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Comparative analysis on growth performance, survival and harvest traits between offspring from wild and mass-selected populations of *Crassostrea nippona*

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ABSTRACT

Crassostrea nippona has recently been recognized as a preferred alternative to *C. gigas* in summer due to its firm flesh and high glycogen content. To promote industrial development, the mass selective breeding program was initiated to improve growth performance of *C. nippona* in 2014. Although a steady genetic gain of approximately 10% for growth traits was achieved in each of the first three generations of the selected strains, little is known about the cumulative genetic progress in desired traits contributed by the selective breeding in the genetic improvement program. In this study, the aquaculture performance of the selected population was comprehensively evaluated by comparing the production performance and reproductive traits of the progeny (WF1 and SF4) from the wild population and the third-generation selected line in commercial farming environments. The results showed that the diameters of eggs from both populations were at similar levels, but the fertilization and hatching rates of the progeny from the selected lines were significantly higher than those of the wild population ($P < 0.05$). At larval stages, the shell height and cumulative survival rate (CSR) of individuals from SF4 were consistently superior to that of WF1, especially on day 6 post-fertilization, in which CSR of the improved *C. nippona* was increased by 25.08% compared to that of wild oysters. Meanwhile, a significant 12.10% enhancement in the metamorphosis rate was observed in SF4 when compared with WF1. During the grow-out stage, SF4 displayed distinct advantages in growth performance and survival compared to the wild strain, with 23.19, 33.48, 34.18 and 79.11% improvement in shell height, body weight, CSR and final yield at harvest on day 800, respectively. Meanwhile, relative brood amount (RBA) associated with initial development of offspring was found to be significantly higher in SF4 compared with WF1 ($P < 0.05$). Remarkably, the gonadal development, sex ratios and biochemical composition of oysters from both populations were at similar levels at final harvest, implying that high-intensity artificial selection had no significant effect on reproductive characteristics and nutritional quality of *C. nippona*. The encouraging results in the present study suggest that the overall genetic progress contributed by genetic breeding is considerable in the fast-growing strain of *C. nippona*, and such gains are expected to continue to increase with further selective breeding.

1. Introduction

Aquaculture, as the fastest-growing sector in global food production, contributes almost half of total worldwide fisheries production in 2020 (FAO, 2022), demonstrating an increasingly critical role in food security and economic stability. Although domestication and genetic improvement of aquatic animals remain at a formative stage relative to terrestrial animals, aquatic species reared in short-term captivity carrying critical features, such as abundant genetic diversity, external fertilization and high fecundity, demonstrate strong potential for achieving high genetic gains in economically important traits via selective breeding schemes (Houston et al., 2020). Meanwhile, the improvement of artificial breeding techniques coupled with the ease of transportation of eggs and juveniles have permitted the rapid and widespread dissemination of improved stocks to producers, thereby immediately generating a significant positive impact on aquaculture production (Gratacap et al., 2019).

Pacific oyster (*Crassostrea gigas*), naturally distributed along the

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coast of northwest Asia, is one of the most important commercial shellfish species worldwide and has been introduced with permanent establishment in at least 27 countries due to its fast growth performance and strong environmental adaptability (Krassoi et al., 2008; Martínez-García et al., 2022). In recent years, various breeding methods such as mass selection, ploidy breeding, crossbreeding and genomic selection have been applied alone or in combination to improve the growth rate, shell shape and disease resistance to address the demands of the rapidly expanding oyster industry (Li et al., 2011; Dégremont et al., 2015; Gutierrez et al., 2018; He et al., 2022; Qin et al., 2023). Among them, the application of polyploid manipulation on *C. gigas*, which expands the summer marketing season by inhibiting individuals spawning, has become the main driver for the continuous expansion of the commercial farming scale (Nell, 2002; Matt and Allen Jr, 2014). However, the theoretically sterile triploid *C. gigas* has most recently been frequently reported to exhibit high levels of gametogenesis in farming populations (Hermabessiere et al., 2016; Houssin et al., 2019; Yang et al., 2022). In addition, the prevalence of chromosomal instability in tetraploid oysters poses a serious challenge for breeders to continuously maintain the progeny of polyploid populations (Guo and Allen, 1994). Therefore, cultivating an oyster species suitable for sale in the summer may provide another promising solution to accommodate the rising consumer demand for oysters.

Iwagaki oyster (*C. nippona*), which has a restricted distribution range along the coastal areas of East Asia, is frequently utilized as a preferred alternative to *C. gigas* with low meat quality in summer due to its peak spawning activity in early autumn when water temperatures drop (Okumura et al., 2005; Lu et al., 2017; Hu et al., 2023). In Japan, *C. nippona*, which enjoy great popularity since ancient times owing to the firm flesh and high glycogen content in summer, can fetch up to five times the market price of *C. gigas* during the warm season (Itoh et al., 2004; Okumura et al., 2005). To alleviate the contradiction between limited natural resources and increasing market demand, the artificial seed rearing technology of *C. nippona* was successfully established in Oki Island, Shimane Prefecture in 1992 (Eguchi and Chijiwa, 2012), which produced over 1 million oysters in 2017 with total annual sales of 2.1 million USD (Sasaki, 2022). The great economic potential and unique biological characteristics of *C. nippona* has attracted widespread interest among farmers in initiating the large-scale commercial culture. However, massive mortalities of reared larvae derived from wild or cultured populations as well as the slow growth performance of unimproved individuals in commercial offshore mariculture seriously hinder the rapid development of the oyster industry (Sasaki et al., 2016; Xu et al., 2019a, 2019b, 2019c). Therefore, the selective breeding program was initiated to improve production performance of *C. nippona* through three successive generations of high-intensity mass selection since 2014, and approximately 10% genetic gains in each generation for shell height were obtained at harvest (Hu et al., 2022a, 2022b). Although the degree of improvement for growth traits observed in the selected line was encouraging, little is known regarding the overall genetic progress in target traits contributed by the selective breeding. In addition, considering the intentional and unintentional effects of artificial selection and domestication on certain life history traits, such as viability and gonadal development, of farmed species under aquaculture conditions (Teletchea and Fontaine, 2014), it is necessary to systematically analyze the production and reproductive performance of the selected strain in actual rearing processes.

One of the traditional methods for the quantification of gains in characters of commercial interest is through the comparison of phenotypic differences in the target trait among different generations or strains (Carlberg et al., 2018), but such analyses are generally complicated by a range of uncontrollable variations in farming parameters, such as environmental conditions and food availability (Moran et al., 2023). In contrast, wild populations growing in natural conditions are considered the appropriate standard of comparison to conveniently estimate the contribution of genetic breeding to desired traits in selective breeding programs (Li et al., 2018). Hence, in this study, growth performance, survival, shell shape, biochemical composition and reproductive traits of progeny from mass-selected and wild populations were compared to quantify the cumulative genetic progress of economically important traits in the genetic improvement program and to comprehensively assess the aquaculture performance of the fast-growing strain.

2. Materials and methods

2.1. Experimental strains and broodstock conditioning

In November 2020, wild *C. nippona* harvested from Geoje Island, South Korea (34.90°N, 128.62°E), were transported to the oyster farming area located in Qingdao, China (35.91°N, 120.16°E), for acclimatization. The fast-growing strain of *C. nippona* originated from the wild population in Niigata Prefecture, Japan (37.92°N, 139.04°E), was cultivated in the same coastal area, which was established by selecting the individual with the largest shell height from the candidate population using the truncation method with a certain selection intensity (Hu et al., 2022a, 2022b). In 2019, the third-generation mass selected line was constructed via selection of the 88 oysters with the best growth performance from the second-generation selected group. In May 2021, the wild population and the third-generation selected line of *C. nippona* were simultaneously transferred to a hatchery located in Yantai for broodstock conditioning, in which oysters were fed a mixed diet of *Nitzschia closterium* and *Spirulina* powder until gonadal maturation.

2.2. Experimental design and offspring cultivation

After determining the sex of each oyster under a light microscope, 20 healthy females and 20 healthy males were randomly selected from both the wild and selected populations for dissection to independently collect gametes. Eggs and sperm from the same population were first filtered individually by 100 μm mesh sieves, and were suspended in separate containers with sand-filtered seawater (temperature: 27 °C; salinity: 30 psu). Then, the eggs of all females in each group were mixed and placed in a single 5-L plastic bucket, and the sperm of all males in each group were placed into another plastic bucket. Following a recheck to confirm that the eggs remained in an unfertilized state, the offspring of the wild population and third-generation selected line were separately established by artificial insemination using the corresponding gametes from each population as described above (20♂ × 20♀), which were labeled as WF1 and SF4, respectively. Afterwards, a small subset of fertilized eggs was utilized to count fertilization and hatching rates, while the remaining portion were suspended in a 20 m^3 hatching tank with a density adjusted to 30 eggs/mL for subsequent experiments. The above operations were repeated three times in different tanks with different oysters from each population.

The aquaculture procedures for *C. nippona* larvae and spat are conducted primarily with reference to the methods described in Li et al. (2011). Briefly, 24 h post-fertilization D-larvae were collected with a 50 μm mesh sieves and then placed in a new rearing tank at a density of 5 larvae/mL. Sand-filtered seawater was used to perform 30% water changes twice a day, while the amount of mixed algal diet of *Isochrysis galbana* and *Chaetoceros calcitrans* fed three times daily increased as the larvae grew. The larvae were not subjected to any artificial screening or grading throughout the farming period. When 40% of individuals reach the stage of eyed larvae, strings of scallop shells were hung in the tanks for the larvae to complete attachment and metamorphosis. Afterwards, the spat that reach a size of 2–4 mm was transferred to outdoor ponds for 2 weeks of temporary cultivation to improve their environmental adaptability. Finally, the oyster from each group was placed individually in the 10-layer lantern net at similar densities and transferred to Rushan for farming, during which dead individuals and attached organisms were regularly removed and the culturing densities of each group were readjusted to similar levels (about 4 months apart), and three biological replicates were set up in the wild (including 241, 259 and 286 individuals) and selected groups (including 235, 248 and 302 individuals) to count the survival performance of *C. nippona* during the grow-out stage. All spat were retained on scallop shells and allowed to grow naturally to reduce the impact of artificial operations on the growth and development of oysters.

2.3. Routinely measurements

The embryo development index (egg diameter, fertilization and hatching rates) and metamorphosis rate for each combination were assayed by performing the methods previously employed by Zhang et al. (2017) and Rico-Villa et al. (2006), respectively. At days 1, 6, 12, 18 and 24 post-fertilization, 30 larvae from each population stored in Lugo reagent were randomly selected for determination of shell height using a light microscope with an ocular micrometer. During the grow-out stage, 30 oysters randomly selected from each population were measured for shell height with an electronic vernier caliper (0.01 mm accuracy) on days 60, 170, 290, 410, 540, 680 and 800, while the body weight (total weight of living oysters) of the oysters were individually measured using electronic balance (0.01 g accuracy) beginning at day 410. Meanwhile, the cumulative survival rate (CSR) of *C. nippona* from each biological replicate of both groups at the above sampling time points were calculated by the following equation:

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

where $\mathrm{CSR_t}$ represents the cumulative survival rate of oysters at time t; N_t and N_0 represent the number of living oysters at time t and at the initiation of the experiment, respectively.

2.4. Measurement of production traits at harvest

After 800 days of culture, the two experimental populations were harvested at the end of August 2023 and the economically important traits of *C. nippona* were measured individually. To perform comparisons of yield-related traits, 30 individuals from each population were dissected after completing the data collection of shell height (SH), shell length (SL), shell width (SW) and body weight (BW), and the wet weight of soft tissues were recorded following careful removal of adhering seawater using soft paper towels. Using these measurements, meat yield (wet meat weight/body weight), fan ratio (SL/SH) and cup ratio (SW/SH) were calculated for each group, respectively. Subsequently, the soft tissues of each oyster were freeze-dried for 48 h before determination of the dry meat weight. Freeze dried tissues were ground into powder individually, then 5 oysters per group were randomly chosen and pooled together for analysis. The pooled powdered tissue was used to determine the glycogen content, total protein content, and total lipid content based on the NIRS calibration model established by Wang et al. (2015).

Final yield (FY) was expressed as the final biomass harvested for each population per 1000 individuals initially cultured, which was calculated according to the following formula described by Proestou et al. (2016):

$$\mathrm{FY}\ (\mathrm{g}) = CSR_{Final} \times BW_{Final} \times 1000$$

2.5. Measurement of reproductive traits at harvest

To compare differences in reproductive traits, an additional 30 individuals were randomly chosen from each of the two populations at harvest on day 800. A small piece of gonad tissue from each oyster was separately fixed in Bouin's fluid for 24 h and then washed with 70% alcohol for histological analysis. Oyster sex and stage of gonadal development were determined by observing tissue sections under an optical microscope according to Normand et al. (2008). Afterwards, the wet weight of the soft body as well as the number and diameter of eggs carried by 10 random females in each population were recorded successively to calculate the relative brood amount (RBA) as follows (Wu et al., 2023):

$$\mathrm{RBA}\ (\mathrm{million/g}) = \frac{\text{Egg number}}{\text{Wet meat weight}}$$

2.6. Statistical analyses

In this study, all data analysis was performed using the IBM SPSS Statistics 25.0 software, and the analyzed results were presented as in the format of mean ± standard deviation (SD). To examine the effects of artificial selection on production traits of *C. nippona*, phenotypic differences between groups at the same age were analyzed using *t*-test, which was widely applied in comparative studies of production performance for bivalves (Lebata-Ramos et al., 2021; Wang et al., 2022), and the significance level was set at $P < 0.05$. When a significant difference was observed, the relative gain (RG) of the mass-selected line in the corresponding trait compared to the wild population was calculated using the following equation (Walton et al., 2013):

$$\mathrm{RG}\ (\%) = \frac{M_S - M_W}{M_W} \times 100$$

where M_S and M_W represent the mean phenotypic value of the mass-selected and wild populations on the same trait, respectively.

3. Results

3.1. Hatching index and larvae development

No significant difference was observed in the diameter of eggs spawned by the third-generation selected line and the wild population ($P > 0.05$), while both fertilization and hatching rates in SF4 were considerably higher than those in WF1 ($P < 0.05$), with relative gains of 6.38 and 18.89%, respectively (Table 1).

During the larval rearing period, significant differences in the cumulative survival rate between the two experimental groups were detected at all sampling dates ($P < 0.05$), and larval CSR of SF4 was improved by 25.08, 30.94, 47.09 and 52.07% on days 6, 12, 18 and 24, respectively, compared to WF1 (Table 1). Notably, at the early larval stage, individuals from WF1 suffered a severe mortality event (43.33%) on day 6, after which the number of surviving larvae gradually stabilized with the duration of the rearing time. In terms of the growth performance (Table 2), the shell height of larvae from the mass-selected strain was observed to be remarkably higher than that of the wild population throughout the larval stages except for day 1 ($P < 0.05$), with RG of 10.20, 13.79, 23.22 and 21.88% on days 6, 12, 18 and 24, respectively. Ultimately, the mean metamorphosis rate of larvae from WF1 and SF4 reached 69.40 and 77.80%, respectively, implying a 12.10% improvement in SF4.

3.2. Comparison of survival and growth at the grow-out stage

At grow-out stages, survival of oysters from SF4 was consistently numerically superior to that of WF1 at all sampling time points (Table 3), and the variation between the two populations became incrementally greater with the duration of the culture. *C. nippona* spat from both the breeding and wild populations initially exhibited similar high-level CSR on day 60, 170 and 290 (> 85%). However, a recognizable decrease (about 10%) in survival was found in both groups during high summer temperatures from day 290 to day 410, and a significant difference in CSR between SF4 and WF1 was first observed on day 410 ($P < 0.05$). A similar decline in the cumulative survival rate was subsequently observed when *C. nippona* was exposed to the second summer heat wave from day 680 to day 800, and the remarkable difference in the CSR between the two groups was detected at harvest on day 800 ($P < 0.05$). Overall, the CSR of SF4 was significantly enhanced

Table 1
Mean ± SD hatching index and cumulative survival rate of larvae from the wild and mass-selected populations (WF1 and SF4) of *Crassostrea nippona*.

Items	Hatching index			Cumulative survival rate (%)			
	Egg diameter (μm)	Fertization rate (%)	Hatching rate (%)	Day 6	Day 12	Day 18	Day 24
WF1	48.14 ± 2.85^{a}	88.67 ± 2.52^{a}	65.33 ± 5.86^{a}	56.67 ± 5.77^{a}	49.78 ± 3.67^{a}	36.78 ± 4.83^{a}	31.67 ± 6.01^{a}
SF4	48.25 ± 2.68^{a}	94.33 ± 2.08^{b}	77.67 ± 4.73^{b}	70.88 ± 6.03^{b}	65.18 ± 2.45^{b}	54.10 ± 6.16^{b}	48.16 ± 8.05^{b}
RG (%)	–	6.38	18.89	25.08	30.94	47.09	52.07

Different letters in each column indicate significant difference ($P < 0.05$). The relative gain (RG) obtained in SF4 compared to WF1 is only displayed for significant indicators.

Table 2
Mean ± SD shell height and metamorphosis rate of larvae from the wild and mass-selected populations (WF1 and SF4) of *C. nippona*.

Items	Shell height (μm)					Metamorphosis rate (%)
	Day 1	Day 6	Day 12	Day 18	Day 24	
WF1	63.04 ± 2.65^{a}	92.22 ± 8.02^{a}	117.09 ± 11.46^{a}	193.50 ± 12.32^{a}	303.22 ± 20.87^{a}	69.40 ± 5.91^{a}
SF4	63.13 ± 2.27^{a}	101.63 ± 6.45^{b}	133.24 ± 8.43^{b}	238.43 ± 13.50^{b}	369.56 ± 15.01^{b}	77.80 ± 3.08^{b}
RG (%)	–	10.20	13.79	23.22	21.88	12.10

Different letters in each column indicate significant difference ($P < 0.05$). The relative gain (RG) obtained in SF4 compared to WF1 is only displayed for significant indicators.

Table 3
Mean ± SD cumulative survival rate, shell height and body weight of *C. nippona* from the wild and mass-selected populations (WF1 and SF4) during grow-out stage.

Items	Cumulative survival rate (%)			Shell height (mm)			Body weight (g)		
	WF1	SF4	RG (%)	WF1	SF4	RG (%)	WF1	SF4	RG (%)
Day 60	93.47 ± 2.09^{a}	96.42 ± 2.60^{a}	–	4.01 ± 0.73^{a}	4.24 ± 0.56^{a}	–	–	–	–
Day 170	88.66 ± 2.86^{a}	94.05 ± 2.77^{a}	–	31.05 ± 5.05^{a}	35.74 ± 4.75^{b}	15.10	–	–	–
Day 290	86.59 ± 3.05^{a}	92.37 ± 3.16^{a}	–	31.69 ± 5.92^{a}	35.88 ± 5.03^{b}	13.22	–	–	–
Day 410	72.63 ± 4.75^{a}	83.96 ± 3.89^{b}	15.60	47.37 ± 7.13^{a}	54.91 ± 5.87^{b}	15.92	13.98 ± 4.90^{a}	16.85 ± 3.80^{b}	20.53
Day 540	64.97 ± 5.29^{a}	77.27 ± 3.11^{b}	18.93	52.96 ± 8.11^{a}	64.22 ± 5.64^{b}	21.26	22.13 ± 7.68^{a}	28.51 ± 6.29^{b}	28.83
Day 680	57.71 ± 5.33^{a}	71.18 ± 5.28^{b}	23.34	55.16 ± 11.45^{a}	69.38 ± 8.44^{b}	25.78	39.94 ± 13.60^{a}	49.86 ± 10.93^{b}	24.84
Day 800	46.70 ± 6.52^{a}	62.66 ± 6.01^{b}	34.18	59.30 ± 11.92^{a}	73.05 ± 8.60^{b}	23.19	46.80 ± 15.65^{a}	62.47 ± 12.43^{b}	33.48

Different letters within the same row indicate significant difference ($P < 0.05$). The relative gain (RG) obtained in SF4 compared to WF1 is only displayed for significant indicators.

by 15.60, 18.93, 23.34 and 34.18% compared to that of WF1 on days 410, 540, 680 and 800, respectively.

In terms of growth traits, oysters from the mass-selected population exhibited faster growth rates at all ages compared to that of the wild population in this study (Table 3). The shell height and body weight of the oysters from SF4 first appeared significantly different from those of WF1 on days 170 and 410, respectively ($P < 0.05$). Subsequently, the relative gains associated with shell height (ranging from 13.22 to 25.78%) and body weight (ranging from 20.53 to 33.48%) displayed an overall increasing trend as the culture time increases. At harvest on day 800, the mean shell height of oysters from WF1 and SF4 reached 59.30 and 73.05 mm, and the mean body weights of oysters from WF1 and SF4 reached 46.80 and 62.47 g, respectively.

Table 4
Mean ± SD yield traits, shell shape and biochemical composition of *C. nippona* from the wild and mass-selected populations (WF1 and SF4) at harvest on day 800.

Items	WF1	SF4	RG (%)
Yield traits			
Final yield (g)	2185.54 ± 305.09^{a}	3914.51 ± 375.52^{b}	79.11
Wet meat weight (g)	7.17 ± 2.49^{a}	11.60 ± 2.29^{b}	61.79
Meat yield (%)	15.34 ± 1.13^{a}	18.62 ± 1.03^{b}	21.38
Dry meat weight (g)	1.63 ± 0.49^{a}	1.87 ± 0.43^{b}	14.72
Shell shape			
Cup ratio (shell width/shell height)	0.34 ± 0.10^{a}	0.39 ± 0.09^{b}	14.71
Fan ratio (shell length/shell height)	0.65 ± 0.13^{a}	0.64 ± 0.11^{a}	–
Biochemical composition			
Glycogen content (% DW)	6.52 ± 0.60^{a}	7.05 ± 0.53^{a}	–
Total protein content (% DW)	52.12 ± 1.83^{a}	53.25 ± 1.54^{a}	–
Total fat content (% DW)	23.84 ± 1.04^{a}	22.72 ± 1.17^{a}	–

Different letters within the same row indicate significant difference ($P < 0.05$). The relative gain (RG) obtained in SF4 compared to WF1 is only displayed for significant indicators.

3.3. *Comparison of yield traits, shell shape and biochemical composition at harvest*

To further quantitatively evaluate the degree of improvement in yield traits of the selected strain, several essential production-related metrics were compared between populations at the final harvest (Table 4). On day 800, the final yield and wet meat weight of SF4 (3914.51 and 11.60 g) were significantly heavier than that of WF1 (2185.54 and 7.17 g), increasing by 79.11 and 61.79%, while the meat yield and dry meat weight of SF4 (18.62% and 1.87 g) were significantly larger than that of WF1 (15.34% and 1.63 g), increasing by 21.38 and 14.72%, respectively ($P < 0.05$). For shell shape, there was no significant difference between the breeding and wild populations in the fan ratio (0.64–0.65), but oysters from SF4 (0.39) were significantly more cupped than that of WF1 (0.34), with RG of 14.71% ($P < 0.05$). In respect of the biochemical composition, the glycogen content as a percentage of dry meat weight of *C. nippona* from the WF1 and SF4 were 6.52 and 7.05%; total protein content of *C. nippona* from the WF1 and SF4 were 52.12 and 53.25%; total fat content of *C. nippona* from the WF1 and SF4 were 23.84 and 22.72%, respectively. No significant differences were detected in the statistical analysis performed on each nutritional indicator ($P > 0.05$).

3.4. Comparison of reproductive traits at harvest

Reproductive traits were determined to assess the influence of artificial selection on reproductive capacity and gonadal development of *C. nippona*. At harvest on day 800, the RBA of oysters from SF4 (0.71 million/g) was significantly higher than that of WF1 (0.58 million/g) ($P < 0.05$), with a relative gain of 22.41% (Table 5). Moreover, there was no dramatic difference in the diameter of eggs produced by WF1 and SF4 ($P > 0.05$). Histological analyses showed that males and females from each mating combination normally developed with mature oocytes and spermatozoa (Fig. 1), and the number of oysters in each stage of gonadal development were similar across populations. Approximately 73 and 67% of oysters from WF1 and SF4 were in the mature stage respectively, while the remaining individuals (about 27% and 33%) from WF1 and SF4 were in the spawning stage. Sex determination identified that the sex ratios (female: male) of oysters in SF4 and WF1 were 2:1 and 2.33:1, respectively.

4. Discussion

In selective breeding programs, one or several economically important traits are typically targeted for genetic improvement of closed breeding populations carrying abundant available genetic variation to rapidly reach breeding goals. However, given the behavioural plasticity of species by domestication and the correlations between different traits, the considerable genetic gains achieved through selection for the target trait may be accompanied by positive or negative pleiotropic effects on the potential favorable traits (Houston et al., 2020; Fradgley et al., 2023). For example, individual size and reproductive stage are two critical parameters that determine the marketability of aquatic animals, while a moderate to high negative correlation between early maturity and fast growth was widely detected in fish and crabs (Gjerde, 1984; Jiang et al., 2020). By contrast, in spite of the great economic value derived from the reproduction properties of *C. nippona*, little is known about how the reproductive and production performance of *C. nippona* varies under high-intensity artificial selection. Fortunately, progeny from undomesticated wild populations is considered the appropriate control to further quantify the cumulative genetic progress in commercial characters related to yield and quality in *C. nippona* following three successive generations of mass selection for fast growth (Li et al., 2018), which contributed to the systematic evaluation the aquaculture potential of the selected population. Although there are certain differences in genetic diversity between different wild populations of the same oyster species (Zhang et al., 2018), the impact of this discrepancy on phenotypic differences among offspring from different populations appears to be limited, especially in terms of growth (Li et al., 2011; Ma et al., 2022), which provides an important theoretical premise for carrying out the current research.

Table 5
Relative brood amount, sex ratio and gonadal development of *C. nippona* from the wild and mass-selected populations (WF1 and SF4) at harvest on day 800.

Items	WF1	SF4	RG (%)
Relative brood amount (million/g)	0.58 ± 0.08^a	0.71 ± 0.05^b	22.41
Egg diameter (μm)	48.36 ± 2.64^a	48.39 ± 2.62^a	–
Sex ratio (Female: Male)	2:1	2.33:1	–
Gametogenic stages			–
Mature stage	22	20	–
Spawning stage	8	10	–

Different letters within the same row indicate significant difference ($P < 0.05$). The relative gain (RG) obtained in SF4 compared to WF1 is only displayed for significant indicators.

In the present study, *C. nippona* from the fast-growing strain unsurprisingly showed obvious growth advantages in terms of shell height throughout the rearing process by comparison with that of WF1, and the differences in phenotypic values between populations followed an overall upward trend with the duration of the farming time, indicating the increasing accumulated expression of additive genetic effects provided by the intense selection in SF4 (Liu et al., 2015). Relative gains of the growth trait reached 21.88 and 23.19% at the end of the larval and grow-out stages respectively, which was similar to the previously reported results of a theoretically estimated genetic gain of approximately 30% for *C. nippona* after three consecutive generations of mass selection (Hu et al., 2022a, 2022b), suggesting that the cumulative genetic progress in the growth performance of *C. nippona* contributed by current breeding procedures were considerable. Notably, similar advancements were identified in the comparison of body weight between populations, with offspring from the selected line achieving a 33.48% increase in body weight compared to the control at harvest day 800, although *C. nippona* were completely separated from the substrate for individual weighing at about 410 days on account of slow growth characteristics. Similarly, the yield traits closely related to body weight in SF4 at harvest, such as final yield, wet meat weight, meat yield and dry meat weight, were significantly increased by 79.11, 61.79, 21.38 and 14.72%, respectively ($P < 0.05$). These encouraging findings implied a potential positive correlation between shell height and body weight in *C. nippona*, suggesting that genetic gains could be obtained by indirect selection between two traits (Hu et al., 2022a, 2022b), and similar conclusions were revealed in genetic improvement programs of other oyster species, such as *C. gigas* and the Portuguese oyster (*C. angulata*) (de Melo et al., 2019; Vu et al., 2020). Interestingly, although the variances of shell height and body weight in the wild group were higher than those in the selected group, the variances between the two groups were homogeneous at all growth stages ($P > 0.05$), which might be consistent with our previous findings that the selected line could maintain high levels of genetic variation (Hu et al., 2022a, 2022b), showing that the selective breeding population still had high potential for further genetic improvement (Zhang et al., 2019). Similar results were also found during the establishment of fast-growing strain and black-shell strain in *C. gigas* (Li et al., 2011; Zhang et al., 2018; Xu et al., 2019a, 2019b, 2019c). Furthermore, it is worth noting that the final yield, the trait of greatest interest to farmers, is not only related to the average growth performance of individuals, but also depends on the overall survival in the rearing population from planting spat to harvest.

Overall, along with substantive progress in growth traits of *C. nippona* by genetic selection for shell height, an unanticipated and significant enhancement (52.07% for larvae and 34.18% for adults) in the survival of the offspring from the selected strain was noticed when compared to that of the wild strain. Analogously, de Melo et al. (2019) reported positive moderate-to-high genetic correlations among group traits, such as yield, individual weight and survival, and concluded that the remaining traits can be indirectly improved when selection is performed for one of the traits. Indeed, the survival advantage demonstrated in SF4 might be the consequence of combined actions of high-intensity artificial selection and domestication in this study (Houston et al., 2020). In the selective breeding program of *C. nippona*, the parents (about 700 days old) of the second- and third-generation mass-selected populations underwent natural selection from two consecutive high summer temperatures in field conditions prior to spawning (Hu et al., 2022a, 2022b), meaning that beneficial alleles related to survival might be progressively accumulated in recurrent cycles of breeding (Dégremont et al., 2010; Houston et al., 2020), thus accelerating adaptation of *C. nippona* from the breeding population to captive environments. Interestingly, despite the fact that commercial aquaculture environments are more stable than complex and fluctuating natural surroundings, the maladaptation of wild individuals to intensive farming systems, such as elevated stocking densities and handling practices, was probably a leading cause of the poor production performance in the current study (Nascimento-Schulze et al., 2021). For

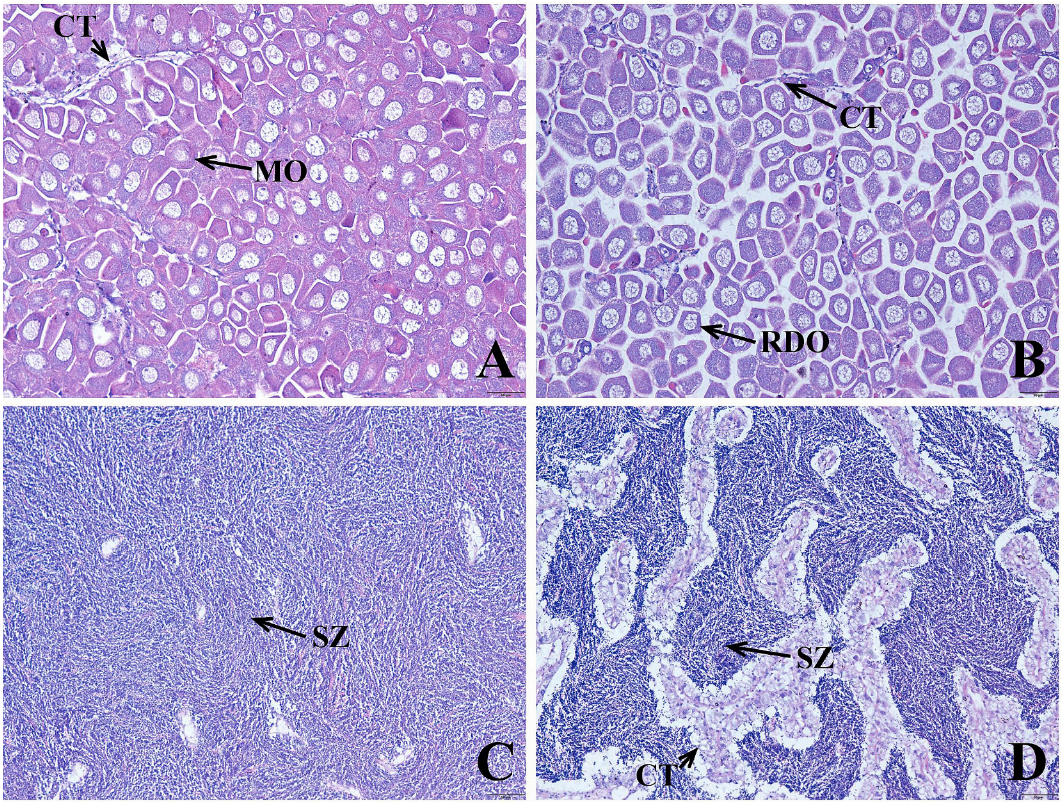

Fig. 1. Gonadal development stages of *C. nippona* in female (upper panels) and male (lower panels) using 40× light microscope. A&C. mature stage; B&D. spawning stage. CT: connective tissue; MO: mature oocyte; RDO: residual oocyte; SZ: spermatozoa.

example, a severe larval mortality event with cumulative survival rate plummeting to 56.67% on day 6 post-fertilization was observed at the early umbo stage in WF1, which was similar to the mass mortality of larvae described within hatchery-based seed production of *C. nippona* in Japan (Sasaki et al., 2016). Given that the rearing environment was consistent between groups in this study, differences in larval survival performance might indicate that wild individuals were less adaptable to the captive environment and the egg quality between the two populations was different (Nascimento-Schulze et al., 2021; Wu et al., 2023). By contrast, inferior early mortality (29.12%) and dramatic survival improvement (RG: 25.08%) were observed in the same developmental stages of the larvae from SF4, which might be related to artificial selection and domestication promoting the enrichment of genetic variation related to larval survival, hinting that the improved *C. nippona* was more suitable for commercial seed production. High early mortality rates not only lead to serious negative effects on the breeding willingness of farmers, but also cause high variance in reproductive success among individuals and low effective population sizes, which in turn contributes to the reduction of genetic variability in the candidate population and even to inbreeding depression (Hedgecock and Pudovkin, 2011). To address these problems, multiple interventions, such as initiating genetic improvement programs focused on larval performance, increasing the scale of broodstock and implementing the multi-line breeding strategy, were expected to be applied to rapidly increase the seed production and prevent the reduction of valuable genetic diversity of *C. nippona* (Nascimento-Schulze et al., 2021; Hu et al., 2022a, 2022b).

In high fecund marine animal, female reproduction traits, such egg diameter, relative brood amount (RBA) and egg weight, are generally considered to be strongly associated with the quality of offspring (Coldebella et al., 2011; Bombardelli et al., 2017). Of these, RBA is recognized to be an essential indicator that can more precisely assess reproductive potential than the egg number by closely correlating with the initial development of the offspring (Wu et al., 2023). In this study, the egg diameters between groups were at similar levels in the statistical analysis, but significantly lower RBA was observed in WF1 by comparison with SF4 at harvest ($P < 0.05$), which might be associated with differences in reproductive management strategies of different populations and the heritable variation accumulated by successive generations of artificial selection and domestication (Bombardelli et al., 2017), partly explaining the suboptimal embryo development and poor larval performance in the wild population, suggesting that female reproductive traits in *C. nippona* might be improved through genetic improvement (Wu et al., 2023). Furthermore, similar sex ratios between WF1 and SF4 were found at the same time that gonadal development of *C. nippona* from both populations was nearly synchronous, implying that high-intensity artificial selection had no significant effect on the reproductive characteristics of *C. nippona* while enhancing growth performance. The findings were consistent with the field experiments of the fast-growing strain and the non-selected population in Sydney rock oysters (*Saccostrea glomerata*) (Dove and O'Connor, 2012). Different from growth traits under strong genetic control, gonadal development in shellfish is commonly determined by temperature thresholds for gametogenesis and food abundance in living environments (Han et al., 2022). Another important aspect that the nutritional content of oysters usually follows seasonal variations driven by the gametogenic cycle, especially glycogen content (Qin et al., 2021). In this study, the similar biochemical composition between the two populations at the final harvest indicated that the nutritional quality of *C. nippona* from the mass-selected strain was of similar magnitude as that of wild individuals, which might indirectly support the conclusion of no significant effect imposed by selection on gonadal development of *C. nippona* in the selective breeding program. Notably, the low glycogen content (6.52–7.05%) and partial spawning behavior presented in both populations might imply that *C. nippona* farmed in northern China were unsuitable for consumption in the late summer, in agreement with previous reports by Japanese scholars (Okumura et al., 2005; Sasaki, 2022). In the unpublished comparative study of biochemical composition between *C. gigas* and *C. nippona*, we also found that non-spawning *C. nippona* had obvious nutritional advantages in terms of glycogen content, protein and lipid quality during the season when *C. gigas* released gametes.

The shell dimensions of oysters are generally considered to be the result of a combined effect of genetics, environmental conditions and farm husbandry practices (Mizuta and Wikfors, 2019). Nevertheless, experimental individuals from both populations were exposed to the same aquatic environment and rearing system in this study, demonstrating that the observed significant difference in cup ratio between the selected and wild populations was likely to be genetically determined ($P < 0.05$). Although selection for high growth might indirectly result in individuals with a more rounded shape in the long term (Vu et al., 2020), such genetic gains were recognized as quite low due to unpredictable variation of proportion among growth metrics resulting from disproportionate nature by which selection pressure is exerted on component traits (Gunsett, 1984), suggesting that the effect of indirect selection to enhance shell shape in oysters is limited. Therefore, the apparent variation in shell shape observed in this study might be attributed to genetic differences between the different geographic populations. Remarkably, the cup ratio of *C. nippona* from both WF1 (0.34) and SF4 (0.39) was presented well above the 0.25 threshold value of good morphology of oyster shell determined by Brake et al. (2003), exhibiting strong aquaculture potential to satisfy the increased demand for half-shell oysters.

5. Conclusion

In this study, the offspring of the selected strain subjected to three successive generations of high-intensity mass selection showed distinct advantages in growth performance, survival and reproductive capacity compared to those of wild individuals, illustrating that the cumulative genetic progress contributed by selective breeding was considerable in the genetic improvement program of *C. nippona*. At the larval stage, the cumulative survival rate of the descendant from the selected line were significantly higher compared to those of the undomesticated population, especially at early umbo stage. During grow-out stage, the production performance of improved *C. nippona* was consistently outperformed by that of wild oysters, with significant 79.11% increase in final yield at harvest, while relative brood amount associated with initial development of offspring was found to be significantly higher in improved *C. nippona* compared with wild individuals. Notably, histological analysis proved that high-intensity artificial selection had no obvious impact on the gonadal development of *C. nippona*, and the nutritional quality of oysters from the selected strain was comparable to that of wild individuals. Overall, the results presented in this study indicate that the mass-selected population possesses strong aquaculture potential for commercial large-scale farming which will continue to increase with further selective breeding.

CRediT authorship contribution statement

Yiming Hu: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation. **Qi Li:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization. **Chengxun Xu:** Project administration. **Shikai Liu:** Resources. **Lingfeng Kong:** Software. **Hong Yu:** Data curation.

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.

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