



Response to selection for growth in successive mass selected generations of Iwagaki oyster *Crassostrea nippona*

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ARTICLE INFO

Keywords:

Crassostrea nippona
Response to selection
Faster growth
Mass selection
Genetic parameters

ABSTRACT

The Iwagaki oyster (*Crassostrea nippona*) is a potential aquaculture species with delicious taste and edibility in summer when the Pacific oyster (*Crassostrea gigas*) suffer from low meat quality. Although there is an increasing interest in hatchery mass production and field grow-out among farmers, large-scale expansion of *C. nippona* aquaculture has been compromised by unimproved growth performance. To improve the growth rate of *C. nippona*, we initiated the mass selective breeding program for fast growth in 2014 and estimated the genetic parameters of growth traits in the first three successive mass-selected generations. Each selected line exhibited better growth performance than the corresponding control group at all sampling dates. At grow-out stage, the average selection response (SR), realized heritability (h_R^2) and genetic gain (GG) for shell height was 0.45–0.56, 0.27–0.42 and 9.20–11.12% in the three mass selected lines, respectively. A significant increase in GG for shell height was observed in the third-generation selected line compared with that in the first-generation ($P < 0.05$). Meanwhile, the average SR (0.44–0.49) and GG (11.07–12.46%) for body weight of *C. nippona* were observed in the selective breeding program after three successive generations of mass selection. The encouraging results obtained in this study suggest that genetic variances still remain at a relatively high level in the breeding populations of *C. nippona*, and demonstrate the feasibility of improving the growth traits by selective breeding for *C. nippona*.

1. Introduction

China is the largest oyster aquaculture producer worldwide comprising 86% of the global production (Botta et al., 2020). The Pacific oyster (*Crassostrea gigas*) is one of the most important endemic oysters that is commonly cultivated along the coast of northern China (Ma et al., 2021). In 2019, the Pacific oyster production reached 1.2 million metric tons, accounting for 21% of the total yield of oyster aquaculture in China (BOF, 2020). However, the marketability of *C. gigas* is limited in summer, because the relocation of energy from growth to reproduction leads to its unpalatable meat and decreased nutrient components (Liu et al., 2020; Li et al., 2022). In recent years, although triploid *C. gigas* with year-round marketability were massively produced by mating tetraploids and diploids (Guo et al., 1996), the rapid development of the polyploid industry also has raised a primary concern on the potential harm of farmed escapees to natural populations (Piferrer et al., 2009). Therefore, searching for another new oyster species with refined flavor and firm texture to satisfy the demand of consumers during the summer

season, when the diploid Pacific oyster is not marketable, may be a positive and effective attempt.

The Iwagaki oyster (*Crassostrea nippona*) is naturally distributed in coastal areas of East Asia such as China, Japan and South Korea (Itoh et al., 2004; Lu et al., 2017). In natural conditions, spawning events of *C. nippona* occurred from August to September and 25 °C appeared as a critical temperature to trigger spawning (Semura et al., 2010), while the minimal temperature of spawning in *C. gigas* was 18 °C (Mann, 1979; Ubertaini et al., 2017). Because of high glycogen content and delicious taste of *C. nippona* in summer when *C. gigas* suffer from low meat quality (Okumura et al., 2005; Masahiro et al., 2018), its commercial price is estimated to be fivefold that of *C. gigas* in Japan (Itoh et al., 2004). Although an increasing interest in *C. nippona* farming has been prompted among breeders due to the great economic potential and extensive market prospects, the industry has never matured into a large-scale aquaculture producer during long-term domestication. One of the crucial reasons is that the slow growth performance of *C. nippona* at grow-out stage leads to lower yield and higher rearing costs for needing

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<https://doi.org/10.1016/j.aquaculture.2022.738575>

Received 16 April 2022; Received in revised form 27 June 2022; Accepted 29 June 2022

Available online 4 July 2022

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longer time to attain the market size, compared with *C. gigas* (Xu et al., 2019a). In addition, the current production of *C. nippona* is still mainly focused on wild-caught individuals to meet the market demand and hatchery mass production (Fujiwara, 1998; Shirafuji et al., 2008). The small base population and unequal parental contributions could increase the probability of unintentional inbreeding and the expression of harmful alleles in long-term continuous production, which would undermine the sustainable development of the aquaculture industry (Rhode et al., 2014; Matusse et al., 2016; Lu et al., 2017). Therefore, it is important to improve production performance and retain potentially beneficial alleles of *C. nippona* in the breeding process. Promisingly, numerous artificial selected trials focusing on the growth-related traits of oysters have achieved obvious progress (Newkirk and Haley, 1982; Toro et al., 1996; Nell and Perkins, 2005; Li et al., 2011; Dégremont et al., 2015; de Melo et al., 2016), which provide the important reference for genetic improvement of *C. nippona*.

Mass selection is one of the classical approaches widely applied in selective breeding programmes of aquaculture species, and it could be easily implemented by establishing several selected groups and control groups to assess the breeding potential of desired traits (Gjedrem and Baranski, 2009; Chatchaiphan et al., 2019). However, the breeding strategy might lead to inbreeding and reduce the response to selection because this selection scheme cannot provide the information of individual tagging and pedigree records (Bentsen and Olesen, 2002). Encouragingly, most selective breeding programmes through mass selection in oysters not only proved the possibility of applying high selection pressure to improve the growth traits in recent years (Nell and Perkins, 2005; Gjedrem and Baranski, 2009; Li et al., 2011; Zhang et al., 2019), but also confirmed that the loss of genetic diversity in breeding populations could be effectively inhibited during the oyster breeding process (In et al., 2016; Han et al., 2019; Xu et al., 2019b; Chen et al., 2022).

In this study, we initiated a selective breeding program to improve growth of *C. nippona* in 2014, and truncation selection for fast growth was adopted throughout three successive generations of mass selection from 2015 to 2019. The response to mass selection was quantified by estimating temporal changes of genetic parameters in the three mass-selected generations, which will give us a perspective to determine the degree of progress being achieved per generation and the potential for increased gains in future generations.

2. Materials and methods

2.1. Base stock, broodstock conditioning and experimental design

In 2014, 200 wild *C. nippona* collected from Niigata Prefecture, Japan, were conditioned in the oyster farm of Rushan, Shandong Province, China (36.4°N, 121.3°E). In May 2015, wild oysters were transported to a hatchery in Yantai to begin conditioning broodstock oysters. The water temperature was gradually raised from ambient temperature to 27 °C and fed daily with a mixed algal diet of *sochrysis galbana* and *Nit schia closterium* until sexual maturity.

The standard shell height of all oysters was measured to determine their size-frequency distribution before each selection. On August 1, 2015, 20 wild oysters were first randomly selected to produce the control line (C1), and then 60 individuals from the top end of the size distribution were selected to establish the first-generation selected line (S1). Finally, the S1 and C1 were transferred to Sanggou Bay in Shandong Province for cultivation and growth data were recorded until the next generation of selection. On August 5, 2017, 40 two-year-old individuals were randomly selected from the S1 to construct the control group (C2) before removing the selected parents. Then, according to the selection intensity of 1.64, 90 oysters with the longest shell height were selected to produce the second-generation selected line (S2). On July 20, 2019, following the above method, 100 and 80 mature oysters from the S2 were applied to as broodstocks for the third-generation selected and

control lines (S3 and C3), respectively. Information on selected parents, the cutoff point and selection intensity of each generation are presented in Table 1.

2.2. Fertilization, larval rearing and spat grow-out

Mature broodstocks were induced to spawn in a 20 m³ cultivation pond using a sudden increase in temperature from 23 °C to 27 °C after air exposure for 7 h in the shade. Observed the density and proportion of sperm and eggs in the water under the microscope, and removed the broodstock in time. When 60% of the fertilized eggs were observed to release the first polar body, embryos were transferred to a new 20 m³ tank for hatching at 27 °C.

The procedures of cultivating *C. nippona* larvae and spat were mainly based on the standard breeding methods by Li et al. (2011). In brief, after 26 h incubation, the upper D-larvae were siphoned onto 50 µm mesh sieves and transferred to the new rearing tank. Adjusted the initial density of D-larvae to 4 larvae/ml, and the feeding amount of *S. galbana* and *Chaetoceros calcitrans* continued to increase with larval growth. The sand-filtered seawater (temperature: 27 °C; salinity: 31 psu) was used to exchange 30% of the pool water twice a day. When 40% of the larvae appeared with eyespots, strings of scallop shells were hung in the tanks for larvae to attach. After about a week of cultivation, the eyed larvae metamorphosed to spats, and then they were transferred to the outdoor pond for a 14 days of temporary cultivation. Finally, the spats were transported to Sanggou Bay and put into 10-layer lantern nets hanging on long-lines for farming. The individual numbers of each layer were adjusted monthly to maintain similar levels between selected and control groups per generation.

2.3. Sampling and growth measurement

At days 1, 5, 10, 15, 20 and 25 post-fertilization, each group of larvae was sampled and stored in Lugo reagent, and 30 larvae were randomly selected to measure their shell height using a light microscope with an ocular micrometer. During the grow-out stage, the shell height of 30 oysters randomly selected from each group was measured with an electronic vernier caliper (0.01 mm accuracy) on days 60, 180, 300, 420, 530, 650 and 780. Meanwhile, the body weight of 30 oysters randomly selected from each group was individually measured by electronic balance (0.01 g accuracy) on days 420, 530, 650 and 780.

2.4. Estimation of genetic parameters

According to Falconer and Mackay (1996), the intensity of selection (i) was calculated as the difference in mean shell height between the selected parents and the base stock divided by the standard deviation of the stock. The realized heritability (h_R^2) was calculated following Hadley et al. (1991) as:

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

To compare the improvement degree of growth traits at different life-history stages of oysters, the standard response to selection (SR) and genetic gain (GG) was estimated following the equation (Zheng et al., 2006):

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

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

where \bar{X}_S and \bar{X}_C are the mean phenotypic value of offspring in the selected and control lines, respectively; σ_C is the standard deviation of control progeny; i is the intensity of selection.

2. . *Statistical analyses*

All data are presented in the format of mean \pm standard deviation (SD), and statistical analyses were performed using the IBM SPSS Statistics 25.0 software. Differences in phenotypic values in shell height and body weight at different ages were analyzed by *t*-test in the selected and control groups of each generation. The significance level of genetic parameters

Table 4

Mean ± SD standardized response to selection (SR), realized heritability (h_R^2) and genetic gains (GG) of shell height and body weight in different selected lines of *C. nippona*.

Genetic parameters	Shell height			Body weight		
	S1	S2	S3	S1	S2	S3
Larvae						
SR	0.40 ± 0.19 ^a	0.34 ± 0.18 ^a	0.52 ± 0.29 ^a	-	-	-
h_R^2	0.30 ± 0.14 ^a	0.21 ± 0.14 ^a	0.30 ± 0.21 ^a	-	-	-
GG (%)	3.67 ± 1.88 ^a	3.60 ± 1.97 ^a	4.13 ± 2.43 ^a	-	-	-
Grow-out						
SR	0.56 ± 0.13 ^b	0.45 ± 0.07 ^a	0.53 ± 0.04 ^{ab}	0.44 ± 0.05 ^a	0.49 ± 0.06 ^a	0.47 ± 0.06 ^a
h_R^2	0.42 ± 0.10 ^b	0.27 ± 0.04 ^a	0.31 ± 0.02 ^{ab}	0.33 ± 0.04 ^a	0.30 ± 0.04 ^a	0.28 ± 0.04 ^a
GG (%)	9.20 ± 1.72 ^a	10.48 ± 0.87 ^{ab}	11.12 ± 0.54 ^b	12.05 ± 1.08 ^a	11.07 ± 2.11 ^a	12.46 ± 0.60 ^a

Different letters within the same row indicate significant differences among means for the same trait ($P < 0.05$).

compared with that obtained in S1 ($P < 0.05$). The genetic gains of shell height within S1-S3 at harvest on day 780 were 9.43, 11.50 and 11.16, respectively.

The range in the mean SR of body weight of selected lines at grow-out stage was from 0.44 to 0.49; the mean h_R^2 was in the range of 0.28 to 0.33; the mean GG was in the range of 11.07 to 12.46% (Table 4). There were no significant differences in SR, h_R^2 or GG among different selected lines. The genetic gains of body weight within S1-S3 at harvest on day 780 were 11.47, 13.80 and 10.21%, respectively.

4. Discussion

When the breeding populations contain abundant available genetic variance, the selection index can generally achieve a high level of selection response under high selection pressures. In this study, the positive standard response to selection observed in the first generation of mass selection also appeared in the subsequent two generations, and the mean SR of shell height ranged from 0.45 to 0.56 for S1, S2 and S3 at grow-out stage. Similarly, the SR of 0.506 to 0.547 was also observed in the Pacific oyster selected for ninth- to tenth-generations (Zhang et al., 2019). Since the selected and control lines were cultured under consistent rearing conditions at all stages in this study, the significant differences in shell height between the two groups of each generation could be considered as responses to different selection levels (Liang et al., 2010), and could be the result of the change of gene frequency of selected traits (Falconer and Mackay, 1996), indicating that the selection for the fast growth of *C. nippona* was effective. Although mass selection without individual tagging and pedigree information is considered to lead to inbreeding and reduce the selection response (Bentsen and Olesen, 2002), most studies to enhance growth of shellfish species have not detected a significant decline in the genetic diversity of selected lines (Han et al., 2019; Xu et al., 2019b; Chen et al., 2022), and the positive response to selection for fast growth observed in this study suggests that the breeding populations of *C. nippona* contain a relatively high level of genetic variation to obtain further improvement in the future breeding program. In addition, relatively low growth performance was observed in the S2 and C2 compared to other generations during grow-out stage, which could be caused by the deficiency of available food and the high

stocking density in coastal aquaculture area.

In this study, the mean h_R^2 of 0.27 to 0.42 for shell height of three selected groups at grow-out stage were at moderate-to-high level, which was consistent with previous expectations in other oyster species (Li et al., 2011; Zhang et al., 2019). In addition, heritability estimated for the growth trait at grow-out stage not only increased with age but was also more representative than the overall average heritability due to decreasing the influence of environmental (and error) effects on phenotypes and increasing accumulated expression of additive genetic effects over time (Domingos et al., 2013; Liu et al., 2015). Thus, the heritability of shell height at harvest reached 0.42, 0.26 and 0.33 for S1, S2 and S3 respectively, suggesting that the selective line of *C. nippona* still has a high potential for sustained genetic selection to achieve further gains (Falconer and Mackay, 1996; Zhang et al., 2019; Wang et al., 2020). Simultaneously, the simulation study also showed that compared with high heritability, relatively low heritability can effectively reduce the probability of broodstocks from the full-sibling family and prevent accumulation of inbreeding in offspring (Bentsen and Olesen, 2002). In mass selection, the selected broodstocks with the satisfactory performance from the breeding candidates generally carry the best genotypes to produce greater genetic gain. Although the parental selection that reduces the opportunity of mating with other individuals is generally considered to decrease genetic variation and heritability in mass selection (Zheng et al., 2006), most selective breeding researches have demonstrated that these parameters are hardly affected by providing a mass of breeding candidates (Li et al., 2011; Wang et al., 2020). For example, the realized heritability for shell height of *C. gigas* was still maintained at the range of 0.275–0.420 after ten successive mass-selected generations for fast growth, and the shell height also achieved remarkable gains of 7.2–15.2% at harvest compared with the control group (Zhang et al., 2019).

Ensuring that positive genetic progress can be continuously obtained is crucial to improving characters of commercial interest in the breeding program. In the present study, the average genetic gains for shell height of the first to third generation selected lines at grow-out stage continued to increase and reached 9.20, 10.48 and 11.12%, respectively. Some mass-selected programs focused on the improvement of the growth rate of oysters had also achieved genetic gains similar to this study, and finally obtained encouraging results. Toro et al. (1996) reported the average genetic gain of shell length in Chilean native oysters (*Ostrea chilensis*) reached 13.56% with the first generation of selection. In Sydney rock oysters (*Saccostrea glomerata*), the genetic gain of 4% and 18% in growth traits was achieved and the average time for the progeny of fourth-generation breeding lines grew to market size was shortened by 12.5 months (Nell and Perkins, 2005). In the mass-selected *C. gigas*, the genetic gain of 8.8–15.2% in shell height was observed in the selected lines within 450 days after seven to nine generations of selection (Zhang et al., 2019). The selective breeding results of aquatic species had concluded that the genetic gain of 10–20% in growth traits would achieve the doubling of growth rates after six generations of selection (Gjedrem and Rye, 2018). In this study, selected lines of *C. nippona* reached the level of genetic gain similar to other oyster species after 780 days of cultivation, indicating that *C. nippona* with a longer generation interval may take more time to achieve the goal. Promisingly, the third-generation selected line with the highest selection intensity obtained the greatest genetic gain among different selected lines, suggesting that continuous high-intensity selective breeding could improve the desired gene frequency and achieve a higher growth improvement of the breeding line. In addition, the average genetic gains of three selected lines at the larval stage were relatively low and ranged from 3.60 to 4.13%. It was also found that selective breeding had little effect on larval growth even if the heritability remained at the medium-to-high level in other shellfish studies, illustrating that the genetic improvement of larval shell height was difficult to obtain improvement (Zheng et al., 2004; Barros et al., 2018).

Indirect selection is considered to be a high-efficiency selective

breeding method for genetic improvement of both traits by utilizing the correlation between the desired trait and selected trait (Falconer and Mackay, 1996). Studies on the direct selection of body weight closely related to yield have been initiated in oysters. Newkirk and Haley (1982) and Toro et al. (1996) reported that the genetic gain of body weight in European oysters (*Ostrea edulis*) and Chilean oysters was 23% and 10–13% after one generation of selection, respectively. Although the genetic correlation coefficient between the shell height and body weight of *C. nippona* has not been reported, positive correlations have been demonstrated in previous selective breeding programmes for important economic shellfish, such as Pacific oyster and bay scallop (*Argopecten irradians*) (de Melo et al., 2019; Zhang et al., 2019; Wang et al., 2020). In this study, due to the slow-growing characteristic and a shortened optimal growing season (Mao et al., 2006; Xu et al., 2019a), *C. nippona* was completely stripped off from the substrate for accurate weight measurement at about 420 days and the average genetic gain of body weight in three selected lines was 11.07–12.46%. The results showed that the body weight also obtained positive progress during artificial selection for fast growth, implying that there was a positive correlation between the two growth traits. Because the correlation between traits can be divided into genetic correlation and environmental correlation, and the results of the indirect selection are determined by genetic correlation, environmental conditions and developmental stages (Falconer and Mackay, 1996). Therefore, it is necessary to further explore the correlation between economic characters to apply indirect selection in the mass selection and improve the efficiency of the breeding programmes in *C. nippona*.

5. Conclusion

In short, applying the intense selection pressures to genetic improvement for fast growth of *C. nippona* can obtain continuous and stable progress over three mass-selected generations, which can achieve an average improvement of approximately 10% per generation in the growth trait. Simultaneously, the estimates of genetic parameters in shell height are all at the relatively high level in three breeding populations indicating that a large amount of genetic variation is still maintained in the selected line and there is a potential for the future selective breeding in growth traits. In addition, the body weight of *C. nippona* also obtains a remarkable improvement during successive mass selection for fast growth implying that there is a positive correlation between the two production traits. The results obtained in this study further demonstrate the feasibility of improving the growth traits through mass selection for *C. nippona* and provide important information for further genetic improvement.

Credit author statement

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

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

The authors do not have permission to share data.

Acknowledgments

This work was supported by the Agriculture Research System of China Project (CARS-49), and Fund for Agriculture Seed Improvement Project of Shandong Province (2020LZGC016, 2021LZGC027).

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