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Effects of Salinity and Temperature on Growth and Survival of Juvenile Iwagaki Oyster *Crassostrea nippona*

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Abstract Iwagaki oyster *Crassostrea nippona* occurs naturally along the coasts of Japan and Korea. Because of its unique flavor, delicious taste, edibility during the summer and high commercial value, it has been identified as a potential aquaculture species. To determine the optimum aquaculture conditions and provide necessary information for mass production of the juvenile, the effects of six salinities (15, 20, 25, 30, 35 and 40) and five temperatures (16, 20, 24, 28 and 32℃) on growth and survival of juvenile *C. nippona* were examined in this study. In the salinity experiment, the largest values of mean shell height and growth rate were observed at salinity 25 (20.96 ± 0.36 mm and 172.0 μm d^{-1}, respectively), which were significantly different ($P<0.05$) with those of other treatments, except at salinity 30 (20.56 ± 1.05 mm and 160.3 μm d^{-1}, respectively) ($P>0.05$). The maximum survival rate 84.44% was always observed at salinity 20, and there was no significant difference ($P>0.05$) in survival rate among salinities varying between 15 and 35. In the temperature-related experiments, the highest growth and survival rates of juvenile were observed at 24℃ (180.8 μm d^{-1} and 84.4%) and 28℃ (190.7 μm d^{-1} and 83.3%), respectively, on day 20, and showed significantly ($P<0.05$) larger size and higher survival rate than any other groups. Both juvenile survival and growth were significantly depressed at extreme salinities (15, 40) and temperatures (16℃, 32℃). Based on the results of the present study, a salinity range from 25 to 30 and a temperature range from 24 to 28℃ are considered optimal conditions for survival and growth of juvenile *C. nippona*.

Key words *Crassostrea nippona*; juvenile; salinity; temperature; survival; growth

1 Introduction

Iwagaki oyster *Crassostrea nippona* belongs to Bivalvia, Ostreidae, and is a large sessile oyster inhabiting intertidal hard grounds and reefs along the coast of East Asia including Japan and Korea (Itoh *et al*., 2004). Because of its unique flavor, delicious taste and marketability in summer when Pacific oyster *C. gigas* is unavailable, the commercial price of *C. nippona* is estimated as high as five folds of *C. gigas* in Japan (Itoh *et al*., 2004). Thus this species has a market prospect and high potential value of large scale farming. Traditionally, *C. nippona* farming is largely dependent on natural seeds (Tanaka *et al*., 2010). However, such seed collection is labor intensive, often unreliable and available for a short season. Moreover, with the increasing interest of *C. nippona* culture, natural seed collection may not satisfy the demand of aquaculture, and seed production is always in short supply due to the deficiency of facilities (Tanaka *et al*., 2010; Fujiwara, 1995). These issues have significantly constrained the cultivation of *C. nippona*, hence, developing proper breeding, nursery, and aquaculture techniques for mass production of juveniles are crucial for meeting the success of *C. nippona* farming.

Autecological study of bivalves has clearly demonstrated that environmental factors play an important role in the development, growth and survival of aquatic animals (Tang *et al*., 2012). Among them, salinity and temperature are considered to be the most important physical parameters affecting the physiological responses and survival of aquatic organisms, which have been described as ‘master factors’ (Re *et al*., 2005; Kinne, 1964). Salinity imposes an additional metabolic load (Bao and You, 2004) and affects biological activities, including those related to immune responses (Taylor *et al*., 2007), fertilization (Verween *et al*., 2007), development of embryos (Davis and Calabrese, 1969), survival and growth of larvae and juvenile (Huo *et al*., 2014). Temperature modifies energy flow, which regulates the rate of biological processes (Scheltema, 1967). Accordingly, the effects of these two factors have been described for numerous marine species (Ko *et al*., 2014; Huo *et al*., 2014; Xu *et al*., 2011). For *C. nippona*, although previous studies have documented seasonal variations in reproductive activity and bio-

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chemical composition, karyotype, and culture method (Itoh *et al*., 2004; Okumura *et al*., 2005; Adachi *et al*., 2014), and information pertaining to *C. nippona* for spat production in hatchery is still limited. Large-scale production of an aquaculture species must be based on adequate knowledge of its ecological requirements for optimum development. Moreover, *C. nippona* inhabits the low intertidal and shallow sub-tidal zones at depth of 10–20 m (Li, 2007), making them susceptible to changes in environmental conditions. Therefore, evaluating the effects of exogenous factors, especially temperature and salinity, on the growth and survival of *C. nippona*, not only improves our understanding of ecological habits of this species, but also provides basic data for the conservation and restocking of natural populations.

This study investigated the effects of different salinity and temperature levels on the growth and survival of juvenile *C. nippona*, aiming to determine the optimum rearing conditions of *C. nippona* in hatcheries. The information obtained in this study will be valuable for the further development of *C. nippona* aquaculture industry.

2 Materials and Methods

2.1 Maintenance of Juveniles Prior to the Experiments

At the beginning of the experiments, a total of 1200 juvenile *C. nippona* cultivated at Rushan Bay (36˚43′–37˚36′N and 121˚28′–121˚39′E), Shandong Province, China, were collected randomly in February 2016. The animals were transported to Haiyi Hatchery in Yantai, and acclimatized in 80-L plastic containers with static aerated seawater at a salinity of 33 and a temperature of 3℃ for two weeks. The acclimation conditions represented the general *C. nippona* cultivation conditions of the northern Chinese aquaculture areas in winter. Initial mean shell height (SH) each group at the beginning of temperature and salinity experiments were showed in Table 1. Juveniles were fed daily a mixture of *Isochrysis galbana* and *Nitzschia closterium* (1:1) at a final concentration of 10 cells $\mu L^{-1} d^{-1}$. All containers were aerated with air diffusers and seawater was changed daily. The temperature of seawater was increased to the desired level with thermostatic immersion heaters before being added to the containers. A constant room temperature was maintained with an air conditioner. Only juveniles showing healthy signs and normal behavior were used in the experiments.

Table 1 Initial mean shell height for each group of juveniles at the beginning of temperature and salinity experiments

	Experimental group	Shell height (mm)
Temperature	16℃	14.89±2.07
	20℃	15.03±2.27
	24℃	14.33±2.75
	28℃	15.63±2.83
	32℃	14.85±2.28
Salinity	15	14.87±2.50
	20	15.56±2.49
	25	15.36±2.27
	30	14.24±2.01
	35	15.05±1.69
	40	14.55±1.69

2.2 Experimental Design

Based on water temperature and salinity data along the coast of Shandong Province, juvenile *C. nippona* were acclimated at six salinities (15, 20, 25, 30, 35 and 40) and five temperatures (16, 20, 24, 28 and 32℃). Salinity was adjusted at a rate of $2\,d^{-1}$ with dechlorinated freshwater (dilution) or with artificial sea salt (increase) until the required salinities (15, 20, 25, 30, 35, 40) with deviations ±0.5 were reached. Temperature was adjusted at a rate of 2℃ d^{-1} by holding the experimental containers in a water bath with thermostatic immersion heaters until the test temperatures (16, 20, 24, 28 and 32℃) were reached. Salinity treatments were carried out at a constant temperature of 22℃, when ambient seawater temperature in the field site was 20 to 24℃, while the salinity was kept stable at 32 in temperature treatments. The salinity of the experimental solutions was checked using a portable refractometer. Thermostatically-controlled heaters were used to maintain the temperature of seawater to be within 0.5℃. Each treatment group included three replicates to ensure the accuracy of the results, and each replicate consisted of 30 individuals.

2.3 Measurement of Daily Growth Rate and Survival Rate

The daily growth rate was the ratio of the difference of the measured shell height (SH) and initial height divided by the number of days:

$$R_H\,(\mathrm{mm\,d^{-1}}) = (H_1 - H_0)/(t_1 - t_0)\,.$$

Survival rate (R_S) was the ratio of the measured survival and the initial stocking amount:

$$R_S = \frac{\text{The measured survival amount}}{\text{The initial stocking amount}} \times 100\%.$$

Oysters were exposed for five weeks to the different temperatures and salinities, while the growth and survival were measured every five days in each group of juveniles. Criteria for mortality of individuals were with a permanent wide valve gape and showing no response to the touch of a glass rod or fine brush. Individuals that did not survive the duration of the experiment were removed immediately.

2.4 Statistical Analysis

One-way ANOVA was used to test the effects of salinity and temperature on the survival and growth of *C. nippona*, and differences between means were compared using the least significant difference (LSD) test and Duncan's multiple range test in SPSS (V.18) software. Significance level for all analysis was set at $P<0.05$.

3 Results

3.1 Effects of Salinity on the Survival and Growth of Juvenile

Stock salinity had a significant effect on the size of juvenile *C. nippona* in terms of shell height at the end of the experiment (Fig.1). In general, the shell height of juvenile progressively increased from salinities 15 to 25 and decreased from salinities 25 to 40. The largest value of shell height occurred at salinity 25 (20.96±0.36 mm), and was significantly ($P<0.05$) different from the values in other treatments, except at salinity 30 (20.56 ± 1.05 mm) ($P>0.05$). At salinities higher than 30, differences in the shell height were not statistically significant ($P>0.05$). The lowest value of SH was observed at extreme salinity of 40 (16.16±0.78 mm), and was significantly ($P<0.05$) lower than the values in other treatments, except at salinity of 15 (17.36±0.35 mm) and 35 (17.06±0.91 mm) ($P>0.05$).

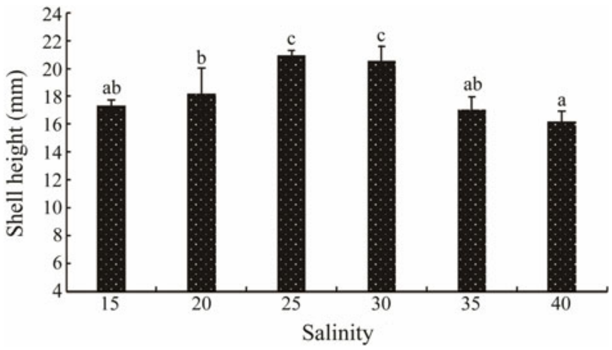

Fig.1 Effect of salinity on shell height of *Crassostrea nippona* juveniles at the end of the experiments. Each bar represents the mean ± SE of three replicates. The same lowercase of same trait mean no significant difference ($P>0.05$).

Daily growth rate of *C. nippona* was significantly affected by salinity (Table 2). With increasing salinity, the value of growth rate increased until the salinity was 25 (172.0 μm d^{-1}), while they were not significantly different from that at salinity 30 (160.3 μm d^{-1}), and then decreased. The lowest growth rate (35.0 μm d^{-1}) was observed at the highest salinity 40, was significantly ($P<0.05$) different from the values in other treatments.

Table 2 Survival and growth rates of *Crassostrea nippona* juvenile held at different salinities

Salinity	Growth rate (μm d^{-1})	Survival rate (%)			
		5 d	10 d	15 d	20 d
15	71.2±11.8^{c}	87±3.3^{a}	82±3.9^{a}	78±1.9^{a}	77±3.3^{ab}
20	93.6±13.4^{b}	91±3.8^{a}	86±1.9^{a}	84±1.9^{a}	84±1.9^{b}
25	172.0±17.2^{a}	91±5.1^{a}	84±6.9^{a}	83±6.8^{a}	81±3.9^{ab}
30	160.3±12.8^{a}	86±8.5^{a}	82±6.9^{a}	79±10.3^{a}	77±8.8^{ab}
35	57.7±5.1^{c}	86±1.9^{a}	84±6.9^{a}	80±8.8^{a}	74±6.9^{ab}
40	35.0±5.3^{d}	87±3.4^{a}	74±1.9^{a}	62±5.2^{b}	61±3.8^{c}

Notes: Within a column, values followed by different letters are significantly different ($P<0.05$, $n=30$). Data are given as Mean±SE.

The survival rates of juvenile were not significantly different among salinities 15 to 40 on days 5 and 10, but a significant effect of salinity was observed after day 15 (Table 2). The survival rate at salinity 40 was high during the first 5 days and dropped sharply from day 10 to day 15. Therefore, salinity 40 was not included in the comparison between treatments in relation to optimum survival rates and optimum growth. Survival rate on day 15 was the highest at salinity 20, but there was no significant ($P>0.05$) differences in survival between different salinities during any trail period, except at salinity 40 ($P<0.05$). Survival rate decreased progressively from salinities 35 to 40 on day 20.

3.2 Effects of Temperature on the Survival and Growth of Juvenile

From 16 to 28℃, the shell height of *C. nippona* increased progressively as temperature increased, and decreased sharply at temperature of 32℃ (Fig.2). The maximal shell heights were observed at temperatures of 24℃ (21.27 ± 0.41 mm) and 28℃ (22.31 ± 0.69 mm), which were significantly ($P<0.05$) different from those measured at all other temperatures. The minimum shell heights were observed at temperatures of 16℃ (17.45±0.35 mm) and 32℃ (17.38 ± 1.35 mm), which were significantly different from the values measured at other temperatures ($P<0.05$).

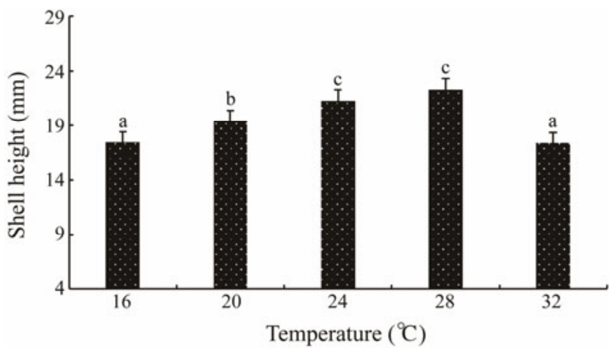

Fig.2 Effect of temperature on shell height of *Crassostrea nippona* juveniles at the end of the experiments. Each bar represents the mean ± SE of three replicates. The same lowercase of same trait mean no significant difference ($P>0.05$).

In general, daily growth rate of the shell height progressively increased from 16 to 28℃ and decreased from 28 to 32℃ (Table 3). Daily growth rate was the highest temperatures of 24℃ (180.8 μm d^{-1}) and 28℃ (190.7 μm d^{-1}). At temperature of 32℃, daily growth rate was 73.0 μm d^{-1}, significantly ($P<0.05$) slower than any other groups, except at temperature of 16℃ (73.3 μm d^{-1}) ($P>0.05$).

The survival rate of juvenile decreased progressively with increasing experiment period (Table 3). The survival rate at 16℃ was high during the first 5 days and dropped sharply from day 10 to day 20. Therefore, 16℃ was not included in the comparison between treatments in relation to optimum survival rates and optimum growth. Table 3 showed that there was no significant difference among different temperatures of 20℃ to 32℃ on day 5, 10 and 15, but a significant effect of temperature was observed after day 20. Among all treatments, it was noted that on day 20, survival rate was significantly ($P<0.05$) higher at 24 and 28℃ than that at 20 and 32℃, ranging from 83% to 84%. Survival rate of the juvenile was longer at 20℃ than that at 32℃, whereas survival rate displayed no significant ($P>0.05$) difference between these two treatments.

Table 3 Survival and growth rates of *Crassostrea nippona* juvenile held at different temperatures

Temperature (℃)	Growth rate (μm d^{-1})	Survival rate (%)			
		5 d	10 d	15 d	20 d
16	73.3±8.7^{c}	86±5.1^{a}	52±15.8^{a}	50±15.3^{a}	43±12.0^{a}
20	124.3±18.7^{b}	88±3.9^{a}	81±10.2^{b}	73±8.8^{b}	70±5.8^{b}
24	180.8±14.7^{a}	91±5.1^{a}	88±5.1^{b}	87±6.7^{b}	84±5.1^{c}
28	190. 7±18.6^{a}	91±6.9^{a}	89±6.9^{b}	86±3.9^{b}	83±3.3^{c}
32	73.0±5.6^{c}	92±1.9^{a}	92±1.9^{b}	76±1.9^{b}	69±1.9^{b}

Notes: Within a column, values followed by different letters are significantly different ($P<0.05$, $n=30$). Data are given as Mean±SE.

4 Discussion

According to the observed mortalities and daily growth rates, different salinities have significant effects on juvenile *C. nippona.* Our findings agree with those of Yao *et al.* (2015) who studied the effect of salinity on larval growth, survival and development of the oysters *C. gigas* and *C. ariakensis*. Previous studies have demonstrated that oysters are generally euryhaline species, although different species may differ in the optimal salinity that they prefer (Xu *et al.*, 2011). For example, European flat oyster *Ostrea edulis* larvae had the high growth rates and survival at an optimum salinities of 24–33 (Walne, 1956). Sydney rock oyster *Saccostrea glomerata* larvae had the highest growth rates at salinities 23–39, and the highest survival rates at salinities 27–39 (Nell and Holliday, 1988). Mangrove oyster *C. rhizophorae* had the highest embryo development rates at salinities of 25–37 (Dos Santos and Nascimento, 1985). For *C. nippona*, there was no significant difference among different salinities of 15–35 after 20 days of cultivation, indicating that 15–35 is the optimum salinity for the survival of juvenile. However, the highest growth rates were observed at salinity of 25 and 30. When the salinity were adjusted to be lower or higher than this range, growth rate of juvenile was significantly affected, resulting in a decreased shell length. Therefore, a salinity range of 25–30 is the optimum salinity for the growth of juvenile. Similarly, in Kumamoto oyster *C. sikamea*, salinities higher than 25 had significant negative effects on the larvae development and growth (Xu *et al*., 2011). The survival and growth of spat, juvenile and adult *C. virginica* were observed to be depressed directly with lower salinity. Combined with suboptimal elevated temperature, lower salinities have negative effects in all size classes of *C. virginica*, resulting in dramatically greater mortality (Rybovich *et al*., 2016).

In the present study, rearing *C. nippona* in the lowest (15 and 20), medium (25 and 30), and the highest salinities (35 and 40) had significant effects on juvenile growth. A similar result was also observed in *C. nippona* larvae, in which the lowest growth rate and survival rate were measured at salinities of 14, 18 and 34 on day 13 (Wang *et al*., unpublished data). The mechanisms through which salinity affects marine mollusks remain unclear. However, previous studies indicate that there were two possible reasons for decreased growth rates and increased mortality under extreme salinity conditions. Firstly, reduced feeding rates could be a major driver for reduced growth rates in extreme salinity since oysters completely seal themselves off by closing their shells when salinity drops too low or increases too high (Berger and Kharazova, 1997). For example, in the European flat oyster *Ostrea edulis*, salinity were found to significantly affect absorption efficiency, filtration rate, and metabolic response, further lend to retarder growth of larvae. (Hutchinson and Hawkins, 1992). Low energy utilization efficiency of the body could be the other reason. Like other marine molluscs, *C. nippona* was an osmotic conformer (Berger and Kharazova, 1997). Additional energy is required for the maintenance of water and mineral balance in body fluids and cells when juvenile *C. nippona* were cultivated at suboptimal salinity conditions (Berger and Kharazova, 1997). This conclusion agrees with that of Forcucci and Lawrence (1986), who unequivocally demonstrated that either additional energy is required for the maintenance or energy is used inefficiently that can cause the slow growth of *Luidia clathrata* when salinity was adjusted to a level that was lower or higher than optimal salinity ranges.

Temperature is another important environmental variable that can influence juvenile survival and growth. In the present study, minimum growth and survival rate were observed at 16℃, and as temperature increased, these values reached the maximum at 24 and 28℃. This agrees with many other studies on the growth of mollusks that juveniles generally grew more quickly at the higher temperatures (Klinzing and Pechenik, 2000; Pechenik and Heyman, 1987). Davis and Calabrese (1969) suggested that the failure of marine bivalve species to grow at low temperatures appears to be a result of their inability to digest the available food, although they can survive and ingest food for a long time. The appropriate improvement of cultivation temperature could promote the activities of certain digestive enzymes, and increase the assimilation efficiency of the organism, subsequently leading to an increase in somatic growth (Bayne *et al*., 1999). However, with the temperature rising from 28 to 32℃, survival and growth of juvenile decreased sharply. This has been explained by the increase in growth of potential pathogenic ciliates and bacteria in cultures, leading to the proliferation of invaders or fouling organisms that bring their own microflora and severe disease problems, and further reducing oxygen availability (Gruffydd and Beaumont, 1972). Moreover, along with the increase of temperature, more energy was allocated to the osmoregulation and less to the protein synthesis, which resulted in the depletion of glycogen reserves and protein content in tissues, and eventually led to less favorable energy balance and smaller maximum size (Ivanina *et al*., 2013). Therefore, the temperature of 24–28℃ is optimum for the growth and survival of juvenile *C. nippona*. Similarly, optimum larval development and settlement of the oyster *C. gigas* occurred at 27℃ (Rico-Villa *et al*., 2009). For *O. edulis*, the average rate of growth of larvae increased progressively as the temperature increased from 10 to 25 or 27.5℃ and then decreased at 30 and 32.5℃ (Davis and Calabrese, 1969).

Another notable result in the present study is that juvenile cultivated at 16℃ displayed lower survival rates than at 32℃, even though there was no significant difference between these two extreme temperatures on growth rates. The present result indicated that juvenile C. *nippona* had greater adaptability to high temperature than low temperature. This may be related to the summer breeding habits of C. *nippona*. The parental *C. nippona* has a sexual maturation between August and September, which is the period with the highest water temperature of the year (Okumura *et al*., 2005). Similar phenomenon also shown in other marine bivalves, including *Saccostrea glomerata* and *Tegillarca granosa* (Parker *et al*., 2009; You *et al*., 2001).

In summary, the results of this study clearly demonstrate that juveniles of *C. nippona* can tolerate a wide range of salinities (15–35) and a wide range of temperatures (20–32℃), while exhibit larger shell height and better survival at salinities 25–30 and 24–28℃. Hence, culturing this species at lower salinities of 25–30 and higher temperatures of 24–28℃ will likely produce a high yield and benefit juvenile *C. nippona*.

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