each strain was separately weighed using an electronic balance (0.01 g) at the sampling endpoint. At the end of the summer period in October, the number of live oysters and oysters of all strains were counted to determine the incremental survival rate (IS) in summer according to the following formula:

$$IS\ (\%) = \frac{N_s}{N_0} \times 100$$

where N_s = number of live spat per cage at the end of the summer period in October; N_0 = total number of oysters per cage.

At the sampling endpoint, dead and live oysters were counted for cumulative survival rate (CS) assessment according to the following
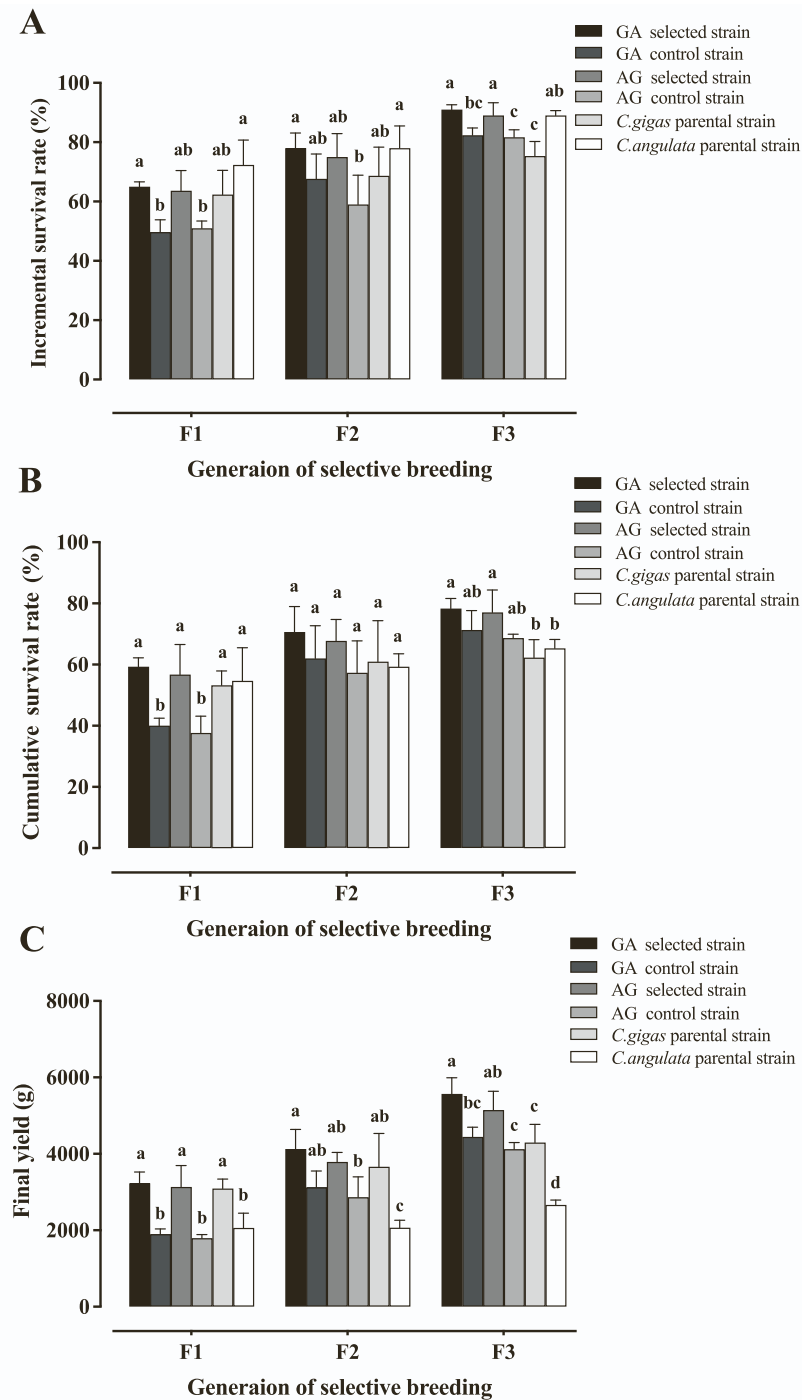
Fig. 4. Incremental survival rate, Cumulative survival rate and final yield of the GA selected strain, GA control strain, AG selected strain, AG control strain, *C. gigas* parental strain and *C. angulata* parental strain for all three generations. The differences were not statistically significant ($P > 0.05$) when the same superscript letters were shared for survival rate in the same generation.

formula:

$$CS\ (\%) = \frac{N_t}{N_0} \times 100$$

where N_t = number of live spat per cage at the sampling endpoint; N_0 = total number of oysters per cage.

The final yield from each cage was defined as follows (Rawson and Feindel, 2012):

$$Y = CS_{Final} \times N_{Initial} \times TW_{Final}$$

where CS_{Final} = cumulative survival rate of oyster at the sampling endpoint; $N_{Initial}$ = initial number of spats for each cage; TW_{Final} = average total weight of each strain of oyster at the sampling endpoint.

2.5. Thermal tolerance

The thermal tolerance was assessed as previously described with a slight alteration (Camara et al., 2017). For each generation, about 700 oysters (11 months old) of each strain were reared in an indoor tank (30 m^3) with filtered, aerated seawater (salinity 30 psu, temperature 20 °C)

Table 4
Production increase (*PI*) of incremental survival rate in summer (IS), cumulative survival rate (CS) and final yield (Y) in different selective breeding strains.

Traits	Items	GA selected strain			AG selected strain		
		GAF1	GAF2	GAF3	AGF1	AGF2	AGF3
IS	$PI_{(S/C)}$ (%)	30.87	15.27	10.53	24.84	27.12	8.98
	$PI_{(S/G)}$ (%)	4.28	13.59	20.80	2.14	9.22	18.14
	$PI_{(S/A)}$ (%)	− 10.14	0.00	2.25	− 11.98	− 3.85	0.00
CS	$PI_{(S/C)}$ (%)	48.33	13.98	9.81	50.44	18.02	12.14
	$PI_{(S/G)}$ (%)	11.25	15.85	25.67	6.25	10.93	23.53
	$PI_{(S/A)}$ (%)	8.54	19.1	19.90	3.66	14.04	17.86
Y	$PI_{(S/C)}$ (%)	70.38	31.96	25.27	74.69	32.26	24.76
	$PI_{(S/G)}$ (%)	4.56	12.57	29.63	1.40	3.38	19.78
	$PI_{(S/A)}$ (%)	56.77	99.37	109.09	52.04	83.10	93.20

for 7 days before the experiment. Oysters were fed with fresh *Phaeodactylum tricornutum* Bohlin three times per day. The heat shock experiment consisted of with 42 treatments using a factor design (7 temperature × 6 strains) with 3 replicates. One polyethylene barrels (300*L*) was used for each temperature and each barrel was divided into 6 equal trial blocks by polyethylene mesh. 30 individuals of each strain were placed in a trial block. The temperature was increased at a rate of 0.5 °C h^{-1} from the control temperature (20 °C), until the target temperatures were reached (41.0, 41.5, 42.0, 42.5, 43.0 and 43.5 °C). The temperature of each barrel was maintained using a water bath unit with heaters or water chiller. No food was provided during experiment. Oysters were kept at the target temperature for 2 h and then transferred to tanks (20 °C) to recover for 96 h. The surviving oysters were fed normally and 30% of the seawater was exchanged daily. Mortalities of the six strains were recorded at one-hour intervals. Dead oysters were identified when they did not respond to gentle touch with a pipette tip and were removed immediately. No death was observed at control temperature (20 °C).

2.6. Environmental parameters

Seawater parameters of the culture sites were downloaded from National Marine Data Center, National Science & Technology Resource Sharing Service Platform of China (http://mds.nmdis.org.cn/). Due to the little variation in seawater temperature and salinity over the same period from 2020 to 2023, Fig. 2 only displayed the average monthly temperature and salinity of the breeding site over the three years. The site showed a clear seasonal pattern throughout the year, with water temperatures varying from 4.1 to 23.2 °C. The average water

temperature in August, September and October exceeded 19 °C.

2.7. Statistical analyses

All statistical analyses of the phenotypic data were carried out using the SPSS 26.0 software. Arcsine transformation for survival rate and logarithmic transformation for growth, total weight and final yield was performed to improve the normality and homoscedasticity of raw data. To compare the differences in thermal tolerance among the six strains over three generations, the median LT_{50} value was calculated using Probit analysis (Finney, 1971). In each generation, the statistical significance of any differences in strain phenotype was evaluated with one-way analysis of variance (ANOVA) followed by multiple comparison Tukey test. To determine the effects of the strain origin (SO) and selective generation (SG) on the phenotypic parameters, a two-way analysis of variance was used as follows (Zhang et al., 2017):

$$Y_{ijk} = \mu + SO_i + SG_j + (SO \times SG)_{ij} + e_{ijk}$$

where Y_{ijk} = phenotypic parameters of the k replicate from the i strain origin (GA and AG) and the j selective generation (F1, F2 and F3); μ = overall mean; SO_i = strain origin effect; SG_j = selective generation; $(SO \times SG)_{ij}$ = interaction effect between strain origin and selective effect; e_{ijk} = residual error. Differences were declared significant at $P < 0.05$.

The selection intensity (*i*) was estimated as the average phenotypic difference between the selected brooders and the base population divided by the standard deviation of base population (Falconer and Mackay, 1996). The realized heritability (h_R^2) (Falconer and Mackay, 1996), selection response (*SR*) (Zheng et al., 2006) and genetic gain (*GG*) (Zheng et al., 2006) for each successive generation were calculated as follows:

$$h_R^2 = \frac{X_S - X_C}{i\sigma_C}$$

$$SR = \frac{X_S - X_C}{\sigma_C}$$

$$GG = \frac{X_S - X_C}{X_C} \times 100$$

To estimate the increase in production traits of the selected strains compared with that of the control strains and parental strains, the production increase (*PI*) (Wang et al., 2011) was calculated as follows:
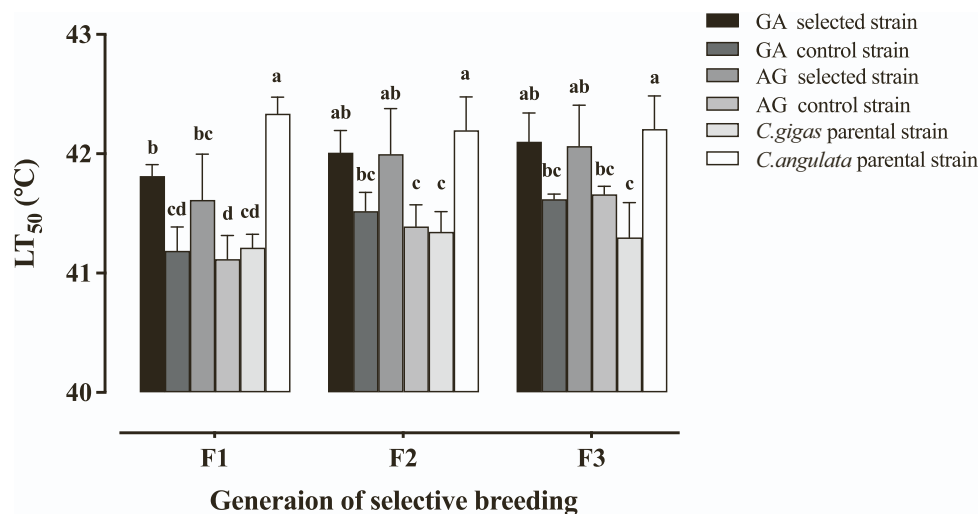

Fig. 5. LT_{50} of the GA selected strain, GA control strain, AG selected strain, AG control strain, *C. gigas* parental strain and *C. angulata* parental strain for all three generations. The differences were not statistically significant ($P > 0.05$) when the same superscript letters were shared for LT_{50} in the same generation.

$$PI = \frac{X_S - X_i}{X_i} \times 100$$

where X_S = average phenotypic value (shell height and total weight) of selected strains; X_C = average phenotypic value of control strains; σ_C = standard deviation of control strains; Xi = average phenotypic value of control strains or parental strains.

3. Results

3.1. Growth

Two-way ANOVA showed a significant effect of strain origin ($P <$ 0.001) and selection generation ($P <$ 0.001) on shell height and total weight (Table 2). At the sampling endpoint, for all three breeding generations, the shell height and total weight of the selected strains were significantly ($P <$ 0.05) greater than that of the control strains (Fig. 3). The GA selected strains performed somewhat better overall than AG selected strain for all three generations, with significant differences ($P <$ 0.05) in total weight in the F3 generation. Shell height and total weight of the two selected strains were similar to those of the *C. gigas* parental strains and much better than those of the *C. angulata* parental strains. In shell height, the production increase value $PI_{(S/A)}$ of GA and AG selected strains were 30.35–43.28% and 28.41–39.74%. In total weight, the $PI_{(S/A)}$ of GA and AG strains were 43.84–74.12% and 46.22–63.90% (Table 3). A reduction of the coefficient of variation (CV) of shell height and total weight was observed with increasing generation number in the GA (SH: 8.52–12.50%; TW: 8.65–12.13%) and AG (SH: 10.24–14.39%; TW: 9.89–15.18%) selected strains.

The standardized response to selection (SR) of shell height ranged from 0.83 to 0.91 for the GA selected strain, while ranged from 0.67 to 0.82 for the AG selected strain (Table 3). The SR of total weight ranged from 0.78 to 1.06 for the GA selected strain, while ranged from 0.73 to 0.85 for the AG selected strain. For all three generations, the genetic gains of shell height and total weight ranged from 7.77% to 14.85% for the two selected strains. Moderate levels of realized heritability (h_R^2) were observed in the GA selected stains (shell height: 0.53–0.57; total weight: 0.39–0.51) and AG selected strains (shell height: 0.57–0.67; total weight: 0.41–0.59).

3.2. Survival

Two-way ANOVA indicated that there were significant effects of strain origin ($P <$ 0.001) and selection generation ($P <$ 0.001) on incremental survival rate in summer, but the effect of the strain origin × selection generation interaction on survival was not significant ($P >$ 0.05; Table 2). Throughout the three generations, the majority of the mortality occurred during the summer period, between July and October each year (Fig. 4A), with an average temperature exceeding 23 °C (Fig. 2). The summer survival rate of the GA and AG control strains were 49.67–82.33% and 51.00–81.67%, respectively. In general, summer survival of the GA (65.00–91.00%) and AG (63.67–89.00%) selected strain exceeded those of the control strains at all breeding generations. The production increase values $PI_{(S/G)}$ of GA selected stain (4.28–20.80%) and AG selected stain (2.14–18.17%) increased with continued selection (Table 4).

The selection generation ($P <$ 0.001) was the major contributing factor in cumulative survival rate, while strain origin ($P <$ 0.01) had secondary influence (Table 2). At the end of the three testing periods, the cumulative survival rate was consistently higher in the selected strains than in the control strains and parental strains (Fig. 4B). As selection continued, the value of PI increased, reaching a bottleneck on the F3 generation for GA ($PI_{(S/G)}$: 25.67%; $PI_{(S/A)}$: 19.90%) and AG ($PI_{(S/G)}$: 23.53%; $PI_{(S/A)}$: 17.86%) selected strains (Table 4).

3.3. Yield

There was no evidence of an interaction between strain origin and selection generation for final yield, suggesting that the relative performance of each strain was consistent across generations (Table 2). Both the strain origin and selection generation main effects on total yield were significant ($P <$ 0.001). Yield was highest for the GA (3230.27–5566.37 g) and AG (3132.72–5143.23 g) selected strains at all breeding generations (Fig. 4C). Despite no significant differences ($P >$ 0.05) between the two selected strains, the GA selected strain overall performed somewhat better than the AG selected strain in final yield. By the F3 generation, the both selected strains were significantly ($P <$ 0.05) higher than their corresponding control strain and the two parental strains. Notably, the production increased value of the selected lines ranged from 24.76% to 74.69% for $PI_{(S/C)}$, 1.40% to 29.63% for $PI_{(S/G)}$ and 52.04% to 109.09% for $PI_{(S/A)}$ (Table 4).

3.4. Thermal tolerance

Two-way ANOVA showed that the LT_{50} was strongly affected by strain origin ($P <$ 0.001) and selection generation ($P <$ 0.01; Table 2). After exposure to heat shock with 96 h recovery, the *C. angulata* parental strain exhibited the greatest thermal tolerance among all strains with an average LT_{50} of 42.25 °C (Fig. 5). In both selected strains, thermal tolerance increased with continued selection, with LT_{50} values ranging from 41.81 °C to 42.10 °C in the GA selected strain and from 41.61 °C to 42.06 °C in the AG selected strain. By the F3 generation, thermal tolerance in terms of LT_{50} was ranked in the following order: AAF3 > GAF3 > AGF3 > AGFC3 > GAFC3 > GGF3.

4. Discussion

Mass selection has been by far the most common method of selective breeding in aquaculture because of its simplicity (Doyle, 2002; Donato et al., 2005). Although its results were not consistently successful, most responses in shellfish, especially oysters, were positive and encouraging. In this study, we proved that mass selection was effective for improving production traits and thermal tolerance, which contributed to higher final oyster yields.

Successive selection of top-performing hybrids has been proposed as a means of combining genomes and selecting for best traits from initial parents (Lasley, 1987; Argue et al., 2014), potentially providing a practical source of improved strains for the breeding industry. On the one hand, genomic recombination may generate individual genotypes that do better or worse than the average mid-parent value, leading to the emergence of extreme (negative or positive) phenotypes, which is known as transgressive segregation (Rieseberg et al., 1999; Albertson and Kocher, 2005). These extreme phenotypes might facilitate new adaptations that possibly enable hybrid progenies to form ecological niches distinct from their original parents, making them stable introgressants or hybrid species (Burke and Arnold, 2001; Seehausen, 2004). On the other hand, there is a large amount of phenotypic difference and genetic variation in hybrid offspring which could be exploited in a selection program to produce strains with desirable traits (Toro and Newkirk, 1991; Argue et al., 2014). In this study, the F1 generation of strains showed high coefficients of variation for growth traits, which provided abundant variation for selection and could be used to select fast-growing oysters.

The shell height and wet weight of two selected strains were clearly improved after three consecutive generations of selection in this study. This change in population mean caused by selection is achieved by changes in the gene frequencies at loci affecting the selected trait (Falconer and Mackay, 1996), as successive directed selection increases the gene frequency of the target trait in the population. However, more generations of selection would need to be implemented for certain trait to be genetically comparable to F1 hybrid (Dunham et al., 2008). This is

because hybrid F2 and hybrid F3 often exhibit lower performance due to the presence of hybrid breakdown (also described as outbreeding depression) (Qin et al., 2021). As shown in this study, the F1 and F2 generations of the selected strains grew slightly lower than the *C. gigas* parental strain, despite continued selection being performed. Several studies have also reported promising results in mass selection based on hybrid populations. For example, in a study on a backcross population between *A. irradians irradians* and *A. purpuratus*, mass selection through four successive generations led to a 43.6% increase in whole body weight (Xu et al., 2019b). In another study on the hybrid of these two scallops, after 4 generations of mass selection, the adductor muscle weight and orange adductor muscle ratio of the selected strain increased by 80.8% and 94.9%, respectively (Chen et al., 2020). These results suggest that mass selection has great potential for application in shellfish breeding. In addition, we found that the coefficient of variation for shell height and total weight of both selected strains decreased with continued selection. This indicated that the degree of variation in growth traits within populations decreased with increasing generations, tending to genetic stability after continuous selection (Sun et al., 2022). It should be mentioned, however, that the reduction of the coefficient of variation of the population was also likely associated with a joint effect of inbreeding and genetic drift (Donato et al., 2005).

In the three selective generations, the genetic gains for shell height and total weight of 7.77–14.85% have been observed for GA and AG selected strains. This level of improvement is comparable to that reported for other aquatic animals. For example, a genetic gain of 7.5–12.1% for weight was obtained after three generations of mass selection in a hybrid population produced from three genetically differentiated silver barb (Hussain et al., 2002). In this study, mass selection based on hybrid oysters yielded a larger selection response (0.73–1.06) in comparison to the responses recorded in other aquatic animals, e.g., 0.69–0.83 in the third generation of Manila clam (Liang et al., 2019) and 0.27–0.68 in one generation of Pacific oyster (Li et al., 2011). In addition, the realized heritability (h_R^2) of the three breeding generations of the two selected strains ranged from 0.53 to 0.67 for shell height and 0.39 to 0.59 for total weight, indicating moderate to high heritability. This suggested that, after three generations of selection, the hybrid oyster population changed rapidly and showed considerable genetic improvement. In sockeye salmon, h_R^2 for body weights has been estimated as 0.36–0.62 (Crozier et al., 2011); in bay scallop, the realized heritability of shell length was between 0.43 and 0.45 (Wang et al., 2020); in small abalone, the h_R^2 value for shell length was estimated as 0.44 ± 0.06 (You et al., 2010); in Pacific oyster, the h_R^2 values for shell height were found to be 0.275–0.420 (Zhang et al., 2019). The relatively high realized heritability indicated that abundant additive genetic variation existed in both populations (Chatchaiphan et al., 2019), and thus further mass selection should be practiced to achieve genetic progress (Toro and Newkirk, 1991).

For industrial oyster farming, survival to harvest is paramount. However, *C. gigas* industry has frequently suffered from massive mortality episodes in northern China (Mao et al., 2005; Lian et al., 2010; Bai et al., 2021). In this study, 24.67–37.67% mortality rates were observed in *C. gigas* parental strain during the summer months. Temperatures above 19 °C are considered to be directly or indirectly associated with oyster mortalities (Soletchnik et al., 2007; Malham et al., 2009). The water temperatures (19.5–23.2 °C) of the breeding site exceeded this threshold from July to October, indicating a potential risk of heat stress. In marine bivalves, heat shock induces oxidative stress and impairs immune defenses (Chen et al., 2007), which can lead to metabolic exhaustion and susceptibility to pathogenic infections (Li et al., 2007). In this study, mass selection targeting thermotolerance was conducted on the hybrids of *C. gigas* and *C. angulata* with different thermal adaptations (Jiang et al., 2023). After three-generation selection, the LT_{50} values of GA (42.10 °C) and AG (42.06 °C) selected strains were clearly higher than those of *C. gigas*. Thus, it appears that genetic selection has enhanced the capacity of these oyster strains to cope with high temperatures. The resistance of *C. gigas* to high temperatures has been the focus of numerous studies. On the west coast of USA, a selective breeding program based on improving the thermal tolerance of *C. gigas* suggested that the survival of all selected families in the third generation (81%) was significantly higher than the control (38%) (Hershberger et al., 1984). A breeding program in China observed a 11.86% increase in survival of heat-tolerant lines of *C. gigas* compared to controls during a summer mortality episode (Ding et al., 2020). These results were consistent with our field experiment, indicating a corresponding increase in summer survival of the heat-tolerant strains compared with controls. Therefore, selection for heat tolerance may be used as an indirect means of improving summer survival in oysters.

There were differences in the responses to selection between the two groups, despite the adoption of similar selection intensities. Among all three breeding generations, the GA selected strain was overall superior to the AG selected strain in terms of growth, survival and thermal tolerance. This asymmetric response to selection can be attributed to differences in genetic variance between the two base populations (You et al., 2010; Zhang et al., 2017), that is, the base population of GA selected strain may have a higher level of genetic variation than the base population of AG selected strain. In general, populations with more abundant additive genetic variation for the desired trait have a greater selection response. Another factor may be the existence of a maternal effect, which is regarded as a source of environmental differences (Toro and Newkirk, 1991). Nevertheless, maternal effects usually appear early and its effects usually diminish with ageing of progeny (Falconer and Mackay, 1996). In addition, the potential impacts of random genetic drift, inbreeding depression, major gene effects, scalar asymmetry, G × E interactions, natural selection and stocking density on selection responses should be considered (Falconer and Mackay, 1996; Evans and Langdon, 2006; Van et al., 2014; Chatchaiphan et al., 2019).

5. Conclusion

This study conducted a three-generation mass selection on the reciprocal hybrids between two closely related oyster species (the fast-growing *C. gigas* and the warm-adapted *C. angulata*) for improving growth and thermal tolerance. The current results are satisfactory because the average estimated increase of growth is approximately 12.68% per generation. Selection based on heat stress also improved the thermal tolerance of the two strains, with corresponding increases in summer survival of 18.14% and 20.80%. The total yield of the two strains increased by 29.63% and 19.78% respectively compared to *C. gigas* strain. Overall, the GA selected strain outperformed the AG selected strain in terms of yield traits. The establishment of superior intercross strains will free hatcheries from the need to maintain, improve and synchronize reproduction of two parental species for production of F_1 hybrids. This study provides germplasm resources for the sustainable development of the oyster industry and can be used as a reference for shellfish breeding strategies.

CRediT authorship contribution statement

Gaowei Jiang: Investigation, Conceptualization, Formal analysis, Data curation, Writing – original draft. **Yong Chi:** Methodology. **Jianmin Zhou:** Methodology. **Geng Cheng:** Methodology. **Lijie Du:** Data curation. **Chengxun Xu:** Supervision, Resources. **Qi Li:** Supervision, Conceptualization, Resources, Writing – review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest.

Data availability

Data will be made available on request.

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