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A Comparative Study on the Difference in Temperature and Salinity Tolerance of *Crassostrea nippona* and *C. gigas* Spat

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Abstract: Although *Crassostrea nippona* and *C. gigas* are occasionally found to be sympatric, little is known about the differences in tolerance against environmental stresses between species, which may potentially result in severe economic losses due to the neglect of species-specific biological characteristics in farming practices. Therefore, two independent and consecutive experiments were performed to evaluate the differences in temperature and salinity tolerance between *C. nippona* and *C. gigas* spat and determine the optimal environmental conditions for the aquaculture of *C. nippona* spat. The experimental results of dynamic treatment showed that the accumulative survival rate (ASR) of *C. nippona* spat was generally lower than that of *C. gigas* throughout temperature changes and salinity decreases, while the ASR of *C. nippona* spat was superior to that of *C. gigas* with increasing salinity. In addition, the daily growth rate (DGR) of both species was significantly inhibited at 18 °C and peaked at 25 °C with increasing temperature ($p < 0.05$) at each experimental salinity. At 32 °C, the survival rate (SR) of *C. nippona* spat was significantly lower than that of *C. gigas* on day 10 at all salinities tested ($p < 0.05$), whereas the difference in the SR between species at 35 psu gradually disappeared with prolonged stress time. The data indicated that *C. gigas* spat was suitable for rearing at temperatures of 25 and 32 °C at all salinity levels, except the combination of 32 °C and 35 psu. By contrast, combinations of 25 °C and salinities of 21–35 psu were considered as the optimal environmental conditions for the long-term culture of *C. nippona* spat. These present findings contribute to a better understanding of the tolerance of *C. nippona* spat to environmental stresses and suggest that particular attention should be paid to the inferior adaptability of *C. nippona* when farming in locations outside their natural habitats.

Keywords: oyster farming; environmental tolerance; dynamic treatment; optimum condition; combined effects; survival; growth

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1. Introduction

Iwagaki oyster *Crassostrea nippona* is a large sessile oyster naturally distributed in the low tidal positions of East Asia [1,2]. In Japan, *C. nippona* caught from the Sea of Japan has been traditionally consumed in summer since ancient times due to its high glycogen content and firm texture [3,4], especially when the Pacific oyster *C. gigas* suffers from low meat quality during the reproductive season, which also enhances the commercial value of *C. nippona* [1], hence the name "summer oyster" [5]. Although triploid *C. gigas* with year-round marketability has been extensively farmed worldwide, problems such as triploid gametogenesis have become increasingly prominent in the process of rapid development of the polyploid industry [6,7]. Therefore, cultivating an oyster species that is naturally edible during the warm season may provide another promising solution. With the development of the aquaculture industry, Shimane Prefecture, which is the largest producer of *C. nippona* in Japan, marketed more than 1 million oysters with a gross sales revenue of 2.1 million USD in 2017 [8]. In recent years, there has been increasing interest

among Chinese farmers to initiate large-scale farming of *C. nippona* because of its enormous economic value and commercial potential [9–11]. Currently, however, the publicly available information on specific environmental conditions for the commercial-scale field cultivation of *C. nippona* is sparse [12]. Despite the similarities between *C. nippona* and *C. gigas*, which are widely distributed and farmed along the coast of Japan [13–15], commercial-scale field cultivation of these two species should be conducted with caution on account of their differences in habitat conditions and different physiological responses to environmental changes [13,16,17].

Species-specific environmental tolerances are generally understood to be the consequence of long-term adaptive evolution in distinct habitats, especially for sessile/sedentary species [18–20]. In natural environment, the Pacific oyster inhabiting primarily in many estuarine intertidal zones is frequently forced to experience highly dynamic and stressful environments, such as high temperature (>40 °C) and low salinity, due to tidal fluctuations and surface freshwater inputs [21,22], which has allowed it to evolve a powerful environmental adaptability in response to environmental stress [23]. However, unlike *C. gigas*, *C. nippona* generally inhabits stones and reefs below the bottom of the intertidal zone and expands everywhere offshore up to the 20 m depth, where the environmental conditions are relatively constant [5,13,24]. Although significant growth differences between *C. nippona* and *C. gigas* under the same cultivation system have been reported [9], little is known about the differences in tolerance against environmental stresses between species, which may mislead decision-making on suitable aquafarming locations for *C. nippona*.

Among the environmental factors, temperature and salinity are recognized as the most important physical parameters impacting the performance, primarily the growth and survival, of aquatic animals [25–27]. For *C. nippona*, Chinese scholars have reported the optimal farming conditions for juveniles according to the survival and physiological responses at different temperatures and salinities [12,28]. The influence of the above abiotic factors on the growth performance and viability of post-larvae or early stage spat of *C. nippona* is, nevertheless, poorly known. In many commercial farms, the hatchery-bred spat is transferred directly to rearing conditions different from that of the original farm for further growth [29], whereas environmental variations beyond the ecological tolerance limit are observed to be lethal for oysters [30]. Noro [31] found that extremely low winter temperatures (below 5 °C) could result in mass mortality of the early stage spat of *C. nippona* (shell height 10 mm) in grow-out culture, but had limited effect on larger size individuals (shell height 40 mm). Regrettably, it is difficult for *C. nippona* spat obtained by the natural spawning of broodstock to reach the required size at the onset of winter minimum temperatures because of its late gonadal maturation and slow growth rate as well as a continuous decline in the natural sea temperature [9–11]. Hatchery conditioning by artificially manipulating environmental conditions to initiate gametogenesis in broodstock may be an efficient and feasible method to allow a 1- to 2-month earlier transfer of spat from the hatchery [18,32]. However, such a farming strategy will result in the inevitable exposure of hatchery-produced spat to seasonally harsh conditions caused by heavy rainfall and high temperatures leading to potential mortality risks, especially given the limited understanding of *C. nippona* spat. It is therefore necessary to continue attempts to define the environmental tolerance of *C. nippona* spat before conducting large-scale farming.

Against this background, two independent and consecutive experiments were performed to evaluate the differences in temperature and salinity tolerance between *C. nippona* and *C. gigas* spat and determine the optimal environmental conditions for the aquaculture of *C. nippona* spat. The information obtained in this study may provide a valuable reference for the further development of the *C. nippona* aquaculture industry.

2. Materials and Methods

2.1. Experimental Animal

Spat used in the study were the progeny of wild *C. nippona* collected from Geoje Island, Korea (34.81° N, 128.74° E) and wild *C. gigas* sampled from Rongcheng, Shandong

(37.04° N, 122.47° E), and were produced separately at the hatchery located in Yantai (Shandong Province) in July 2022 following the larviculture protocols of Li [33]. Oyster species identification was confirmed by sequencing the ITS1 and COI gene fragments using previously developed primers prior to spawning [34,35]. After larval settlement and metamorphosis, early stage spat were fed daily with a standard diet of *Isochrysis galbana* at a concentration of 10 cells μL^{-1} d^{-1} until the shell height of both *C. nippona* (3.26 ± 0.61 mm) and *C. gigas* (3.34 ± 0.67 mm) reached the appropriate size for the studies. Then, nearly 2700 individuals randomly selected from each species were initially maintained in the culture tank with ambient conditions (temperature: 25 °C; salinity: 28 psu) prior to transfer to the experimental environment.

2.2. Experimental Design

Two independent and consecutive experiments were designed to assess the performance of survival and growth of *C. nippona* (NN) and *C. gigas* (GG) spat under different temperature and salinity changes. For each experiment, spat from different treatment groups of different species were allocated individually in the 50 L polyethylene bucket with three replicates. Oysters that either failed to close valves or detached from the substrate after gentle touching were identified as conforming to the criteria for death and removed immediately. Experimental water temperature was maintained by water bath with thermostatically controlled immersion heaters or water chiller (HC-150A, 33ILEA, China) and errors were limited within ±0.5 °C, while the required salinity was achieved by diluting seawater with filtered fresh water or adding aquarium salt and regularly monitoring using the refractometer (ATAGO). The water in each treatment tank was fully aerated and replaced daily by half of the sand-filtered seawater with the same environmental condition.

2.2.1. Experiment 1: Thermal and Salinity Tolerance Limits of Spat

The thermal and salinity tolerance limits of *C. nippona* and *C. gigas* spat was determined by the dynamic methodology described in previous studies [20,36,37]. In the temperature treatment, 40 individuals from *C. nippona* or *C. gigas* in each treatment tank were exposed to a constant rate of temperature increase or decrease by 2 °C d^{-1} based on ambient temperature, and dead individuals were recorded and removed daily, until all spat reached an upper or lower end-point. The salinity in each treatment group was kept stable at 28 psu during temperature changes. The maximum and minimum temperatures at which all spat in each treatment tank survived were denoted as STMax (survival thermal maximum) and STMin (survival thermal minimum), respectively, while the corresponding temperatures of death of all individuals were recorded as CTMax (critical thermal maximum) or CTMin (critical thermal minimum). The temperature at which half of the *C. nippona* or *C. gigas* spat survived with heating or cooling is recorded as 50% CTMax or 50% CTMin, which was estimated through Probit analysis using IBM SPSS Statistics 25.0 software.

Similarly, in the salinity treatment, the salinity in each treatment tank was increased or decreased at a rate of 2 psu d^{-1} based on ambient salinity until all spat died, and the temperature was maintained at 25 °C during salinity changes. The SSMax (survival salinity maximum), SSMin (survival salinity minimum), CSMax (critical salinity maximum), and CSMin (critical salinity minimum) were calculated separately according to the survival response of spat from the NN or GG group. The 50% CSMax or 50% CSMin, which caused 50% mortality in each tank when the salinity was increased or decreased, was determined using IBM SPSS Statistics 25.0 software with Probit analysis.

2.2.2. Experiment 2: Growth and Survival Rates of Spat

A completely randomized 3×3×2 factorial design was implemented to examine the combined effects of temperature and salinity on the daily growth rate (DGR) and survival rate (SR) of *C. nippona* and *C. gigas* spat for a period of 30 days. The temperature (18, 25 and 32 °C) and salinity (21, 28 and 35 psu) levels used in the experiment were determined based on the survival thermal (STMax and STMin) and salinity (SSMax and SSMin) limits

of spat in Experiment 1 as well as the range of natural environmental conditions along the coast of northern China [38]. Eighty individuals randomly selected from each species were directly assigned to each of nine environmental regimes with three replicates, and were fed daily with *I. galbana* throughout the experimental period. Dead individuals were removed from each container in time and the survival rate of each treatment group was statistically counted every 10 days. Shell height of 30 randomly selected individuals in each replicate tank was measured using an electronic vernier caliper (0.01 mm accuracy) at the beginning and end of the experiment.

The daily growth rate (DGR) and survival rate (SR) of each treatment group were calculated according to the following formula:

$$\text{DGR (mm d}^{-1}) = \frac{(SH_f - SH_i)}{t}$$

$$\text{SR (\%)} = \frac{A_t}{A_i} \times 100\%$$

where SH_i and SH_f are mean value of the initial and final shell height, respectively; t is the number of days; A_i and A_t are the initial stocking amount and the measured survival amount at time t, respectively.

2.3. Statistical Analyses

Multifactor experimental data were analyzed by a three-way ANOVA that first assessed the interaction among temperature, salinity and species. If a significant interaction was identified, the effects of a single factor, except for species, on the DGR and SR of spat were analyzed using one-way ANOVA followed by Tukey's Multiple Comparison Test at each level of the other factors. In all experiments, differences in growth and survival between *C. nippona* and *C. gigas* at the same environmental conditions were analyzed by *t*-test. Data were presented as the mean ± standard deviation (SD) and the significance level for all data analysis was set at $p < 0.05$. All statistical analyses were conducted with the IBM SPSS Statistics 25.0 software.

3. Results

3.1. Thermal and Salinity Tolerance Limits of Spat

In the temperature treatment, *C. gigas* showed less sensitivity to decreasing and increasing temperatures than *C. nippona* according to variations in accumulative survival rates (ASR) of spat (Figure 1). Spat from the NN and GG groups acclimated in ambient conditions started to die at 16.33 and 15.67 °C with decreasing temperature and at 31 and 32.33 °C with increasing temperature, respectively. The ASR of the NN group was consistently lower than that of the GG group during temperature changes, and statistically significant differences in ASR between species were observed at cooling (9, 11, 13 and 15 °C) and heating (35, 37 and 39 °C) of the water, respectively ($p < 0.05$). The estimated STMin, CTMin, STMax, and CTMax of *C. nippona* spat were all within the range of variation of the corresponding physiological indicators of *C. gigas* (Table 1) and there were significant interspecies differences in 50% CTMin and 50% CTMax ($p < 0.05$). The relationships among 50% CTMax, 50% CTMin and temperature (*Te*) in the NN and GG groups are shown separately as follows: 50% CTMin-NN = 4.438–0.382*Te* ($R^2 = 0.903$); 50% CTMin-GG = 4.062–0.396*Te* ($R^2 = 0.989$); 50% CTMax-NN = −18.280 + 0.503*Te* ($R^2 = 0.997$); 50% CTMax-GG = −17.348 + 0.462*Te* ($R^2 = 0.989$).
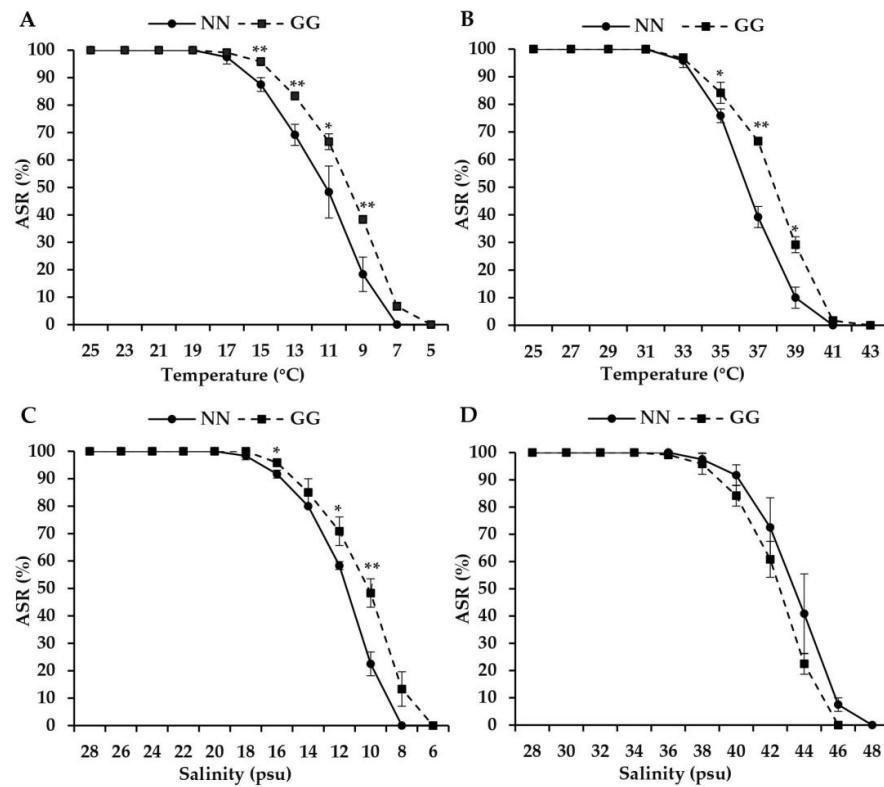
Figure 1. Comparison of accumulative survival rate (ASR) of *C. nippona* (NN) and *C. gigas* (GG) spat under constant changes (2 °C d^{-1} or 2 psu d^{-1}) in (**A**,**B**) temperature and (**C**,**D**) salinity. Single asterisk indicates $p < 0.05$, double asterisk indicates $p < 0.01$.

Table 1. Physiological indices of *C. nippona* and *C. gigas* spat under constant changes in temperature (2 °C d^{-1}) and salinity (2 psu d^{-1}).

Physiological Indices	Temperature (°C)		Salinity (psu)	
	NN	GG	NN	GG
CMin	7	5	8	6
50% CMin	11.61 ± 0.42 [b]	10.24 ± 0.09 [a]	12.03 ± 0.08 [b]	10.78 ± 0.40 [a]
SMin	19.33	18	18.33	17.67
CMax	41	42.33	48	46
50% CMax	36.32 ± 0.19 [a]	37.51 ± 0.14 [b]	43.02 ± 0.54 [b]	42.06 ± 0.23 [a]
SMax	32.33	33	36.67	36

CMin, critical temperature or salinity minimum; 50% CMin, 50% critical temperature or salinity minimum; SMin, survival temperature or salinity minimum; CMax, critical temperature or salinity maximum; 50% CMax, 50% critical temperature or salinity maximum; SMax, survival temperature or salinity maximum. Different lowercase letters denote significant difference in the same physiological index between species ($p < 0.05$).

In the salinity treatment, *C. gigas* demonstrated stronger adaptability to decreased salinity, while *C. nippona* seemed to show greater tolerance to increased salinity. Spat of the NN and GG groups subjected to decreasing salinity were initially detected dead at 17.33 and 16 psu (Figure 1), after which the ASR of the NN group was lower than that of the GG group until all individuals died at 6 psu, and significant interspecies differences in ASR were detected at 10, 12 and 16 psu ($p < 0.05$). Meanwhile, the 50% CSMin in *C. nippona* (12.03 ± 0.08 psu) was significantly higher than that in *C. gigas* (10.78 ± 0.40 psu) ($p < 0.05$). In addition, *C. nippona* and *C. gigas* spat started to die at 38.67 and 38 psu, respectively, due to the increased salinity stress (Figure 1), and then *C. nippona* consistently had higher ASR than *C. gigas* before the last individual died at 48 psu. The difference in accumulative survival rates between species was significant at 42 and 46 psu ($p < 0.05$). The NN group

(43.02 ± 0.54 psu) had significantly higher 50% CSMax than the GG group (42.06 ± 0.23 psu) ($p < 0.05$). The relationships among 50% CSMax, 50% CSMin and salinity (Sa) in the NN and GG groups are shown separately as follows: 50% CSMin-NN = 4.906–0.408Sa (R^2 = 0.973); 50% CSMin-GG = 4.084–0.379Sa (R^2 = 0.960); 50% CSMax-NN = −20.005 + 0.465Sa (R^2 = 0.759); 50% CSMax-GG = −19.459 + 0.463Sa (R^2 = 0.805).

3.2. Growth and Survival Rates of Spat

Three-way ANOVA for assessing the effect of the temperature (Te), salinity (Sa), species (Sp) and their interaction on the daily growth and survival rates of spat cultivated for 30 days is represented in Table 2. In terms of growth (Figure 2), the overall average daily growth rate of all treatment groups in *C. gigas* (321.33 ± 120.64 μm d^{-1}) was significantly higher than that in *C. nippona* (216.48 ± 72.56 μm d^{-1}), and the maximum difference in DGR (219.67 μm d^{-1}) between species was observed at the temperature–salinity regime of 25 °C and 28 psu. Among different environmental conditions, the DGR of *C. nippona* was significantly higher than that of *C. gigas* at the regime 18 °C and 21 psu, while the DGR of *C. nippona* was significantly lower than that of *C. gigas* at all other test conditions except the combination of 32 °C and 21 psu ($p < 0.05$). The DGR of both species at each of the three salinity levels reached a maximum at 25 °C, while low temperature (18 °C) resulted in significant growth inhibition compared to ambient temperature ($p < 0.05$). The spat from the NN and GG groups achieved the uppermost DGR at salinities of 35 (309.33 μm d^{-1}) and 28 psu (478.67 μm d^{-1}) at the temperature of 25 °C, but reached the bottommost DGR at salinities of 28 (94.33 μm d^{-1}) and 21 psu (93.67 μm d^{-1}) at 18 °C, respectively.
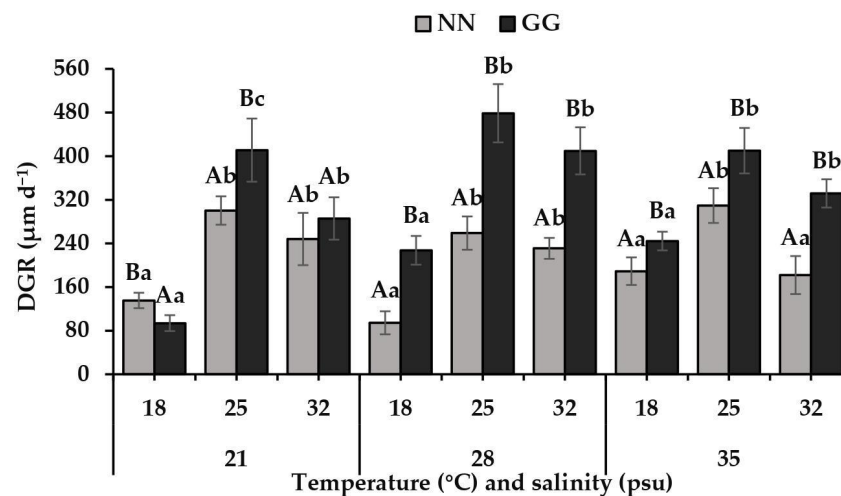

Figure 2. Comparison of the daily growth rate (DGR) of *C. nippona* and *C. gigas* spat exposed to different combinations of temperatures (18, 25 and 32 °C) and salinities (21, 28 and 35 psu) for 30 days. Different lowercase letters indicate significant differences at different temperatures for the same salinity level within the same species, while different uppercase letters represent significant differences between the NN and GG groups at the same environmental condition ($p < 0.05$).

In addition, the survival rate of spat in the GG group was numerically higher than that of the NN group at all environmental regimes except for the ambient condition, and the maximum difference in SR (29.17%) between species occurred at the temperature–salinity regime of 32 °C and 28 psu on day 30 (Figure 3). At 32 °C, species had a significant impact on the SR of spat on day 10 at three salinity levels ($p < 0.05$), but the influence of species disappeared gradually at a salinity of 35 psu with prolonged exposure time ($p > 0.05$). At 18 °C and 35 psu, there was always a significant difference in the survival rate between *C. nippona* and *C. gigas* spat, but both remained above 79% at the conclusion of the experiment ($p < 0.05$). During the experiment, the SRs between species were similar (above 92.50%) and not significantly different ($p > 0.05$) at temperatures of 18 and 25 °C and at salinities of 21 and 28 psu, which was also found at 25 °C and 35 psu.

Table 2. Analysis of variance (three-way ANOVA) testing the factor effects (temperature, salinity and species) on the daily growth and survival rates of spat.

| Source of Variation	Df	Daily Growth Rate (µm d^{-1})			Survival Rate (%)		
		Mean Squares	*F*-Value	*p*-Value	Mean Squares	*F*-Value	*p*-Value
Temperature (Te)	2	177,412.604	151.918	<0.001	4248.727	258.514	<0.001
Salinity (Sa)	2	7432.998	6.365	0.004	639.005	38.880	<0.001
Species (Sp)	1	148,419.893	127.091	<0.001	1204.167	73.268	<0.001
Te × Sa	4	7768.062	6.652	<0.001	37.269	2.268	0.081
Te × Sp	2	11,096.771	9.502	<0.001	250.347	15.232	<0.001
Sa × Sp	2	22,573.074	19.329	<0.001	3.125	0.190	0.828
Te × Sa × Sp	4	1883.387	1.613	0.192	178.472	10.859	<0.001
Error	36	1167.820			16.435		
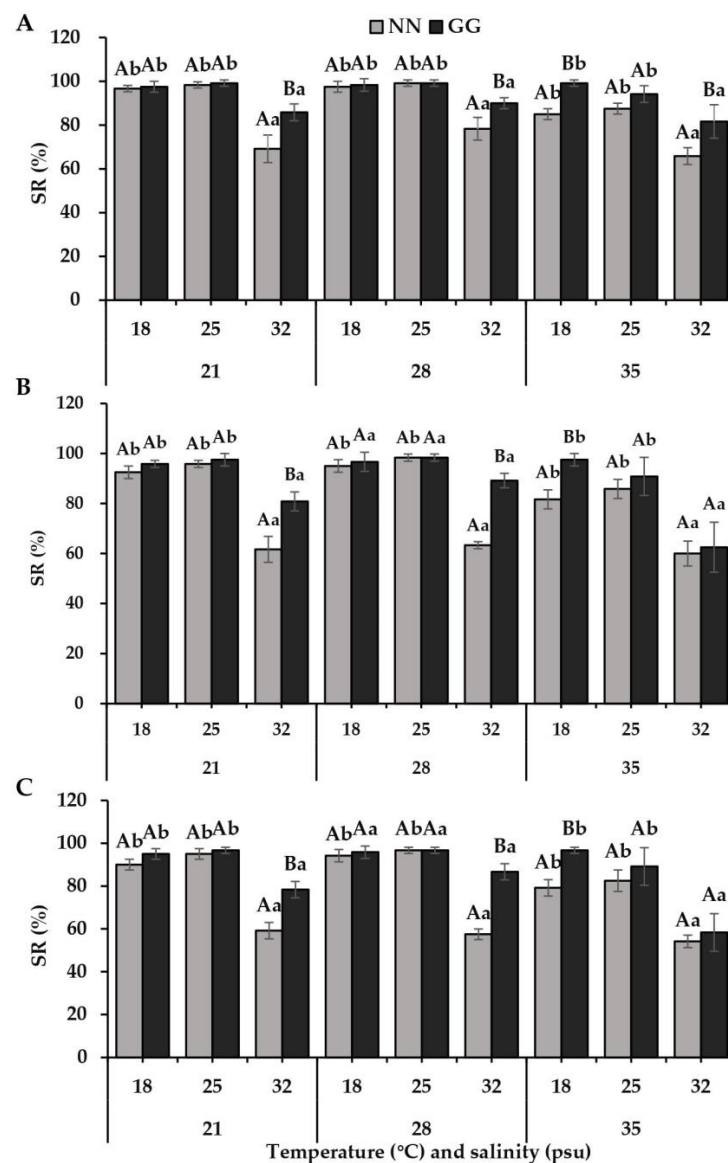
Figure 3. Comparison of the survival rate (SR) of *C. nippona* and *C. gigas* spat exposed to different combinations of temperatures (18, 25 and 32 °C) and salinities (21, 28 and 35 psu) on day (**A**) 10, (**B**) 20 and (**C**) 30. Different lowercase letters indicate significant differences at different temperatures for the same salinity level within the same species, while different uppercase letters represent significant differences between the NN and GG groups at the same environmental condition (*p* < 0.05).

4. Discussion

4.1. Differences in Thermal and Salinity Tolerance Limits

The dynamic approach, characterized by the critical thermal or salinity methodology (CTM or CSM), has been extensively applied to conduct macrophysiological comparative studies and quantify the environmental tolerance in marine organisms [20,36,39–41]. Although the relevant physiological indicators have not been reported in *C. nippona* so far, the tolerance indices of *C. gigas* (STMax: 33.63 °C; 50% CTMax: 36.67 °C; CTMax: 36.67 °C) to continuous temperature changes (2 °C d^{-1}) observed in previous studies were similar to the current experimental results [42]. In the current study, the variation in the accumulative survival rate of the early stage spat between species revealed an overall trend that *C. nippona* spat were more susceptible to temperature changes and hyposalinity stress compared to *C. gigas*. The interspecific differences in ecological characteristics were in accordance with the prior report that the phagocytic activity mediated by hemocytes from *C. nippona* was considerably lower than that of *C. gigas* under hyposalinity (7.5 psu) and hypothermia (5 °C) stress [13]. One possibility for this observation was that subtidal organisms tend to be more sensitive to environmental changes than creatures residing in littoral and estuarine habitats subjected to brackish water or aerial exposure [18,20,40]. Although *C. nippona* and *C. gigas* are sometimes found to be sympatric, the former is commonly grown below the intertidal zone enabling it to avoid direct exposure to environmental stresses caused by drastic changes in abiotic factors [13,24]. Similar interspecific variations caused by habitat niche differences were identified in comparative studies of other bivalves as well [27,43,44], and de Bravo [45] found that *Perna perna* inhabiting shallow water possessed broader thermal scope (CTMax-CTMin) and greater salinity tolerance than *P. viridis* found in deeper zones.

Moreover, species that evolved in little or no seasonal environments generally exhibit narrower environmental tolerance and thus are more vulnerable to alterations in abiotic factors compared to organisms that are widely distributed outside their native range, such as *C. gigas* [16,40,44]. For *C. nippona*, Hokkaido, where the minimum sea surface temperature in winter is below 7 °C, is generally considered to be the northern distribution limit in Japan [13]. Similarly, in China, *C. nippona* are primarily found along the coast of Zhoushan Island in Zhejiang Province [11,17], characterized by relatively moderate seasonal changes in the environmental condition (e.g., temperature ranges from 10 to 30 °C) when compared with the warm temperate and other sub-tropical coasts [38,46]. The increased temperature sensitivity and decreased hyposalinity tolerance exhibited by *C. nippona* spat compared to *C. gigas* might be strongly associated with the limited natural distribution of the oyster species, and low temperature (5 °C) and salinity (14 psu) levels had been proven to induce mass mortality in early life stages of *C. nippona* that might determine the boundary of species distribution range [31,47]. In addition, although no significant difference in the ASR with increasing salinity was detected between *C. nippona* and *C. gigas*, higher CSMax, SSMax and 50% CSMax were observed in the NN group, which was probably species-specific and might suggest that *C. nippona* preferred high-salt conditions compared to *C. gigas*. This might correspond to the previous finding that the optimum growth salinity of Sydney rock oyster (*Saccostrea commercialis*) settled in the more oceanic environment of outer harbors during the larval stage was higher than that of *C. gigas* [27].

4.2. Differences in Growth and Survival Rates

The tolerance of organisms to changes in salinity is not only affected by the ontogenetic and physiological stage, but also by other environmental factors such as temperature, and vice versa [18,25,26]. It is therefore necessary to consider the interaction between temperature and salinity when studying interspecific differences in environmental adaptability. In this study, two-way ANOVA (data not shown) revealed that a significant interaction between the two abiotic factors was found in the daily growth rate and survival rate of each species ($p < 0.05$), while the DGR and SR of early stage spat revealed different responses of

C. nippona and *C. gigas* to nine environmental regimes. In general, the overall performance of *C. gigas* spat was superior to that of *C. nippona* under acute temperature and salinity challenges, potentially implying that the physiological flexibility of *C. gigas* was higher compared to that of *C. nippona*. In particular, the overall average growth rate of *C. gigas* spat ($321.33 \pm 120.64 \ \mu m \ d^{-1}$) from all treatment groups was close to 50% faster than that of *C. nippona* ($216.48 \pm 72.56 \ \mu m \ d^{-1}$), which was consistent with the result of interspecific hybrids [9]. However, the DGR of *C. gigas* spat was significantly lower than that of *C. nippona* at the temperature–salinity combination of 18 °C and 21 psu ($p < 0.05$). Meanwhile, the salinity fluctuations in this experiment seemed to promote the growth of *C. nippona* except at the high temperature (32 °C), which was contrary to the growth performance of *C. gigas*. These differences between species could be related to species-specific environmental adaptation strategies, and reduced energy input for shell production might facilitate *C. gigas* surviving longer in unfavorable environments [18]. Similar findings were reported by Laing [48], as they found that the growth rates of king scallop spat (*Pecten maximus*) at low temperatures was reduced at low salinity.

In the present study, the fact that *C. nippona* spat showed sensitivity to a sudden increase in salinity in the early stage of the experiment might be due to the inferior adaptability of the stenohaline species to drastic changes in salinity [24,44], but the final survival data remained at high levels (above 79%) at temperatures of 18 and 25 °C suggesting that the adverse effect of hypersalinity stress might be temporary. Unexpectedly, the effect of species on the SR of spat eventually seemed to disappear with extending exposure times at the regime of 32 °C and 35 psu, probably due to the high temperature diminishing the salinity tolerance of *C. gigas* spat [49], which might indirectly support the conclusion that *C. nippona* spat were more suitable to hypersaline environments than *C. gigas*. The long-term adaptation of *C. nippona* to the hypertonic environment of the relatively deeper seabed may be an important reason for its special biological characteristics [13]. In addition, the impact of acute salinity stress used in this experiment on the DGR and SR of *C. nippona* was limited without considering temperatures, demonstrating that *C. nippona* spat could be capable of long-term survival in commercially farmed systems with similar environmental conditions even in the rainy season. In contrast, temperature might be the more important cause of large intra- and interspecific variations in the growth and survival of spat in the current study. The DGR and SR of both *C. nippona* and *C. gigas* spat at each salinity level reached the maximum at 25 °C with increasing temperature, indicating that the optimum farming temperatures of both species were observed near 25 °C which were consistent with previous studies [12,50,51]. The inhibition of the filtration rate and digestibility might be the main reasons for the poor growth performance of both species observed at 18 °C which generally occurred in October or November along the coast of China [12,28,52]. The finding proved strong proof that it is necessary to shorten the maturation period of broodstock by artificially manipulating the environmental conditions to extend the optimal growing season of *C. nippona* spat, whether for improving environmental tolerance or reducing the farming cost [10,32]. However, this will result in the inevitable exposure of hatchery-produced spat to heat stress [12,18,38]. High temperatures can lead not only to a less favorable energy balance and reduced maximum size in shellfish, but also to severe disease problems caused by immune system disorders of organisms and an increase in the growth of potentially pathogenic ciliates and bacteria in subsistence environments [12,28,49,53]. In this study, significant differences in the SR between the NN and GG groups were found at all combinations of 32 °C on day 10 ($p < 0.05$), indicating that *C. nippona* was more sensitive to sudden increased temperatures similar to the findings obtained in the former experiment. Although the DGR of *C. nippona* spat was relatively high at each test condition at 32 °C, the poor survival (below 60%) was observed on day 30, suggesting that sites for field cultivation where this temperature might occur regularly should be avoided, at least for the early stage spat.

5. Conclusions

In conclusion, the present study showed that *C. gigas* spat was more tolerant to variations in temperatures and low-salinity stress compared to *C. nippona* which preferred hypersaline environments, and the latter was more susceptible to acute changes in environmental conditions. Therefore, particular attention should be paid to the inferior adaptability of *C. nippona* when attempting to farm in locations outside their natural habitats. For *C. nippona*, retarded growth occurring at a low temperature(18 °C) implied the need to implement standard conditioning protocols to prolong the optimal growing season, while aquafarming locations exposed to temperatures above 32 °C for an extended period are not to be recommended at least in the early stage spat. Environmental regimes of 25 °C and 21–35 psu were considered optimal conditions for the cultivation of *C. nippona* spat. The information obtained in this study is helpful in deepening the knowledge about the tolerance of *C. nippona* to environmental stresses and provides a valuable reference for the development of the future aquaculture industry.

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Data Availability Statement: The data that support the findings of this study are available from the authors upon reasonable request.

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