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Effects of temperature, salinity and body size on the physiological responses of the Iwagaki oyster *Crassostrea nippona*

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Abstract

The physiological responses of the juvenile *Crassostrea nippona* in terms of filtration, oxygen consumption and ammonia excretion to changes in temperature (16–32°C), salinity (15–35 psu) and body size (small, medium and large) were investigated. In this study, the values of filtration rate (FR), oxygen consumption rate (OCR) and ammonia excretion rate (AER) increased with temperature rising from 16°C to 24°C, reaching the highest values at 24°C and 28°C; with any further increase in temperature above this limit, these values decrease drastically ($p < .05$). The highest Q_{10} coefficients were 2.75 for large, 3.54 for medium at 16–20 and 3.47 for small size at 20–24°C respectively. Moreover, the responses of FR and OCR were found to be influenced significantly by salinity, tending to increase concomitantly with salinity up to 25–30 psu, though the values of these parameters were diminished dramatically ($p < .05$) above this level, showing a reverse pattern from that observed in AER, which firstly decreased to the lowest level at 25 and 30 psu, and then severely ($p < .05$) increased to the highest level at 35 psu. In addition, the low O:N ratios of all sizes of *C. nippona* at 16°C and 30–35 psu were indicative of a protein-dominated catabolism, whereas the O:N ratios of large size at 20–32°C and all sizes at 20–30 psu, indicating that the metabolic energy from protein diminished and lipid and carbohydrate were used as the energy substrates. Physiological rates of *C. nippona* were well correlated with its size. The average values of mass exponents (*b*-values) estimated in the present study were 0.657 for OCR and 0.776 for AER at different temperatures, and 0.647 for OCR and 0.767 for AER at varying salinities, signifying that physiological process of *C. nippona* becomes relatively slower with increasing body size regardless of temperature or salinity. Finally, our results confirm that the optimal temperature and salinity for juvenile *C. nippona* lie within 24–28°C and 25–30 psu respectively. The results of physiological traits in response to environmental factors of this species are informative in site selection for the cultivation.

KEYWORDS
body size, *Crassostrea nippona*, juvenile, physiology, salinity, temperature

1 | INTRODUCTION

Feeding, respiration and excretion are generally considered to be the critical approaches for evaluating the physiological responses of marine molluscs. Filtration rate and oxygen consumption rate follow a trend similar to that of the growth rate and are associated with scope for growth, which reflects the balance between energy acquisition and metabolic expenditure (Han, Lee, & Wang, 2008; Yin et al., 2013), while the ammonia excretion rate, besides being a good predictor of the production in aquatic species, is also a good indicator of the energy loss and the condition and fitness of individuals (Fernandez & Tanner, 2008). Nevertheless, all of these parameters that comprise the growth equation are capable of variation in response to changes in the exogenous and endogenous stressors, and fluctuations in the balances between these measurements are the substance of physiological acclimation (Kang et al., 2015). Therefore, it is necessary to clarify the limits of these stressors for the validity of a physiological study.

Temperature and salinity have long been recognized as two of the dominant extrinsic factors influencing a host of physiological processes of marine invertebrates (Shumway, 1982). Temperature can affect gametogenesis, reproduction, fertilization, nutritional efficiency, and feeding and metabolic activities in aquatic organisms (Christophersen & Strand, 2003; Saucedo, Ocampo, Monteforte, & Bervera, 2004; Vélez & Epifanio, 1981), and salinity is a limiting factor in the distribution and affects biological activities, including those related to immune responses, osmoregulation and morphological development (Berger & Kharazova, 1997; Nicolini & Penry, 2000; Taylor et al., 2007). The intrinsic factor, which mainly refers to body size, is derived from the sampled population and has been established the fundamental allometric relationship with the rates of physiological processes (Kang et al., 2015). Based on this allometric equation, probabilistic estimates and comparisons of physiological responses among individuals can be made for different environmental conditions (Schurink & Griffiths, 1992). By now, the importance of studying the effects of temperature, salinity and body size has been increasingly highlighted and the positive relationship between these factors and biological processes has been well documented not only among but also within ecologically or commercially relevant species (Han et al., 2008).

The ecologically and economically important bivalve mollusc Iwagaki oyster *Crassostrea nippona* is commonly inhabiting in stones and reefs in the low tidal positions and the intertidal zones, along the coast of East Asia (Itoh, Tun, Komiyama, Ueki, & Ogawa, 2004; Yoon, Jung, & Choi, 2008). This species is appreciated by consumers for its unique flavour, and delicious and tender taste, and is recognized as an important fishery resource to complement with the unavailability of the Pacific oyster *C. gigas* during summer on account of low condition index of gonad caused by spawning; hence, the commercial price of *C. nippona* is estimated as high as fivefold that of *C. gigas* in Japan (Itoh et al., 2004). In consideration of the broad market prospect and the potential commercial importance for its exploitation, cultivation and utilization, studies on this species have been concerned with breeding and nursery techniques for hatchery production of seed (Fujiwara, 1995; Wang, Li, Zhang, & Yu, 2018), large-scale culture methods of juvenile (Fujiwara, 1998b), biological and biochemical characteristics of adult (Okumura, Miura, Semura, & Kishimoto, 2005), but not with the effects of environmental factors on physiological activities with the increasing interest of *C. nippona*. Further understanding of its physiological energetics will add to our knowledge on its physiological tolerance as well as adaptation strategy in relation to growth and survivorship to varying environmental conditions (Jiang, Lin, & Wang, 2008b).

The objective of the present study was to determine the effects of temperature, salinity and body size on the physiological components of *C. nippona*, using the rates of filtration, oxygen consumption and ammonia excretion as the physiological stress indicators, to conduct a basic framework for physiological research in *C. nippona*. The results obtained in this study could provide reliable information on the regulation of specific environmental factors for restoration management of natural stocks and cultivation practices for *C. nippona*.

2 | MATERIALS AND METHODS

2.1 | Biological material

The juveniles of *C. nippona* were obtained from oyster farm at Rushan Bay (36°43′–37°36′N and 121°28′–121°39′E), Shandong province, China, transported to the hatchery at Yantai City and held in indoor cement tanks (5°C, 32 psu). They were divided into three different size groups, and each group consisted of 20 individuals (Table 1). Soft tissues of the oysters in each chamber were dissected, and tissue dry meat weight (DW: g) was individually determined after drying the tissues at 80°C for 48 hr after physiological measurements were completed.

2.2 | Experimental design

The juveniles were acclimated at each of the five experimental temperatures (16°C, 20°C, 24°C, 28°C and 32°C) and five

TABLE 1 Biological characteristics of different groups of *C. nippona* used in the experiment

Experimental body sizes	Shell height (mm^{-1})	Shell length (mm^{-1})	Shell width (mm^{-1})	Live weight (g^{-1})	Dry meat weight (g^{-1})	Dry shell weight (g^{-1})	Condition index per %
Large	62.16 ± 6.33	46.62 ± 7.18	22.31 ± 4.14	30.54 ± 7.47	2.25 ± 0.72	18.69 ± 4.54	12.15 ± 2.97
Medium	51.34 ± 5.27	40.62 ± 3.38	21.70 ± 4.38	19.49 ± 5.96	1.42 ± 0.32	11.73 ± 3.46	12.69 ± 2.71
Small	39.90 ± 4.07	28.84 ± 3.71	19.06 ± 4.43	12.18 ± 2.01	1.01 ± 0.18	6.95 ± 1.81	15.90 ± 3.28

salinities (15, 20, 25, 30 and 35 psu) by modifying the temperature and salinity by 1°C/day and 2 psu/day respectively. When the desired temperature or salinity was reached, juveniles were held at that temperature or salinity for two weeks before the physiological measurements were carried out. The salinity was kept stable at 32 psu in temperature groups, and the temperature was kept stable at 20°C in salinity treatments. During accumulation, the animals were fed with an axenic culture of mixed algal diet of Tahitian *Isochrysis* aff. *galbana* (T-ISO) and *Nitzschia closteriuman* (30×10^6 cells/L) at daily rations of 3%–4% (dry algal weight/dry oyster meat weight). Faeces and uneaten food in the tanks were siphoned out before each feeding, and 100% of the seawater was renewed every second day. The duration of the whole experiment was two months.

2.3 | Physiological parameter measurements

Filtration rate (FR) was determined using a static system in which the decrease in algal cell density within the experimental aquarium was monitored in relation to time. Two acclimated oysters were placed in 3-L plastic respiration chamber at test temperature or salinity level for 1 hr. Each treatment had five replicates and three blank chambers with no juvenile as the control. After experimenting, the seawater was siphoned into a brown glass bottle for algal concentration measurement. Each treatment was analysed six times to minimize measurement errors. The value of the FR (L g^{-1} hr^{-1}) was calculated using the following equation:

$$FR = V(\ln Ct_0 - \ln Ct_1)/(DW \times T).$$

where V is the volume of the respiration chamber (L), DW is the dry weight of juvenile (g), and Ct_0 and Ct_1 are the concentrations of algae at the beginning and after time T (hr) respectively. Recorded Ct_0 and Ct_1 for oysters in each chamber were the average of five replicates on the concentrations of unicellular algae over time, and reported values of the FR are means of five of these chamber measurements. The experimental algae concentration was 15×10^6 T-ISO cells/L, and cell concentrations were measured by direct counting with particle (cell) counters at the beginning and at the end of each experiment. FR of *C. nippona* was measured every 12 hr to eliminate the effect of any diurnal rhythm on the metabolic rate of the juveniles.

Closed-chamber respiration method was employed to determine the oxygen consumption rate (OCR) and ammonia excretion rate (AER). In order to assure oxygen saturation before taking experiment, seawater was fully aerated for at least 12 hr in a 100-L tank prior to filling the experimental chambers. To avoid interference with post-prandial metabolism of food and faeces excretion, acclimated oysters were kept unfed for 24 hr and then four similar-sized oysters were transferred to respiration chambers that were placed in a water bath previously calibrated to the respective temperatures and salinities. Five replicates and three blank chambers with no juvenile were performed per treatment. All chambers were sealed with liquid paraffin, to ensure that they were airtight. The experiment lasted for 3 hr, and the oysters were unfed during the whole experiment. Water samples were collected by siphoning, and dissolved oxygen (DO) and ammonia were measured at the beginning and at the end of the experiment using a DO meter (YSI, 600XL, USA) with 0.01 mg/L accuracy and the Nessler's reagent colorimetric method respectively. Blank seawater readings of DO and ammonia were carried out and then subtracted from the experimental units to correct for autogenic trends. OCR and AER (mg g^{-1} hr^{-1}) were calculated using the following equation:

$$OCR = V(DO_0 - DO_t)/(DW \times T).$$

$$AER = V(N_t - N_0)/(DW \times T).$$

the initial and final concentrations of DO and ammonia are denoted by subscripts 0 and t, respectively, V is the volume of the respiration chamber (L), DW is the dry weight of juvenile (g), and T is the time between the initial and final measurements (hr).

FR, OCR and AER of different sizes of oysters were standardized to a common 1 g. Total dry weight was calculated using the expression:

$$Y_{st} = (1/DW_e)^b \times Y_e$$

where Y_{st} and Y_e represent standard and experimental rates, respectively, DW_e is the experimental dry weight, and b is the power value that scales physiological rates to body weight in this species of oyster (0.652 for FR; 0.716 for OCR; 0.707 for AER, own unpublished data). FR, OCR and AER were expressed as dry weight-specific (mg h^{-1} g^{-1} DW) rates in the present experiment.

The O:N atomic ratio (based on the oxygen uptake and ammonium nitrogen excretion) was used to estimate the ratio of proteins, lipids and carbohydrates that were used as energy substrates for the organisms under the different experimental conditions, expressed in atomic equivalents according to the formula $O/N = (mgO_2 hr^{-1}/16)/(mgNH_4 hr^{-1}/14)$.

The Q_{10} (temperature coefficient), a measure of the rate of change of a biological or chemical system as a consequence of increasing the temperature by 10°C, was calculated for *C. nippona* according to the Van't Hoff equation (Bayne & Newell, 1983).

$$Q_{10} = (R_2/R_1)^{10/(t2-t1)}$$

where t_1 and t_2 represent the temperature of two group trials, respectively, and R_1 and R_2 represent corresponding metabolic rates under each temperature group.

2.4 | Data analyses

All data were expressed as mean ± standard deviation (*SD*) and were analysed using SPSS 18.0. The differences of FR, OCR and AER

among different treatments were analysed using one-way analysis of variance (ANOVA), followed by the least significant difference (LSD) test and Duncan's multiple range test for post hoc comparisons. Interactions between oyster size and temperature and between size and salinity were analysed with a two-way ANOVA. Significance levels for all analyses were set at $p < .05$.

3 | RESULTS

3.1 | Effects of temperature on FR, OCR and AER

The effects of different temperatures on FR, OCR and AER in different body sizes of the *C. nippona* are presented in Figure 1a–c. In our

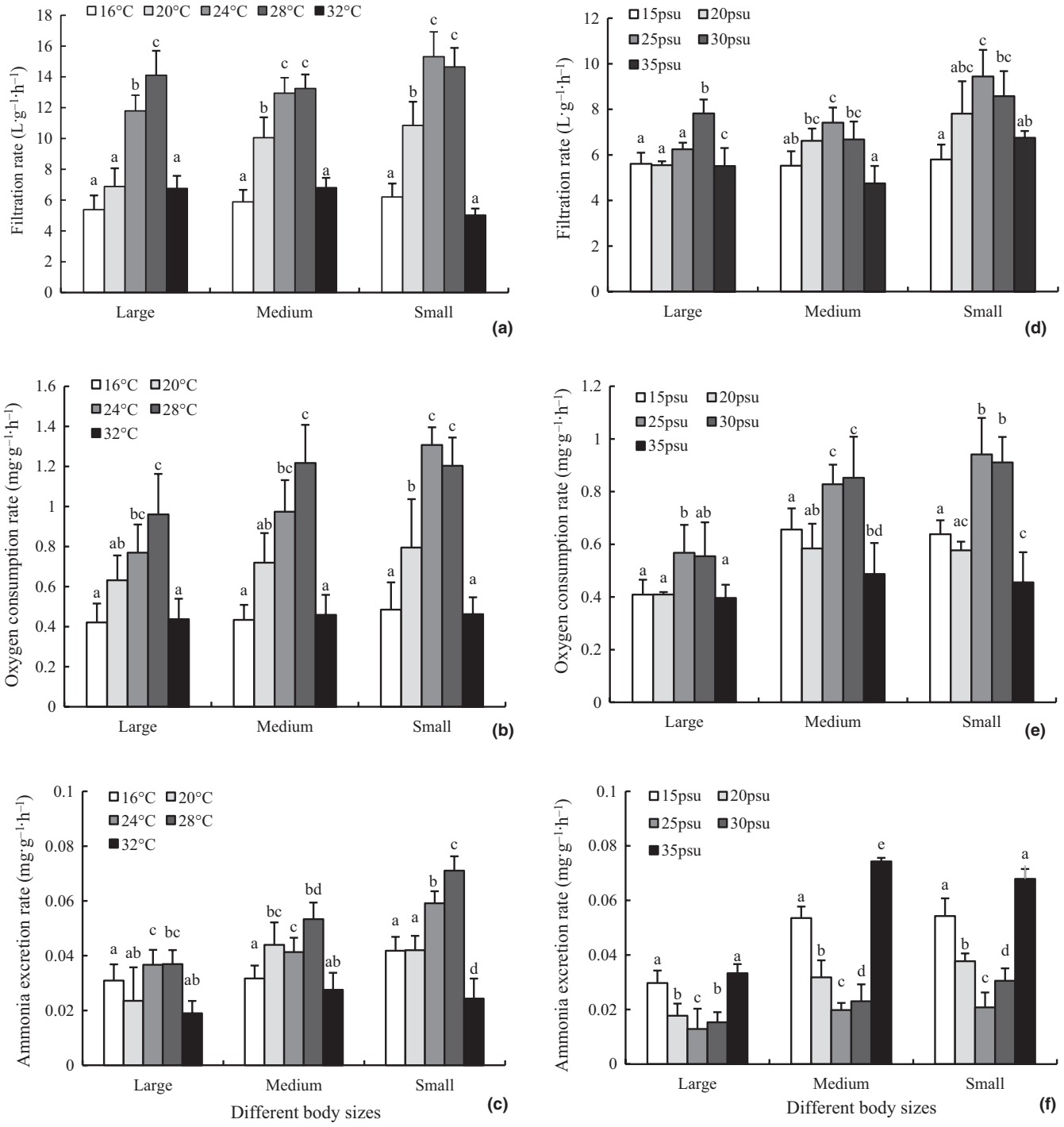

FIGURE 1 Effects of different temperatures on (a) filtration rate, (b) oxygen consumption rate and (c) ammonia excretion rate in different body sizes of the *C. nippona*; effects of different salinities on (d) filtration rate, (e) oxygen consumption rate and (f) ammonia excretion rate in different body sizes of the *C. nippona*. Bars denote standard deviation (five replicates of determinations). Under the same size, different lowercase letters indicate significance ($p < .05$), while identical letters indicate non-significance ($p > .05$)

range of temperatures (16–32°C), small size showed generally higher FR, OCR and AER than medium and large *C. nippona*, showing a curve of ascending to descending with temperature increase. The FR at 28°C was significantly ($p < .05$) higher than those at the other temperatures in large size, and the highest values in small and medium sizes were detected at 24°C and 28°C respectively (Figure 1a). However, there were no significant differences between the FRs at 16 and 32°C, and those at 24°C and 28°C in these two sizes ($p > .05$). For OCR of *C. nippona*, the highest OCR of small size was observed at 24°C (1.307 mg g^{-1} hr^{-1}), whereas the lowest OCR was at 32°C (0.462 mg g^{-1} hr^{-1}) (Figure 1b). Comparably, respiratory metabolism of medium and large size varied from 0.433 and 0.421 mg g^{-1} hr^{-1} (16°C) to 1.217 and 0.960 mg g^{-1} hr^{-1} (28°C) respectively. AERs of different sizes of *C. nippona* firstly increased to the highest level at 28°C and then decreased to the lowest level at 32°C with increasing temperature (Figure 1c). Statistically, temperatures of 16°C, 20°C and 32°C formed a homogeneous group ($p > .05$) in terms of excretion of large size, whereas 28°C yielded the highest relative rate of ammonia excretion in three body sizes (0.037 ± 0.005 mg g^{-1} hr^{-1} for large, 0.053 ± 0.006 mg^{-1} g^{-1} hr^{-1} for medium and 0.071 ± 0.005 mg g^{-1} hr^{-1} for small).

The O:N ratios of the three size ranges of the *C. nippona* at different temperatures are shown in Table 2. The O:N ratios of the large, medium and small size at temperatures of 16 to 32°C ranged from 12.37 to 23.49, from 12.67 to 20.63 and from 9.68 to 19.34 respectively. The Q_{10} coefficients for the different temperatures are shown in Table 3. For large size, the highest value was recorded at 2.75 between 16°C and 20°C, followed by 1.74 between 24°C and 28°C, 1.64 between 20°C and 24°C and 0.14 between 28°C and 32°C. However, the highest Q_{10} value was recorded at 3.54 between 16°C and 20°C in medium size, whereas the highest Q_{10} coefficients were 3.47 for small size at temperatures of 20°C –24°C.

3.2 | Effects of salinity on FR, OCR and AER

The effects of different salinities on FR, OCR and AER in different body sizes of the *C. nippona* are presented in Figure 1d–f. Similarly, the effect of salinity on these values of the juveniles was also significant. The highest FRs in the small size of *C. nippona* were detected at the higher experimental salinities, with values of 9.44 and 8.58 L g^{-1} hr^{-1} at 25 and 30 psu respectively (Figure 1d). Similar trends were also observed in large and medium sizes. The OCR at 25 psu was significantly ($p < .05$) higher than that at the other salinities, except at 30 psu ($p > .05$) in small size, and OCRs at 25 psu were significantly ($p < .05$) higher than those at 35 psu salinity in large and medium sizes (Figure 1e). However, there were no significant differences ($p > .05$) between the OCRs at 20 and 35 psu in three sizes. Different with the trends of effects of salinity on FR and OCR, in our range of salinity (15–35 psu), AERs of large, medium and small sizes firstly decreased to the lowest level at 25 psu and then increased to the highest level at 35 psu (Figure 1f). The AERs at 15 and 35 psu were significantly ($p < .05$) higher than those at the other salinities in large and small sizes, and the significant differences ($p < .05$) between the AERs at different salinity groups were observed in medium size.

The O:N ratios at salinities of 15, 20, 25, 30 and 35 ranged from 10.40 to 38.58 in large size, 5.73 to 36.59 in medium size and 5.87 to 39.65 in small size respectively (Table 2). The atomic O:N ratios of three sizes were the smallest at 35 psu, and O:N ratios were close to each other for different body mass from 15 to 30 psu.

3.3 | Effects of body size on OCR and AER at different temperatures and salinities

The regression equations and parameter estimates of OCR and AER towards body size in *C. nippona* are given in Table 4. At different temperatures and salinities, the relationships between body size and OCR and AER were negatively correlated; these values decreased with increased body size. At different temperatures, the a_1 ranged from 0.585 to 1.573 (average value 1.032) and b_1 ranged from 0.601 to 0.702 (average value 0.657) with the coefficient of determination as high as $R^2 = 0.962$, and the a_3 ranged from 0.032 to 0.065 (average value 0.048) and b_3 ranged from 0.603 to 0.895 (average value 0.776) with the coefficient of determination as high as $R^2 = 0.948$. At varying salinities, a_2 ranged from 0.561 to 1.053 (average value 0.837), b_2 ranged from 0.409 to 0.897 (average value 0.647), while a_4 ranged from 0.024 to 0.063 (average value 0.045), and b_4 ranged from 0.508 to 0.983 (average value 0.767).

TABLE 3 Q_{10} coefficients in different body sizes of *C. nippona* calculated from different temperatures

Temperature (°C)	Large	Medium	Small
16–20	2.75	3.54	3.44
20–24	1.64	2.13	3.47
24–28	1.74	1.75	0.81
28–32	0.14	0.08	0.09

TABLE 2 The effects of different temperatures and salinities on the O:N ratio of different body sizes of *C. nippona*

Experimental body sizes	Temperature (°C)					Salinity (psu)				
	16	20	24	28	32	15	20	25	30	35
Large	12.37	23.49	18.29	22.90	19.43	12.03	12.04	38.58	31.65	10.40
Medium	12.67	14.32	20.63	19.97	14.57	10.73	9.55	36.59	32.40	5.73
Small	9.68	16.56	19.34	14.82	16.64	10.29	9.30	39.65	26.11	5.87

3.4 | The interactive effects of temperature and size, and salinity and size on FR, OCR and AER

The effect of size and temperature on FR was extremely significant ($p < .01$), and the interactive effect of size and salinity on FR was significant ($p < .05$; Table 5). The interactive effects of temperature and size, and salinity and size on OCR were insignificant ($p > .05$), whereas the effects on ARE were highly significant ($p < .01$).

4 | DISCUSSION

4.1 | Effects of temperature on FR, OCR and AER of C. nippona

Feeding activity and metabolic rates normally increase with temperature, up to an optimum or maximum limit beyond which they decrease significantly, which have long been recognized for numerous species of poikilothermic organisms (Sarà et al., 2012). For example, Deng, Yu, Du, Wang, and Fu (2011) reported that the declining point for FR occurred at 25°C (tested range of 15–30°C) in *Pinctada martensii*. The OCR and ARE of juvenile *Ruditapes philippinarum* increased markedly as the temperature increased from 15 to 25°C, but then decreased with further increases in temperature (Nie et al., 2017). The patterns of the increase in the FR, OCR and AER of all sizes of *C. nippona* with increasing temperature are consistent with the results of previous studies. In the present study, the values of these parameters increased with temperature rising from 16°C to 24°C, reaching the highest values at 24°C and 28°C; with any further increase in temperature above this limit, the values decrease drastically. These results suggest that at a certain optimum temperature range, filtration process accelerates, and protein and amino acid catabolism increase, leading to an increase in filtration, oxygen consumption and ammonia excretion. Therefore, an adequate temperature range of filtration and metabolism for *C. nippona* lies within 24–28°C.

Previous studies suggest that the variation in FR with temperature might be mainly due to changes in the beat frequency of the various types of gill cilia (Bernard, 1972). When thermal stress extends the range at which bivalves can physiologically adapt, FR decreases for the beat frequency of the lateral cilia, frontal cilia and

TABLE 4 Estimated parameters in the regression equation of OCR and AER towards body size in *C. nippona* at different temperatures and salinities

	OCR				AER			
Temperature (°C)	a_1	b_1	R^2	n	a_3	b_3	R^2	n
16	0.598	0.601	.966	20	0.041	0.617	.940	20
20	0.993	0.659	.993	20	0.043	0.878	.990	20
24	1.401	0.627	.999	20	0.065	0.895	.989	20
28	1.573	0.698	.853	20	0.060	0.889	.961	20
32	0.595	0.702	.999	20	0.032	0.603	.860	20
	OCR				AER			
Salinity (psu)	a_2	b_2	R^2	n	a_4	b_4	R^2	n
15	0.915	0.827	.914	20	0.058	0.508	.939	20
20	0.685	0.409	.981	20	0.039	0.876	.966	20
25	0.971	0.433	.996	20	0.024	0.983	.961	20
30	1.053	0.897	.926	20	0.042	0.818	.897	20
35	0.561	0.669	.935	20	0.063	0.652	.320	20

TABLE 5 Two-way ANOVA comparing the effects of temperature and size, and salinity and size on FR, OCR and AER of *C. nippona*

		FR (L g^{-1} hr^{-1})			OCR (mg g^{-1} hr^{-1})			AER (mg g^{-1} hr^{-1})		
Source of variation	*df*	MS	*F*	*p*	MS	*F*	*p*	MS	*F*	*p*
Temperature (Te)	4	128.088	103.653	<.001*	0.854	41.875	<.001*	0.001	33.514	<.001*
Size (Si)	2	10.086	8.162	.001*	0.137	6.699	.004*	0.02	38.543	<.001*
Te × Si	8	5.892	4.768	.001*	0.044	2.139	.063	< 0.001	4.333	.001*
Salinity (Sa)	4	9.267	15.790	<.001*	0.197	21.456	<.001*	0.003	114.046	<.001*
Size (Si)	2	11.305	19.262	<.001*	0.264	28.675	<.001*	0.002	85.208	<.001*
Sa × Si	8	1.599	2.724	.022*	0.014	1.511	.195	< 0.001	7.327	<.001*

Note: df , degree of freedom; MS, mean square; asterisks denote significant differences ($p < .05$).

eu-latero-frontal cirri reduction. Moreover, when evaluating the effects of temperature on the FR, valve-opening states of bivalves should be compared (Kittner & Riisgard, 2005). When exposed to low or high temperature (16°C or 32°C) in the present study, oysters closed their valves and the maximal valve opening was stimulated by the increase or decrease in temperature to 24°C or 28°C. In addition, the viscosity of the water, which affects the resistance to water flow in the canal systems, can also explain the variation in FR with temperature. Jørgensen, Larsen, and Riisgård (1990) demonstrated that FR of *Mytilus edulis* increased with temperature, linearly correlated with the decrease in viscosity related to the higher water temperature at a constant salinity. Therefore, changes in the beat frequency of the different types of gill cilia, differences in valve-opening state and the viscosity of the water could be the main reasons for the variation in FR with temperature.

The reduction in oxygen consumption and ammonia excretion in *C. nippona* at 16°C and 32°C may also be caused by the increased duration of valve closure in response to reduced and increased temperature as mentioned above, stopping both the feeding current and ventilator gill irrigation (Hutchinson & Hawkins, 1992). Besides, the fluctuations in the OCR and AER by the oysters in this study caused by changes in temperature might be related to the effect of temperature on the rate of enzymatic activity involved in the normal metabolism (Bougrier, Geairon, Deslous-Paoli, Bacher, & Jonquières, 1995). From an initial situation of low enzyme activity, the increased enzymatic activity would be synchronized with the increase in temperature, and the immediate depression of enzymatic activity would induce when water temperatures are above 28°C, which might explain the declining or turning points for OCR and AER generally occurred at 28°C. Furthermore, previous studies suggest that when thermal stress extends the range of the physiological adaptation of bivalves, metabolic rates might reduce for energy conservation and some maintenance of growth (Laing & Child, 1996), which is consistent with our previous study that juveniles of *C. nippona* exhibit relatively robust physiological condition as well as reduced survival and growth rate at 16°C and 32°C compared with those at 24°C and 28°C (Wang & Li, 2018).

The temperature coefficient (Q_{10}) is a parameter to describe the sensitivity of organisms to temperature increase, and its value reflects the adjustments related to the enzymatic and physiological requirements for energy when temperature increases within the natural range (Kim, Yoon, Kim, Gil, & Lee, 2005). Previous studies have confirmed that the values of Q_{10} were largely close to two for metabolic rates of aquatic animals, indicating that the metabolic increases twofold for a temperature change of 10°C (Zheng, Jin, Li, Bai, & Dong, 2008). In the present study, Q_{10} values obtained from *C. nippona* were 1.64 and 1.74, for large size between 20–24°C and 24–28°C, respectively, which indicated that these populations will be well adapted to these temperatures because there were no strong modifications in metabolism. Similar results were also found in medium size at 20–24°C and 24–28°C. However, water temperature below 20°C represented a stressful situation for *C. nippona*, as Q_{10} was very high (2.75, 3.54 and 3.44 for large, medium and small size, respectively) between 16 and 20°C. It may explain why juvenile *C. nippona* suffered high mortality as well as retarded growth rate at 16°C (Wang & Li, 2018). In contrast, water temperatures above 28°C represented a less sensitive response for *C. nippona*, as Q_{10} values were sharply decreased as temperature increased from 28°C to 32°C, which indicated that *C. nippona* had greater adaptability to high temperature than low temperature. In addition, the value of Q_{10} was 3.47 for small size between 20°C and 24°C, which is higher than large and medium size at the same temperature range. This implied that the tolerance range of temperature increased with the growth of *C. nippona*. Therefore, it is apparent that Q_{10} values vary not only with the particular temperature ranges, but also with the body size of *C. nippona*.

4.2 | Effects of salinity on FR, OCR and AER of C. nippona

Compared with temperature, the responses of FR and OCR were found to be significantly influenced by salinity, tending to increase concomitantly with salinity up to 25–30 psu, though the values of these parameters were severely diminished above this level. Similar results were also reported by other authors for various suspension-feeding bivalves, such as *C. corteziensis* (Guzmán-Agüero, Nieves-Soto, Hurtado, Piña-Valde, & Garza-Aguirre, 2013) and *Argopecten purpuratus* (Navarro & Gonzalez, 1998). In addition, either FR or OCR of the small and medium juveniles at a salinity of 25 psu did not differ in that at 30 psu significantly in this study, indicating that *C. nippona* could well adapt to the changes of salinity between 25 and 30 psu; thus, an adequate filtration and respiratory salinity range for *C. nippona* lie within 25–30 psu based on current studies.

The effect of salinity on the OCR of *C. nippona* follows a similar curve to that of FR, indicating that oxygen uptake seems to be related to feeding activity. Though the mechanisms through which salinity affects marine molluscs remain unclear, mantle cavity hermetization of bivalves could be a major driver for FR and OCR reduce synergistically in suboptimum salinity since oysters seal themselves off by closing their shells to impede water–salt exchange with the external medium and keep steady-state balance in the influx and efflux of water and salts when salinity drops too low or increases too high (Berger & Kharazova, 1997). Moreover, changes in absorption and saturation coefficients of dissolved gases, especially the oxygen solubility of the water (Zheng et al., 2008), which is severely reduced at very high salinities, might be causing the FR and OCR reduction of *C. nippona*.

In this study, AER of *C. nippona* firstly decreased to the lowest level at 25 and 30 psu, and then increased to the highest level at 35 psu, showing a reverse pattern from that observed in FR and OCR. Based on the previous study, the change of osmotic pressure is probably the main factor for metabolism fluctuations caused by different salinities; therefore, the significant increase in AER observed on *C. nippona* with salinity increasing from 25 to 35 psu can be explained by

an increase in activity related to the catabolism of amino acid, where they could be utilized as osmolytes to keep up the osmotic imbalance with the external environment (Navarro & Gonzalez, 1998). Morgan and Iwama (1991) also confirmed that the energetic cost associated with ionic and osmotic regulation was minimal within the salinity range that was normal for the species and life stage; thus, our result suggests that the optimum excretion salinity level for *C. nippona* lies within 25–30 psu.

Previous studies showed that the effect of salinity on AER has been often contradictory because the pattern obtained in *R. philippinarum* (Nie et al., 2017) and *A. purpuratus* (Navarro & Gonzalez, 1998) contradicts with those reported in other molluscs (Barber & Blake, 1985) and our tendency in agreement with the latter studies. Bougrier et al. (1995) argued that this contradictory can be explained by the difference in the process of animal acclimation. Because of the rapid change of rearing environmental factors, the adaptation process of marine animals is apparently shorter in laboratory than the long-term gradual adaptation in nature; thus, the physiological reaction of same species exposed to the same condition is a paradox. For *C. nippona*, the findings of AER in this study might be based on observations of short-term acclimated to reduced or increased salinities, which indicated that the salinity acclimation duration of this organism is likely to be longer than *C. gigas*.

In the present study, we also evaluated the ratios of oxygen consumption to ammonia excretion in atomic equivalents. The O:N atomic ratio is often used as an index of substrate (including carbohydrate, lipids and protein) oxidation that can provide information on changes in energy substrate utilization under various environmental regimes (Rocha, Gomes, Phan, Passos, & Furia, 2005). In spite of the controversy and uncertainty, many scholars agree that O:*N* values up to 16 represent a high level of protein degradation; with a mixture of protein and lipid as substrates, O:N ranges between 16 and 60, while O:N above 60 indicates the elevated energy demand from a predominant lipid substrate (Mayzaud & Conover, 1988). In this study, the low O:N ratio of all sizes of *C. nippona* (ranging from 9.68 to 12.67) at 16°C was indicative of a protein-dominated catabolism, whereas the O:N ratio of large size at temperatures of 20°C, 24°C, 28°C and 32°C was ranging from 18.29 to 23.49, indicating that protein–lipids and carbohydrates were used as the energy substrates. Also, the O:N ratio increased significantly in all sizes of *C. nippona* as the salinity increased from 20 to 25, but decreased slightly from 25 to 30 psu and sharply from 30 to 35 psu, implying that the metabolic energy from protein diminished and that of lipid and carbohydrate increased with an increase in salinity from 20 to 30 psu and metabolic substrates reverse from 30 to 35 psu. Thus, the overall high O:N ratio at high temperature (20–32°C) and low salinity (25–30 psu) was found in all sizes of *C. nippona* might be explained by the oysters rely more heavily on the catabolism of non-protein substrates than of protein to meet the increased demands for energy, and the change in the O:*N* value might be expected by an adaptive strategy of *C. nippona* associated with changes in different temperatures and salinities.

4.3 | Effects of body size on OCR and AER of C. nippona

Besides temperature and salinity, the rates of physiological processes are also closely associated with individual body weight in aquatic organisms. The relations existing between body size (dry-tissue weight, *W*) and OCR or AER (*R*) are expressed as functions by the allometric equation:

$$R = aW^b,$$

where *a* and *b* are constants at specific experimental conditions. A wide variety of factors including animal activity, food availability and temperature influences the variety of the value of a, while the value for the weight exponent, b, is less changeable. In the present study, the average values of b estimated in *C. nippona* were 0.657 for OCR and 0.776 for AER at different temperatures, and 0.647 for OCR and 0.767 for AER at varying salinities respectively. These values are in the middle of b value range of 0.44 to 1.09 and rather close to the mean of 0.75 pooled and summarized for 23 species of bivalves from broad trophic categories (Bayne & Newell, 1983), signifying that metabolic rates of *C. nippona* become relatively slower with increasing body size regardless of temperature or salinity.

Based on previous studies, this tendency can be ascribed to differential metabolic rates of various organs, tissues as well as their mitochondrial membrane content (Zhang, Hu, & Wu, 1982), and differences in growth and circulating rate of the system (Jiang, Wang, & Tang, 1999). The OCR and AER of the internal organs that directly maintain life activities such as hepatopancreas, gonad and gill are much higher than those of maintenance indirectly tissues, such as fat and adductor muscle, and the proportion of the former to body mass in small size was significantly higher than that the latter. Therefore, with the growth of animals and the gradual accumulation of muscle and fat, the OCR and AER of large individuals per unit body mass were markedly lower than those of small individuals. On the other hand, with the increase in body weight of aquatic animals, the circulation speed of the respiratory, excretory and digestive systems tend to decrease concomitantly with lower growth rate for the unit weight; thus, the OCR and AER of small juveniles are relatively high ultimately.

4.4 | Interactive effects of temperature and size, and salinity and size on FR, OCR and AER of C. nippona

The interactive effects between temperature and size, and salinity and size on FR and AER were highly significant without exception, while interactive effects on OCR were insignificant, although the single factor including temperature, salinity and body size had a highly significant effect on OCR in the present study. This result indicated that investigation of single stressor may have the potential to produce misleading inference about physiological responses in a multivariate condition. Also, natural environmental factors, such

as temperature or salinity, and the intrinsic factor (body size) might have an antagonistic effect on OCR, compared with FR and AER of *C. nippona*. Our findings agree with Cao and Wang (2015), who studied the interactive effects of salinity and body weight on OCR and AER of *Boleophthalmus pectinirostris*. Therefore, an essential question for future studies is that it is necessary to define possible influencing factors in terms of variation in environmental complexity at the scale of the local population over which single or multifactor studies should be conducted to make the best predictive inferences (Todgham & Stillman, 2013).

5 | CONCLUSION

The physiological responses of different body sizes of *C. nippona* to different temperatures and salinities have been described for the first time under controlled laboratory conditions in this study. Our results indicate that *C. nippona* have a great ability to adapt to variations of temperature and salinity, and considering the results of FR, OCR and AER all together, it appears that the optimal temperature and salinity for juvenile *C. nippona* lie within 24–28°C and 25–30 psu respectively. The information obtained in this study has implications in the selection of suitable sites for cultivation.

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CONFLICT OF INTEREST

None declared.

AUTHOR CONTRIBUTION

The first author, Tao Wang, contributed a lot to this paper, including design, conducted the whole experiment, contributed to analysis-related experiment data and drafted this manuscript. The corresponding author, Qi Li, also made a great contribution to this paper, including revision of the manuscript, and provided financial support.

DATA AVAILABILITY STATEMENT

The data used to support the findings of this study are available from the first author upon request.

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