



Short communication

Effects of salinity and temperature on growth and survival of diploid and tetraploid larvae of the Pacific oyster, *Crassostrea gigas*

Yongguo Li^a, Chengxun Xu^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

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ABSTRACT

Tetraploid of *Crassostrea gigas* was first reported in the 1990s, but its biological characteristics are seldomly known. To understand the adaptation of tetraploid larvae to salinity and temperature, the effects of salinity treatments (10, 15, 20, 25 and 30 psu) and temperature treatments (18, 23 and 28 °C) on the growth and survival of tetraploid and diploid larvae were comparatively studied. In salinity treatments, the accumulative survival rate (ASR) and relative growth rate (RGR) of tetraploid larvae were generally lower than that of diploid larvae ($P < 0.05$). The maximum ASR and RGR of tetraploid larvae occurred at 30 psu, with the values of 41.09% and 152.91%, respectively. At day 16, the maximum difference in ASR between diploid and tetraploid larvae was 36.41% at 20 psu, and the maximum difference in RGR of diploid and tetraploid larvae was 62.36% at 10 psu. The ASR and RGR of tetraploid larvae were lower than that of diploid larvae under temperature treatments ($P < 0.05$). The maximum ASR and RGR of tetraploid larvae occurred at 28 °C, with the values of 19.67% and 235.40%, respectively. The ASR of both diploid and tetraploid larvae decreased sharply in the first four days. The RGR of larvae at 18 °C was much lower than that at 23 °C and 28 °C ($P < 0.05$). Hence, a salinity range of 25–30 psu and a temperature range of 23–28 °C are suitable culture conditions for tetraploid larvae. These results indicate that the natural environment conditions can meet the temperature and salinity requirements for the survival and growth of tetraploid larvae. However, tetraploid larvae are less adaptable to temperature and salinity than diploid larvae. The environmental adaptability of tetraploid needs further improvement.

1. Introduction

Tetraploid oysters play an important role in the modern oyster industry, especially contribute to the culture of triploid oysters (Guo et al., 1996). Due to the odd number of chromosomes, triploid is sterile in theory. However, there are still a few triploid oysters with a certain degree of gonad development, which provides an effective way to obtain tetraploid oysters (Thorgaard, 1983; Guo and Allen, 1994; Normand et al., 2008). The earliest reported tetraploid oysters were obtained by inhibiting the polar body I in the eggs of the hybridization of the triploid oysters with the diploid male, based on the partial fertility of the triploid oyster (Guo and Allen, 1994). Another method, avoiding the triploid step and inhibiting the polar body I in diploid females cross with diploid males, has also been reported (Benabdelmouna and Ledu, 2015; Qin et al., 2021). Additionally, tetraploid oysters also can be generated by inhibiting the polar body II during hybridization between diploid females and tetraploid males (McCombie et al., 2005). Once tetraploids

are acquired, it is easy to obtain a considerable number of tetraploid progenies by virtue of their fertility.

Tetraploid oysters have become a hotspot in polyploid research, because crossing diploids and tetraploids can produce 100% triploids (Guo et al., 1996). Triploids are usually sterile or partially fertile, which allows them to remain available for market during the reproductive season (Allen and Downing, 1986; Desrosiers et al., 1993; Piferrer et al., 2009; Nell, 2002), which fills the gap when diploid oysters are not available in the market in summer. Although triploid oysters can also be obtained by physical or chemical treatment, these methods have imperfections such as unstable triploid rate and low survival rate, which cannot meet the needs of triploid industrialization (Gérard et al., 1999). Therefore, a stable tetraploid oyster population becomes the important guarantee for commercial production of triploid oyster.

There are no technical obstacles for the production of tetraploid oysters. The research focus of tetraploid oysters has been shifted from how to obtain tetraploid oysters to how to make them survive better. The

* 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).

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characteristics of tetraploid oyster populations have been studied. There is a phenomenon that the genetic diversity of tetraploid population decreases during two successive generations of breeding (Miller et al., 2014). Heteroploid mosaic tetraploids have also been found in tetraploids (McCombie et al., 2005). However, there is no difference between triploids from tetraploids and triploids from aneuploids (Matt and Allen, 2014), which indicates that aneuploid tetraploids can be used in triploid production instead of tetraploid breeding. The application of aneuploid tetraploids in triploid production is particularly important for the triploid industry, because it can reduce the use of tetraploid as broodstock, and save the time to distinguish tetraploid from aneuploid.

Although the tetraploid of Pacific oyster *C. gigas* was first reported in the 1990s (Guo and Allen, 1994), the adaptation of tetraploid larvae to temperature and salinity has not been documented. Environmental factors, especially the salinity and temperature have a significant impact on aquatic animals (Fearman and Moltschaniwskyj, 2010; Fuhrmann et al., 2016; Gagnaire et al., 2006; Pourmozaffar et al., 2020). These factors directly determine whether tetraploid oyster can exist stably and have breeding potential as a new population or not. Although the production performance of triploid oysters is generally superior to that of the diploid oyster (Wadsworth et al., 2019), increased ploidy of polyploidy is not necessarily beneficial to environmental adaptation. The survival rate of tetraploid larvae of *C. gigas* was much lower than that of diploid larvae (Guo et al., 1996). In order to facilitate the development of hatchery techniques for seed production of tetraploid oyster, the present study assessed the temperature and salinity adaptation of tetraploid larvae compared to diploid larvae.

2. Material and methods

2.1. Animals

The parents of diploid and tetraploid larvae were derived from the same selected strain “Haida No. 3” of *C. gigas* (Fig. 1) using black shell color and growth as breeding traits (Xu et al., 2017). The tetraploid broodstock were produced using eggs from triploids that were fertilized with haploid sperm taken from diploids. Both the diploid and tetraploid broodstock were produced in June 2020 in Laizhou, Shandong Province, China.

In June 2021, the diploid and tetraploid broodstock were moved to oyster hatchery in Laizhou for conditioning during three weeks. These broodstock were held in 24-m³ tank of seawater (temperature 24 ± 1 °C; salinity 30 ± 1 psu). The ploidy of tetraploids was identified prior to spawning. Once the oysters were reproductively mature, gametes from 20 individuals (10 males and 10 females) obtained by stripping were mixed in a 5-L bucket at a ratio of 50 spermatozooids per oocyte. The embryos were put into a 24-m³ tank for hatching. After 24-h incubation, the diploid and tetraploid D-larvae were collected from their hatching tank.

2.2. Experiment design

Five levels of salinity (10, 15, 20, 25 and 30 psu) and three levels of temperature (18, 23 and 28 °C) were set to investigate the salinity and temperature adaptation of tetraploid larvae, which were determined according to the range of environmental condition of the Yellow sea (Chu et al., 2005).

The D-larvae of diploid and tetraploid oyster collected from their hatching tank (temperature 25 °C; salinity 30 psu) were transferred into a volume of 60 L tanks with an initial larval density of 1–2 ind/mL. The ploidy of tetraploid larvae was tested by flow cytometry (Beckman CytoFLEX) to ensure that the tetraploid rate of experimental larvae was 100%. Seawater of all temperatures and salinity levels were prepared in advance. Test salinity and temperature were gradually adjusted by 1/3 water changes for four times and a full water change over three days. The salinity was at 30 psu in the temperature treatments, and the temperature was at 25 °C in the salinity treatments. Three replicates were set up. Larvae were fed with *Isochrysis galbana* and 1/3 water was replaced every day.

2.3. Measurement of relative growth rate and accumulative survival rate

The relative growth rate (RGR) and accumulative survival rate (ASR) were measured on day 4, 8, 12 and 16. Thirty randomly selected individuals from each tank were used to estimate RGR. The larval counts and the shell height measurements were taken by Image-ProPlus image analysis software 6.0. The RGR and ASR were calculated according the following formula:

$$\text{RGR (\%)} = \frac{H_t - H_0}{H_0} \times 100\%$$

Where H_t and H_0 are the average larval shell height at the beginning of the experiment and the average larval shell height at time t , respectively.

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

Where N_t and N_0 are the number of larvae at the beginning of the experiment and at time t , respectively.

2.4. Data analysis

SPSS statistical package 23.0 was used for statistical analysis. GraphPad Prism 8 software was used for mapping. Statistical difference was determined at $P < 0.05$.



Fig. 1. The selected strain “Haida No. 3” of *Crassostrea gigas* used in the study.

3. Results

3.1. Effects of salinity on ASR

In terms of ASR, the adaptability of tetraploid larvae to all salinity levels was lower than that of diploid (Fig. 2). The ASR of tetraploid and diploid larvae decreased with the decrease of salinity. The highest ASR in diploid and tetraploid larvae was appeared at 30 psu, with the values of 53.06% and 41.09% respectively. The ASR of tetraploid larvae was most similar to that of diploid larvae at normal seawater salinity, but was significantly lower than that of diploid larvae at day 16 ($P < 0.05$). At day 16, the maximum difference in ASR between diploid and tetraploid larvae was 36.41% at 20 psu. Compared with other salinity levels, the minimum ASR of diploid and tetraploid larvae were 30.84% and 9.70% respectively at 10 psu.

3.2. Effects of salinity on RGR

The RGR of tetraploid larvae was lower than that of diploid larvae at all salinity levels (Fig. 3). At day 16, the RGR of tetraploid larvae was the lowest and the highest at 10 psu and 30 psu, with the values of 78.94%

and 152.91% respectively. However, high salinity does not mean high RGR, and the RGR of tetraploid larvae at 15 psu (105.24%) is higher than that at 20 psu (98.24%) at day 16 ($P < 0.05$). The onset of significant differences in RGR between diploids and tetraploids was not synchronous at different salinity levels. At 10 psu, 20 psu and 30 psu, the RGR of tetraploid and diploid began to differ significantly from day 4 ($P < 0.05$), while at 15 psu and 25 psu, the RGR of tetraploid and diploid began to differ significantly after 12 days ($P < 0.05$).

3.3. Effects of temperature on ASR

The ASR of tetraploid larvae was generally lower than that of diploid larvae at different temperature levels, and the ASR of tetraploid and diploid larvae decreased with decreasing temperature (Fig. 4). Tetraploid larvae can survive to metamorphosis at 23 °C and 28 °C, with the ASR of 9.20% and 19.67% respectively. Diploid and tetraploid larvae have the lowest ASR at 18 °C, and too few larvae survive to be counted at day 16. At 18 °C and 23 °C, the ASR of both tetraploid and diploid larvae decreased sharply in the first four days compared to other periods. However, when the temperature was 28 °C, the ASR of tetraploid and diploid larvae decreased sharply during the first four days and day 12 to

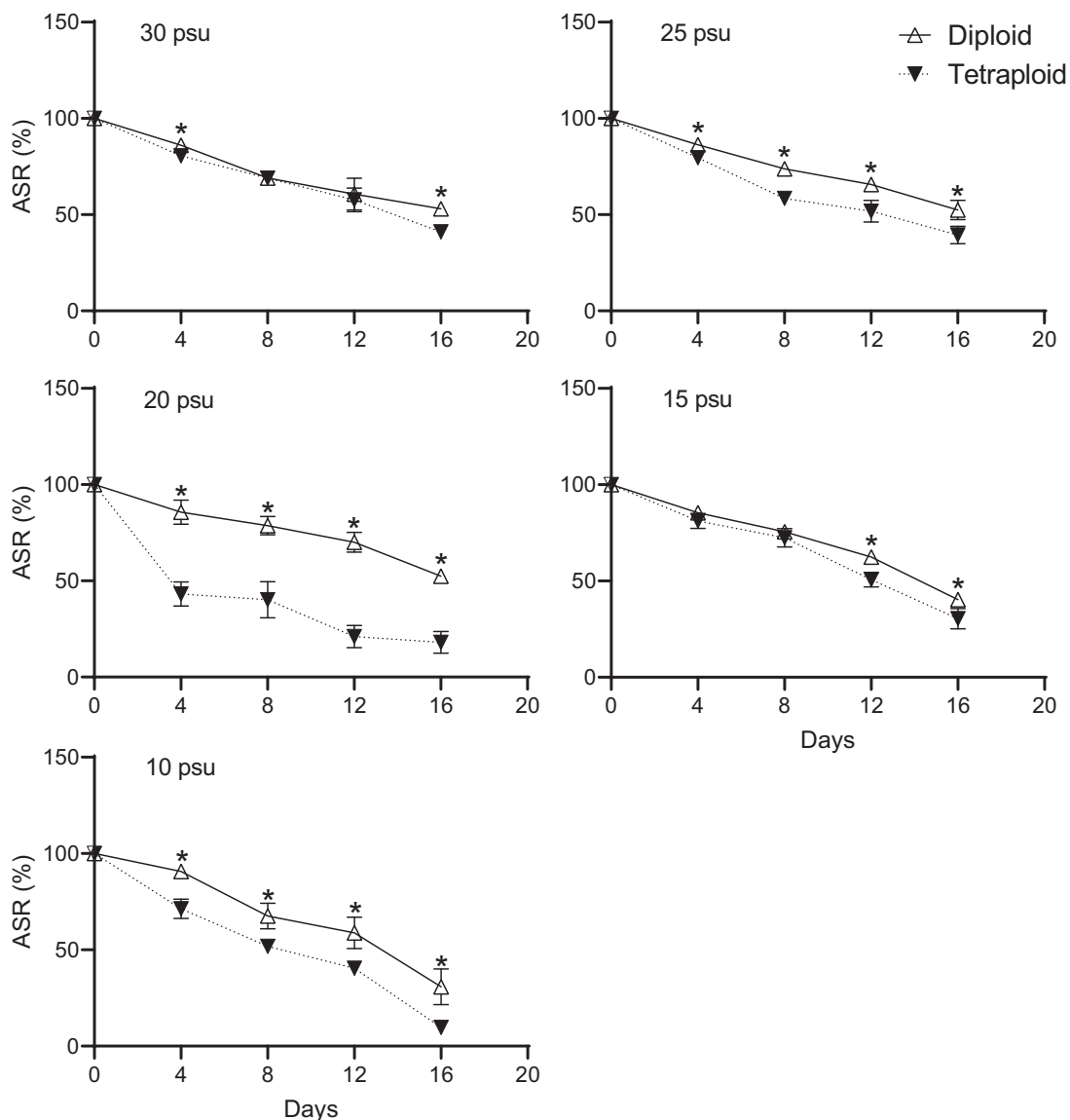


Fig. 2. Comparison of accumulative survival rate (ASR) between diploid and tetraploid larvae of *C. gigas*. Asterisk (*) denotes significant difference ($P < 0.05$).

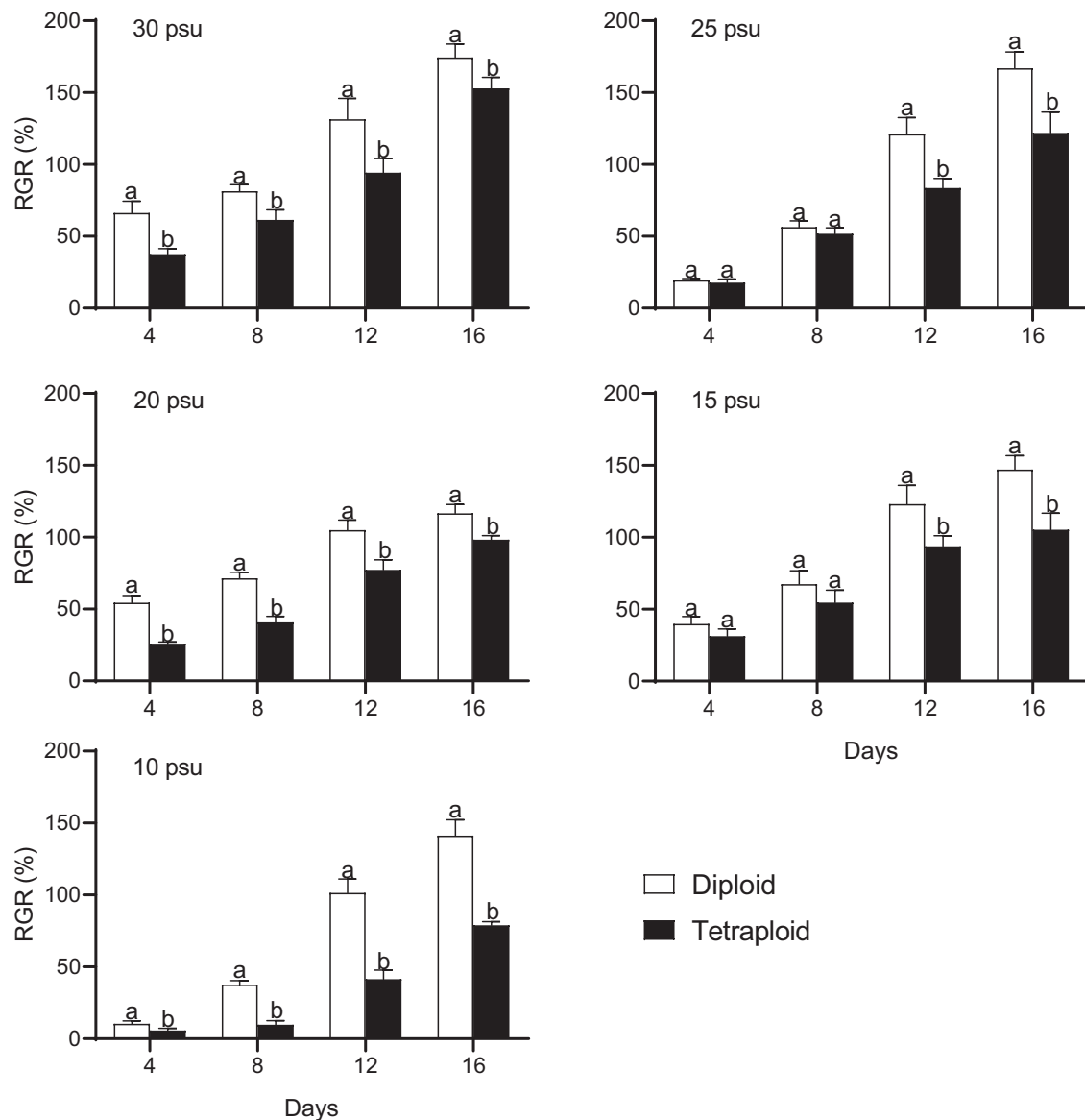


Fig. 3. Comparison of relative growth rate (RGR) between diploid and tetraploid larvae of *C. gigas*. Different lowercase letters denote significant difference ($P < 0.05$).

day 16, respectively.

3.4. Effects of temperature on RGR

The effect of temperature on the growth of tetraploid larvae was shown in Fig. 5. The RGR decreased with decreasing temperature. The RGR of tetraploid larvae at 18 °C was significantly lower than that at 23 °C and 28 °C ($P < 0.05$). At day 16, the maximum value of the RGR for tetraploid larvae was 235.40% at 28 °C. The RGR of tetraploid larvae was significantly lower than that of diploid larvae at different temperature levels ($P < 0.05$). At day 16, the maximum difference between the RGR of tetraploid and diploid larvae occurred at 23 °C, with the values of 80.71%. At 28 °C, both diploid and tetraploid larval growth rates showed a sharp increase during the period of day 4–8.

4. Discussion

Numerous studies have shown that temperature and salinity have important effects on the development of aquatic larvae (Asha and Muthiah, 2005; Kumlu et al., 2000; O'Connor and Lawler, 2004; Ruscoe

et al., 2004; Shi et al., 2010; Wang and Li, 2018), while the effects of temperature and salinity on tetraploid oyster larvae have not been reported. In recent years, triploid oysters have become popular in the world. However, as a crucial part of the triploid industry, it is regrettable that there are few researches on the biological characteristics of tetraploid oysters. It is necessary to study the effects of temperature and salinity on the larvae of tetraploid oyster, either as a neopolyploid of oyster or as the brook stocks of triploid oyster in commercial production.

4.1. Effects of salinity on ASR and RGR

In our study, although tetraploid larvae have a survival disadvantage compared to diploid larvae, tetraploid larvae can survive to metamorphosis under all salinity treatments. The survival disadvantage of neonatal tetraploids is not unique to oysters. Lower tetraploid survival rate than diploid was also found in other aquatic animals (Cassani et al., 1990; Hörstgen-Schwark, 1993; Zhou et al., 2010). Indeed, the phenomenon is not only found in aquatic animals, similar defects were also observed in allopolyploid plants (Comai et al., 2000; Madlung et al., 2005). This phenomenon might be caused by two reasons. One is that,

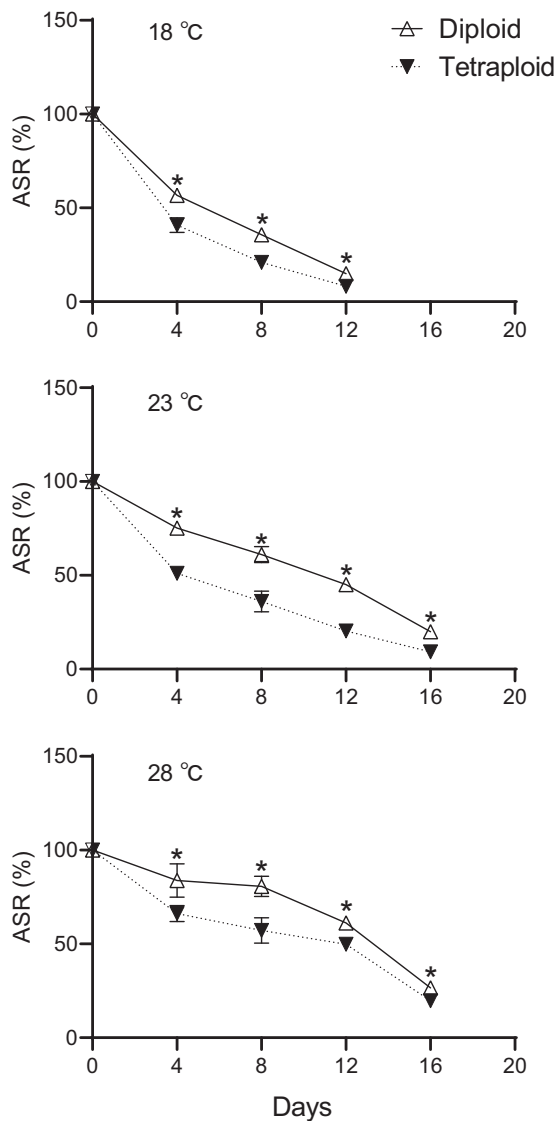


Fig. 4. Comparison of accumulative survival rate (ASR) between diploid and tetraploid larvae of *C. gigas*. Asterisk (*) denotes significant difference ($P < 0.05$).

this may be associated with the increased number of polyploid chromosomes and the greater complexity of their pairing and separation interactions leading to abnormalities during mitosis (Comai, 2005). The other possible reason is that the diploid was a multi-year selective population, and the adaptability to the environment was improved in the selective breeding process (Xu et al., 2020). Compared to diploids, tetraploids as a neopolyploid, its adaptation to the environment has not yet been improved. The ASR of both diploid and tetraploid larvae tended to decrease with the decrease of salinity, which indicated that the diploid and tetraploid larvae had similar pattern of salinity adaptation. The influence of environmental conditions on tetraploids could refer to that on diploids to some extent. Low salinity could lead to a decrease in the ASR of diploid and tetraploid larvae, being consistent with the negative effects of low salinity on hemolymph chemistry and histopathology of oyster (Knowles et al., 2014). In addition, tetraploid and diploid larvae had similar trend of ASR and maintained high ASR in natural seawater (30 psu), which suggested that tetraploids can be cultured under natural seawater conditions like diploids. This result is vital for tetraploid larval culture and subsequent tetraploid selection.

Low salinity could reduce the RGR of diploid and tetraploid larvae,

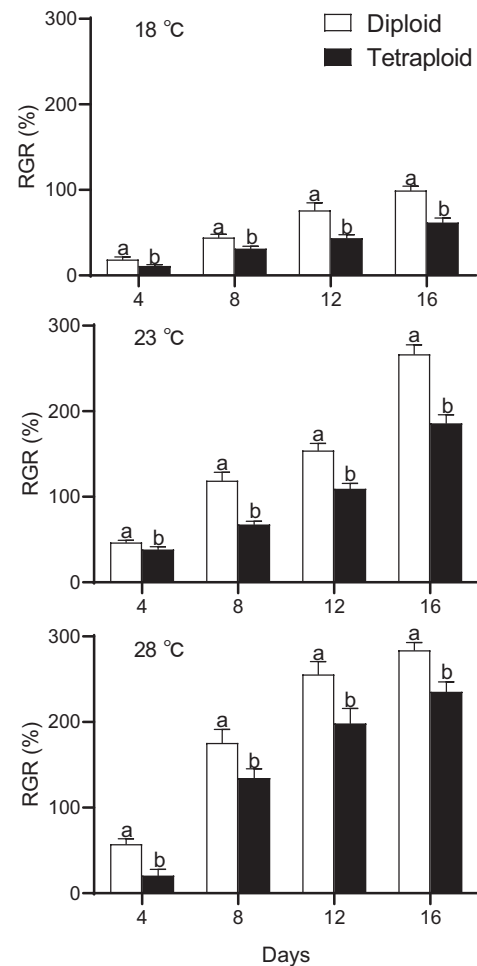


Fig. 5. Comparison of relative growth rate (RGR) between diploid and tetraploid larvae of *C. gigas*. Different lowercase letters denote significant difference ($P < 0.05$).

and had a more negative effect on the latter, which was consistent with the cumulative survival rate, suggesting that tetraploid adaptation to low salinity was lower than that of diploid larvae. In general, the advantage of polyploidy comes from the fact that duplicate copies of genes may evolve new or slightly altered functions, potentially allowing niche expansion or increasing flexibility in an organism's response to environmental changes (Adams and Wendel, 2005; Moore and Purugganan, 2005). However, compared to the diploid population, in the first generation of tetraploid self-breeding populations, the chromosome number of tetraploids increased but the adaptation to low salinity did not increase. This vulnerability to environmental adaptation may be temporary, because evidence from artificially induced tetraploid studies suggested that the allopolyploids may undergo an initial phase of genomic instability, but subsequently could be able to compete with diploids (Comai, 2005). Moreover, the adaptation process of tetraploid oysters can be accelerated by selective breeding. Fortunately, the RGR of tetraploid larvae was the highest at 30 psu (natural seawater salinity). In terms of both growth and survival, natural seawater can meet the salinity requirements of tetraploid larval culture.

4.2. Effects of temperature on ASR and RGR

In terms of the effect of temperature on ASR, the ASR of diploid larvae was higher than that of tetraploid larvae, indicating that the temperature tolerance of diploid larvae was higher than that of tetraploid larvae. And the tetraploids, in addition to adapting to the

challenges of doubling their chromosomes, also face the question of how to adapt better to their environment (Comai, 2005; Zhang et al., 2014). Generally, survival or vigor of neopolyploids was low in synthetic populations (Gaeta et al., 2007; Matsushita et al., 2012). Tetraploid oysters have similar problems, which could be exacerbated by low temperature pressure. The ASR of tetraploid larvae increased with an increase in temperature from 18 °C to 28 °C, demonstrating that increasing temperature in a certain range is favorable for the survival of tetraploid larvae. This may be related to the mass mortality of oyster larvae before the umbo larvae stage. Low temperatures reduce the growth rate of D-larvae and prolong the time for D-larvae to become umbo larvae, leading to an increased risk of larval death.

The RGR of tetraploid and diploid larvae decreased with decreasing temperature, mainly due to the influence of temperature on the physiological process of the larvae (Jiang et al., 2016; Meng et al., 2021). The RGR of tetraploid larvae was lower than that of diploid larvae in temperature treatments, which reflected the difference for temperature adaptation between tetraploid and diploid larvae. The difference may be closely related to the need for tetraploids to adapt to changes in their genomes (Comai, 2005). Fortunately, no matter at 23 °C or 28 °C, the cumulative survival rate and growth rate of tetraploid larvae could be maintained at a certain level, indicating that the cultivation and selection of tetraploid population can be better accomplished in the natural environment during the summer breeding season.

5. Conclusions

In order to understand the salinity and temperature adaptation of tetraploid larvae, the effects of temperature and salinity on the growth and survival of tetraploid and diploid larvae were comparatively studied. In this study, the adaptability of tetraploid larvae to salinity and temperature was generally lower than that of diploid larvae, but the survival and growth of tetraploid larvae could be maintained in the range of natural seawater salinity and temperature in summer, which indicates that the culture of tetraploid larvae and the selection of tetraploid population can be carried out in natural environment. Subsequent studies will focus on improving the environmental adaptability of tetraploid through selective breeding processes.

Credit author statement

Yongguo Li: Completion of the experiment, data analysis, and manuscript drafting. Qi Li: Experimental design, coordination and manuscript revision. Chengxun Xu: Data analysis.

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.

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