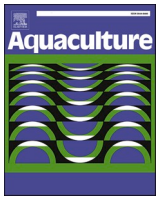
Aquaculture 553 (2022) 738083

Contents lists available at ScienceDirect

Aquaculture

journal homepage: www.elsevier.com/locate/aquaculture



Short communication

The growth, survival and ploidy of diploid, triploid and tetraploid of the Pacific oyster (*Crassostrea gigas*) in larval and juvenile stages

Yongguo Li [a], Qi Li [a,b,*]

[a] Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao 266003, China
[b] Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, Qingdao 266237, China

ARTICLE INFO

Keywords:
Crassostrea gigas
Tetraploid
Survival
Growth
Genomic stability

ABSTRACT

Slow growth and genomic instability are the main problems facing tetraploid oyster breeding. In response to the above problem, we developed tetraploid by using *Crassostrea gigas* 'Haida No.3', a new variety with rapid growth and black shell color, and analyzed the growth, survival and ploidy of diploid ♀ × diploid ♂ (DD), diploid ♀ × tetraploid ♂ (DT: triploid) and tetraploid ♀ × tetraploid ♂(TT) in different periods. The results showed that there was no significant difference between the fertilization rate of DD, DT and TT, but the hatching rate of TT was significantly lower than that of DD ($P < 0.05$). The shell height of TT was significantly lower than DD, and the shell height of DT was significantly higher than that of DD at 13 days ($P < 0.05$). The survival rate of TT was lower than that of DD and DT in larval stage ($P < 0.05$), while there was no significant difference in the mean survival rate of DD, DT and TT in juvenile stage. The tetraploid rate in TT was 100% during the larval and juvenile stages. The shell color of all progeny in TT and DT was black and consistent with *C. gigas* 'Haida No.3'. These results indicated that black shell color of *C. gigas* 'Haida No.3' could be stably passed on to triploid and tetraploid offspring, suggesting that establishing genomically stable tetraploid populations was feasible by screening tetraploid parents.

1. Introduction

Polyploid oysters were first reported in 1981, and the triploid oyster industry has been developing rapidly in the last two decades (Matt and Allen, 2014; Nell, 2002; Stanley et al., 1981). Triploid oysters play an important role in improving oyster yields and economic benefits because of their sterility and better growth performance (Buestel et al., 2009; Hand et al., 2004; Jeung et al., 2016; Nell, 2002; Normand et al., 2008; Piferrer et al., 2009). Triploid was initially produced by physical or chemical induction, but the high mortality and no guarantee of 100% triploid rates made it difficult to apply in commercial production (Gérard et al., 1999; Guo et al., 1992; Quillet and Panelay, 1986; Scarpa et al., 1994; Yang and Guo, 2006; Yamamoto and Sugawara, 1988). The crossing of tetraploid and diploid can produce 100% triploid, which is an ideal way for triploid to be applied in large-scale production (Guo and Allen, 1994a; Guo et al., 1996). However, when oyster producers are immersed in the acquisition of tetraploid oysters, thinking that they can get the tetraploids once and for all, they do not realize that there might be a problem with the tetraploid itself.

The growth rate of tetraploid oyster is not satisfactory (Guo et al., 1996). Tetraploid oysters grow more slowly than diploid oysters, contrary to previous studies that growth performance is usually enhanced by an increase in the genome (Stanley et al., 1984; Wang et al., 2002). For example, triploid production performance is usually better than diploid (Wadsworth et al., 2019). As the core asset of triploid production, slow-growing tetraploid oysters will inevitably affect the growth performance of their triploid offspring. Previous studies showed that the superior traits of the selected population can be passed on to the polyploid offspring (Guo et al., 2002; Leeds and Weber, 2019). However, when oyster producers focused on getting tetraploid oyster as soon as possible, they ignored the influence of breeding population traits on polyploid offspring. Fortunately, tetraploid oysters are fertile (Guo et al., 1996; Guo and Allen, 1997) and their performance can be improved though breeding, though this will undoubtedly require extra time. There is no doubt that the preferred method for producing tetraploids is to use populations with rapid growth, disease resistance or other important traits as base populations.

Even if slow-growing tetraploid oysters are acceptable, genomic instability is intolerable. Mosaicism was first described in oysters more than 20 years ago (Allen et al., 1996) and cytogenetic mechanism of

* Corresponding author at: Key Laboratory of Mariculture, Ministry of Education, Ocean University of China, Qingdao, China.
E-mail address: qili66@ouc.edu.cn (Q. Li).

https://doi.org/10.1016/j.aquaculture.2022.738083
Received 9 December 2021; Received in revised form 20 February 2022; Accepted 25 February 2022
Available online 28 February 2022

transformation from triploid *Crassostrea gigas* to mosaic type has also been described by Zhang et al. (2010). This suggested that genomic instability in oyster polyploids exists. Genomic or chromosomal instability could lead to aneuploidy and mosaic individuals, posing a serious challenge to establishing stable tetraploid lines. The presence of aneuploidy in the offspring of tetraploid x tetraploid might indicate that part of tetraploid oysters suffers from reversion (Benabdelmouna and Ledu, 2015). Therefore, it is worth considering whether the stable tetraploid lines can be established by selecting tetraploids whose chromosomes are not reverted.

As a new variety of multi-generation selection, 'Haida No.3' variety of *C. gigas* with black shell color and rapid growth is an ideal material for producing tetraploids (Xu et al., 2017; Xu et al., 2020), having the potential to improve the tetraploid growth performance. Through our previous work, we have successively produced the tetraploids of *C. gigas* 'Haida No.3' with black shell color. However, the inheritance of production traits in polyploid offspring is not clear. In order to assess the production performance among diploid, triploid and tetraploid ploidy of *C. gigas*, we compared and analyzed their insemination, growth, survival and ploidy. In addition, we improved the detection method of tetraploid parents to establish a stable tetraploid population.

2. Material and methods

2.1. Broodstock

'Haida No.3' variety of *C. gigas* was originally derived from the black-shell individuals discovered in the cultured population in 2010. After seven generations of growth improvement and shell color fixation, 'Haida No.3' variety of *C. gigas* with rapid growth and black shell color was successfully developed (Xu et al., 2017; Xu et al., 2020). The tetraploid broodstock were produced using eggs from triploids of 'Haida No. 3' variety of *C. gigas* that were fertilized with haploid sperm taken from diploids of 'Haida No.3' variety of *C. gigas*.

In May 2021, the one-year-old diploid and tetraploid parents used in this study were transferred to oyster hatchery in Laizhou (Shandong Province, China) three weeks before the experiment started. During the temporary rearing period, all broodstock were conditioned at the temperature of 24 ± 1 °C and salinity of 30 ± 1 psu and fed with *Nitzschia closterium*.

2.2. Cross

The ploidy of all tetraploid oysters was examined before choosing as tetraploid parents. Thirteen males and nine females of each diploid and tetraploid parents were strip spawned. For tetraploid (T), eggs from nine females were pooled in a 5-L bucket at a ratio of 50 spermatozoids per oocyte. For diploid (D), eggs from nine females were mixed divided equally into two 5-L buckets, and then fertilized with a mixture of sperm from thirteen diploid or tetraploid males with a ratio of 50 spermatozoids per oocyte. The crosses consist of three different combinations: diploid ♀ × diploid ♂ (DD), diploid ♀ × tetraploid ♂ (DT), and tetraploid ♀ × tetraploid ♂ (TT). The fertilized eggs were filtered to remove excess sperm, and incubated in a 1000-L tank with a density of 10–20 eggs/mL. Each mating combination was set with three replicates.

2.3. Larval rearing and spat grow-out

The D-larvae of all mating combinations collected from their hatching tank were transferred into 1000 L tanks for larval rearing, respectively, with an initial larval density of 2 larvae mL^{-1}. The water temperature was maintained at 25 ± 1 °C with a salinity of 30 ± 1 psu during the larval stage. Larval rearing was conducted according to the standard procedure described by Li et al. (2011). The D-larvae were fed with *Isochrysis galbana* before the umbo-stage, after which the larvae were fed with a mixture of *I. galbana* and *Platymonas* sp. When 30% larvae reached eyespot-stage, strings of scallop shells were hung as substrates for settlement. The settled spats were transported to a 24-m^3 tank for three-week temporary rearing. During the period of temporary cultivation, the water was completely changed twice a day. Then spats were transferred to Sanggou Bay, Shandong Province (37.11° N, 122.35° E) for farming in July 2021. Three lantern nets with three hundred spats were set for each mating combination.

2.4. Ploidy analysis

Hemolymph (nonlethal) and gills were used to detect tetraploid parental ploidy. Hemolymph was drawn from the adductor muscle using a syringe for the first ploidy test. Gill was used in the second ploidy test after dissection. Tetraploids with consistent results from the two ploidy tests were used in this study. Larvae were tested for ploidy using at least 3000 nuclei. Spats and juveniles were tested for ploidy using gills. The gills and larvae needed to be made into single-cell suspensions, and then the filtered cells were stained in DAPI and detected by flow cytometry (Beckman CytoFLEX).

2.5. Measurement and date analysis

Two hundred diploid eggs and two hundred tetraploid eggs were collected to measure their egg diameters, respectively. Fertilization, hatching rates, survival rate and the shell height in larval stage were measured according to Jiang et al. (2021). The hatching rate is the percentage of the number of D-shaped larvae (24 h of insemination) to the number of fertilized eggs. The larval shell height of 30 larvae were measured by Image-ProPlus image analysis software 6.0. The shell height and survival rate of juvenile were measured according to Han et al. (2020). SPSS statistical package 23.0 was used for statistical analysis. GraphPad Prism 8 software was used for mapping. Statistical difference was determined at $P < 0.05$.

3. Results

3.1. Hatching index

The mean diameter of tetraploid eggs (73.83 ± 3.86 μm) was significantly larger than that of diploid eggs (56.32 ± 2.86 μm), with the value of 31.07% ($P < 0.05$) (Table 1). The mean fertilization rate of DD, DT and TT group was 98.56 ± 0.35%, 97.69 ± 0.29% and 96.64 ± 1.53%, respectively (Table 1). No significant differences in the fertilization were observed among the three combinations ($P > 0.05$). The hatching rate of DD, DT and TT cross was 90.97 ± 1.37%, 50.66 ± 8.09% and 60.34 ± 2.47%, respectively. The hatching rate of DD group was significantly higher than that of DT and TT groups ($P < 0.05$).

3.2. Growth and survival of larvae and juvenile

The mean shell heights of newly hatched D-larvae were 64.38 ± 3.19 μm in DD group, 68.65 ± 2.98 μm in DT group and 87.17 ± 4.45 μm in TT group (Fig. 1). At day 7, the mean shell heights of DD and DT groups were significantly larger than that of TT group, followed the order of DT (149.17 ± 11.41 μm) > DD (146.82 ± 11.55 μm) > TT (105.65 ± 6.13 μm), while no significant difference was observed

Table 1
Hatching index of diploid × diploid (DD), diploid × tetraploid (DT) and tetraploid × tetraploid (TT) crosses of *C. gigas*. Different superscript letters in each column indicate significant difference ($P < 0.05$).

Cross	Egg diameter (μm)	Cleaved rate (%)	Hatching rate (%)
DD	56.32 ± 2.86 [b]	98.56 ± 0.35 [a]	90.97 ± 1.37 [a]
DT	–	97.69 ± 0.29 [a]	50.66 ± 8.09 [b]
TT	73.83 ± 3.86 [a]	96.64 ± 1.53 [a]	60.34 ± 2.47 [b]

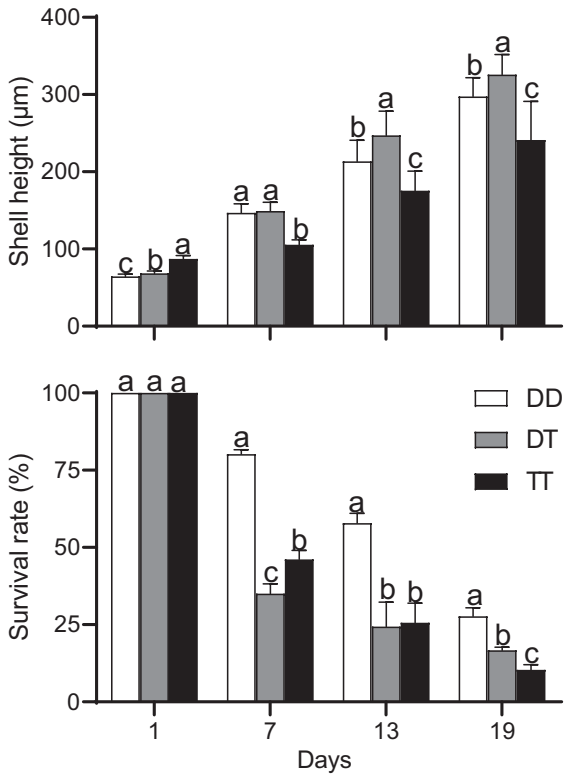


Fig. 1. Survival rate and shell height of diploid x diploid (DD), diploid × tetraploid (DT) and tetraploid × tetraploid (TT) crosses of *Crassostrea gigas* during the larval stage. Different lowercase letters indicate significant differences ($P < 0.05$).

between DD and DT groups ($P > 0.05$). From day 13, the mean shell height of DT (247.29 ± 31.18 μm) was significantly higher than that of DD (213.53 ± 27.22 μm) and TT (175.36 ± 25.42 μm), and that of DD was significantly higher than that of TT ($P < 0.05$). From 50 days to 120 days, the mean shell height of crosses DD, DT and TT increased by 28.26 mm, 31.73 mm and 17.16 mm, respectively (Table 2). The mean shell height of DT cross was significantly higher than that of DD and TT crosses ($P < 0.05$), and the mean shell height of DD cross was significantly higher than that of TT cross ($P < 0.05$).

During the larval stage, the survival rates of DD, DT and TT crosses at day 19 dropped to 27.73%, 16.74% and 10.41%, respectively (Fig. 1). The survival rate of DD cross was higher than that of DT and TT crosses throughout the larval period. At day 7, the survival rate of DT and TT occurred a sharp decrease and the survival rate of TT cross was higher than that of DT ($P < 0.05$), while the survival rate of DT was higher than that of TT at day 19 ($P < 0.05$). In contrast to the low survival rate of larval stage, DD (88.65%), DT (87.74%) and TT (87.84%) had a high survival rate between 50 and 120 days (Table 2).

Table 2
Shell height and survival rate of diploid × diploid (DD), diploid × tetraploid (DT) and tetraploid × tetraploid (TT) crosses of *C. gigas* on days 50 and 120. Different superscript letters in each column indicate significant difference ($P < 0.05$).

Cross	Shell height (mm)		Survival rate (%)
	50 d	120 d	120 d
DD	4.47 ± 0.89 ^{b}	32.73 ± 3.17 ^{b}	88.65 ± 3.72 ^{a}
DT	5.83 ± 1.39 ^{a}	37.56 ± 2.08 ^{a}	87.74 ± 2.46 ^{a}
TT	3.62 ± 0.47 ^{c}	20.78 ± 5.14 ^{c}	87.84 ± 3.50 ^{a}

3.3. Ploidy and shell color

The triploid and tetraploid rates of DT and TT crosses were 100% in larval and juvenile stages, respectively (Fig. 2). Triploid and tetraploid progenies showed black shell color, being the same as diploid *C. gigas* 'Haida No. 3' (Fig. 3).

4. Discussion

4.1. Hatching index

A large number of malformed larvae in DT and TT crosses was the main reason for the low hatching rate, while there were almost no malformed larvae in DD cross, suggesting that the abnormal larvae in DT cross were caused by the increase of intracellular chromosome. The malformed larvae in the TT cross might be the offspring of the first-generation of tetraploid and tetraploid hybrids, which have not yet adapted to the doubling of the chromosome (Comai, 2005). Low hatching rates of tetraploids have also been reported in previous studies (Guo et al., 1996; Zhang et al., 2022). The development of tetraploids based on selective breeding strains might be beneficial to the survival of their offspring, but the adverse effects of chromosome doubling still played an important role in the hatchability of offspring, such as more complex pairing and segregation interactions leading to mitotic abnormalities (Comai, 2005).

4.2. Growth and survival of larvae and juvenile

Considering that almost all larvae in DD cross are D-shaped larvae when DT cross is still largely composed of trochophore larvae, the most likely reason for the difference in the size of newly hatched D-shaped larvae between the DT and DD crosses was that the triploid oyster cells were larger than the diploid ones, or more precisely, it's caused by an increase in cell size without a decrease in cell number (Guo and Allen, 1994b). In oysters, triploid growth usually exceeds diploid growth after a year, when diploids begin to allocate large amounts of energy resources to reproduction (Stanley et al., 1984; Allen and Downing, 1986). In this study, the mean shell height of triploid oysters was always higher than that of diploid oysters from day 13. This might be related to the increased heterozygosity of the triploid gene (Wang et al., 2002). More importantly, triploids might inherit superior growth performance from their parents (Leeds and Weber, 2019). Tetraploid oysters could inherit the excellent production performance of their parents. For example, tetraploid oysters could inherit disease resistance (Guo et al., 2002). In this study, tetraploid offspring still remained black shell color, indicating that black shell color could be inherited. Rapid growth and black shell color are traits specific to 'Haida No.3' of *C. gigas*, which mean that rapid growth may also be inherited, just like black shell color.

Generally, survival or vigor of neopolyploids was low in synthetic populations (Gaeta et al., 2007; Matsushita et al., 2012). It has also been reported that the survival rate of tetraploid aquatic animals was lower than that of diploid animals (Cassani et al., 1990; Hörstgen-Schwark, 1993; Zhou et al., 2010). In this study, the survival rate of tetraploid larvae was found to be higher than that of previous study (Guo et al., 1996). This might mean that selective breeding strain for the development of tetraploids is beneficial to improve the survival performance of tetraploids. In the present study, the differences of survival rate between DD and TT groups in larval and juvenile stages suggested that tetraploids adapted to chromosome changes could survive like diploids.

4.3. Ploidy and shell color

If chromosome loss of tetraploid oysters was real, and not due to the testing method, then tetraploid oyster genome instability could be divided into the following two situations. Firstly, tetraploid oyster genome instability is general, but the timing of chromosomal loss is

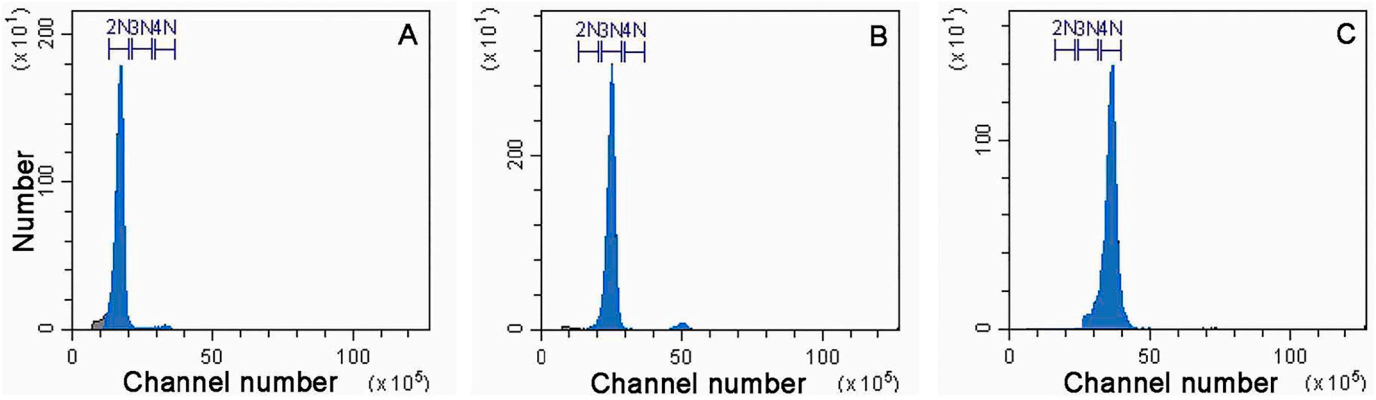


Fig. 2. Flow cytometry graphs of offspring in diploid × diploid (DD), diploid × tetraploid (DT) and tetraploid × tetraploid (TT) crosses of *C. gigas* at day 120. A, DD offspring; B, DT offspring; C, TT offspring.

Fig. 3. The offspring of diploid × diploid (DD), diploid × tetraploid (DT) and tetraploid × tetraploid (TT) crosses of *C. gigas* at day 160.

different in different tetraploids. In this case, the tetraploid oyster genome is relatively stable until the chromosomes started to decay. Secondly, the genome instability of tetraploid oyster is not general and part of the tetraploid oyster genome is always stable. Either way, genomically stable tetraploids exist. Therefore, selecting tetraploids with stable genomes to establish a tetraploid population would be important for improving tetraploid performance. In this study, the tetraploid rate in the TT cross was always 100%, proving that establishing genome-stable tetraploid population by screening tetraploid parents was feasible to some extent. The secretion and distribution of black pigmentation are controlled by two independent loci (Xu et al., 2019). And black pigmentation is identified as the foreground color, while gold and white are the background colors (Ge et al., 2015). The shell color of the offspring of 'Haida no. 3' of *C. gigas* is normally black, but there is a possibility that ploidy changes may affect the expression of genes related to the shell color of the offspring. In this study, Triploids in DT cross and tetraploids in TT cross still retained the same black shell color as *C. gigas* 'Haida No.3', suggesting that the ploidy change did not affect the trait of black shell color. This proves that the polyploid development of selectively bred diploid strain with important economic traits had great potential in the oyster industry.

5. Conclusions

The growth, survival and ploidy of diploid, triploid and tetraploid of *C. gigas* 'Haida No.3' were studied. The significant differences in survival rate of different ploidy only appeared in larval stage. Triploids had a faster growth rate than diploids and tetraploids. Black-shell color trait of diploid *C. gigas* 'Haida No.3'could be inherited stably in both triploid and tetraploid offspring. It is feasible to establish genomically stable tetraploids by screening tetraploid parents. This study provides meaningful implications for the development of tetraploids with important economic traits and the establishment of genomic stability of tetraploids.

Credit author statement

Yongguo Li: Investigation, methodology, completion of the

experiment, software, data analysis, formal analysis, writing-original draft, writing-review. **Qi Li**: Conceptualization, experimental design, coordination and manuscript revision, visualization, supervision, review-editing, funding acquisition, project administration.

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.

Acknowledgments

This work was supported by the grants from National Natural Science Foundation of China (31972789), the China Agriculture Research System Project (CARS-49), Earmarked Fund for Agriculture Seed Improvement Project of Shandong Province (2020LZGC016), Industrial Development Project of Qingdao City (20-3-4-16-nsh), and Science and Technology Development Project of Weihai City (2018NS01).

References

Allen, S.K., Downing, S.L., 1986. Performance of triploid Pacific oysters, *Crassostrea gigas*. I. Survival, growth, glycogen content, and sexual maturation in yearlings. J. Exp. Mar. Biol. Ecol. 102, 197–208.

Allen, S.K., Guo, X.M., Burreson, G., Mann, R., 1996. Heteroploid mosaics and reversion among triploid oysters, *Crassostrea gigas*. Fact or artifact. J. Shellfish Res. 15, 514–522.

Benabdelmouna, A., Ledu, C., 2015. Autotetraploid Pacific oysters (*Crassostrea gigas*) obtained using normal diploid eggs: induction and impact on cytogenetic stability. Genome 58, 333–348.

Buestel, D., Ropert, M., Prou, J., Goulletquer, P., 2009. History, status, and future of oyster culture in France. J. Shellfish Res. 28, 813–820.

Cassani, J.R., Maloney, D.R., Allaire, H.P., Kerby, J.H., 1990. Problems associated with tetraploid induction and survival in grass carp, *Ctenopharyngodon idella*. Aquaculture 88, 273–284.

Comai, L., 2005. The advantages and disadvantages of being polyploid. Nat. Rev. Genet. 6, 836–846.

Gaeta, R.T., Pires, J.C., Iniguez-Luy, F., Leon, E., Osborn, T.C., 2007. Genomic changes in resynthesized *Brassica napus* and their effect on gene expression and phenotype. Plant Cell 19, 3403–3417.

Ge, J.L., Li, Q., Yu, H., Kong, L.F., 2015. Mendelian inheritance of golden shell color in the Pacific oyster *Crassostrea gigas*. Aquaculture 441, 21–24.

Gérard, A., Ledu, C., Phélipot, P., Naciri-Graven, Y., 1999. The induction of MI and MII triploids in the Pacific oyster *Crassostrea gigas* with 6-DMAP or CB. Aquaculture 174, 229–242.

Guo, X.M., Allen, S.K., 1994a. Viable tetraploids in the Pacific oyster (*Crassostrea gigas* Thunberg) produced by inhibiting polar body I in eggs from triploids. Mol. Mar. Biol. Biotechnol. 3, 42–50.

Guo, X.M., Allen, S.K., 1994b. Sex determination and polyploid gigantism in the dwarf surfclam (*Mulinia lateralis* Say). Genetics 138, 1199–1206.

Guo, X.M., Allen, S.K., 1997. Sex and meiosis in autotetraploid Pacific oyster, *Crassostrea gigas* (Thunberg). Genome 40, 397–405.

Guo, X.M., Cooper, K., Hershberger, W.K., Chew, K.K., 1992. Genetic consequences of blocking polar body I with cytochalasin B in fertilized eggs of the Pacific oyster, *Crassostrea gigas*: I. Ploidy of resultant embryos. Biol. Bull. 183, 381–386.

Guo, X.M., DeBrosse, G.A., Allen, S.K., 1996. All-triploid Pacific oysters (*Crassostrea gigas* Thunberg) produced by mating tetraploids and diploids. Aquaculture 142, 149–161.

Guo, X.M., Wang, J., Landau, B.J., Li, L., DeBrosse, G.A., Buono, K.D., 2002. The successful production of tetraploid eastern oyster, *Crassostrea virginica* Gmelin. J. Shellfish Res. 21, 380–381 (abstract).

Han, Z.Q., Li, Q., Liu, S.K., Kong, L.F., 2020. Crossbreeding of three different shell color lines in the Pacific oyster reveals high heterosis for survival but low heterosis for growth. Aquaculture 529, 735621.

Hand, R.E., Nell, J.A., Thompson, P.A., 2004. Studies on triploid oysters in Australia XIII. Performance of diploid and triploid Sydney rock oyster, *Saccostrea glomerata* (Gould, 1850), progeny from a third generation breeding line. Aquaculture 233, 93–107.

Hörstgen-Schwark, G., 1993. Initiation of tetraploid breeding line development in rainbow trout, *Oncorhynchus mykiss* (Walbaum). Aquac. Res. 24, 641–652.

Jeung, H.D., Keshavmurthy, S., Lim, H.J., Kim, S.K., Choi, K.S., 2016. Quantification of reproductive effort of the triploid Pacific oyster, *Crassostrea gigas* raised in intertidal rack and bag oyster culture system off the west coast of Korea during spawning season. Aquaculture 464, 374–380.

Jiang, G.W., Li, Q., Xu, C.X., Liu, S.K., Kong, L.F., Yu, H., 2021. Reciprocal hybrids derived from *Crassostrea gigas* and *C. angulata* exhibit high heterosis in growth, survival and thermotolerance in northern China. Aquaculture 545, 737173.

Leeds, T.D., Weber, G.M., 2019. Effects of triploidy on genetic gains in a rainbow trout (*Oncorhynchus mykiss*) population selectively bred for diploid growth performance. Aquaculture 505, 481–487.

Li, Q., Wang, Q.Z., Liu, S.K., Kong, L.F., 2011. Selection response and realized heritability for growth in three stocks of the Pacific oyster *Crassostrea gigas*. Fisheries Sci. 77, 643–648.

Matsushita, S.C., Tyagi, A.P., Thornton, G.M., Pires, J.C., Madlung, A., 2012. Allopolyploidization lays the foundation for evolution of distinct populations: evidence from analysis of synthetic *Arabidopsis* allohexaploids. Genetics 191, 535–547.

Matt, J.L., Allen, S.K., 2014. Heteroploid mosaic tetraploids of *Crassostrea virginica* produce normal triploid larvae and juveniles as revealed by flow cytometry. Aquaculture 432, 336–345.

Nell, J.A., 2002. Farming triploid oysters. Aquaculture 210, 69–88.

Normand, J., Le Pennec, M., Boudry, P., 2008. Comparative histological study of gametogenesis in diploid and triploid Pacific oysters (*Crassostrea gigas*) reared in an estuarine farming site in France during the 2003 heatwave. Aquaculture 282, 124–129.

Piferrer, F., Beaumont, A., Falguiere, J.C., Flajshans, M., Haffray, P., Colombo, L., 2009. Polyploid fish and shellfish: Production, biology and applications to aquaculture for performance improvement and genetic containment. Aquaculture 293, 125–156.

Quillet, E., Panelay, P.J., 1986. Triploidy induction by thermal shocks in the Japanese oyster, *Crassostrea gigas*. Aquaculture 57, 271–279.

Scarpa, J., Toro, J.E., Wada, K.T., 1994. Direct comparison of six methods to induce triploidy in bivalves. Aquaculture 119, 119–133.

Stanley, J.G., Allen, S.K., Hidu, H., 1981. Polyploidy induced in the American oyster, *Crassostrea virginica*, with cytochalasin B. Aquaculture 23, 1–10.

Stanley, J.G., Hidu, H., Allen, S.K., 1984. Growth of American oysters increased by polyploidy induced by blocking meiosis I but not meiosis II. Aquaculture 37, 147–155.

Wadsworth, P., Wilson, A.E., Walton, W.C., 2019. A meta-analysis of growth rate in diploid and triploid oysters. Aquaculture 499, 9–16.

Wang, Z.P., Guo, X.M., Allen, S.K., Wang, R.C., 2002. Heterozygosity and body size in triploid Pacific oysters, *Crassostrea gigas* Thunberg, produced from meiosis II inhibition and tetraploids. Aquaculture 204, 337–348.

Xu, L., Li, Q., Yu, H., Kong, L.F., 2017. Estimates of heritability for growth and shell color traits and their genetic correlations in the black shell strain of Pacific oyster *Crassostrea gigas*. Mar. Biotechnol. 19, 421–429.

Xu, C.X., Li, Q., Yu, H., Liu, S.K., Kong, L.F., Chong, J.D., 2019. Inheritance of shell pigmentation in Pacific oyster *Crassostrea gigas*. Aquaculture 512, 734249.

Xu, C.X., Li, Q., Chong, J.D., 2020. Combined effect of temperature, salinity, and rearing density on the larval growth of the black shell strain and wild population of the Pacific oyster *Crassostrea gigas*. Aquacult. Int. 28, 335–347.

Yamamoto, S., Sugawara, Y., 1988. Induced triploidy in the mussel, *Mytilus edulis*, by temperature shock. Aquaculture 72, 21–29.

Yang, H.P., Guo, X.M., 2006. Polyploid induction by heat shock-induced meiosis and mitosis inhibition in the dwarf surfclam, *Mulinia lateralis* Say. Aquaculture 252, 171–182.

Zhang, Q.Q., Yu, H.Y., Howe, A., Chandler, W., Allen, S.K., 2010. Cytogenetic mechanism for reversion of triploids to heteroploid mosaics in *Crassostrea gigas* (Thunberg) and *Crassostrea ariakensis*. Aquac. Res. 41, 1658–1667.

Zhang, Y.H., Qin, Y.P., Yu, Z.N., 2022. Comparative study of tetraploid-based reciprocal triploid Portuguese oysters, *Crassostrea angulata*, from seed to marketsize. Aquaculture 547, 737523.

Zhou, X.Y., Abbas, K., Li, M.Y., Fang, L.B., Li, S., Wang, W.M., 2010. Comparative studies on survival and growth performance among diploid, triploid and tetraploid dojo loach *Misgurnus anguillicaudatus*. Aquac. Int. 18, 349–359.