Effects of Seawater Temperature and Salinity on Physiological Performances of Swimming Shelled Pteropod *Creseis acicula* During a Bloom Period

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Creseis acicula is a swimming shelled pteropod species, widely distributed in the world's oceans. In 2020, an unprecedented bloom of *C. acicula* was observed in Daya Bay, and lasted from June to July. To date, there is very limited information on the physiological characteristics of this species, which is essential to understand bloom dynamics. In the present study, the physiological performances of *C. acicula* in response to temperature (17–35°C) and salinity (18–38 ppt) were investigated. The oxygen consumption (OCR) and calcification rates (CR) of *C. acicula* peaked at 32 and 26°C, respectively, while ammonia excretion rate (AER) significantly increased with increasing temperature. The thermal coefficient Q_{10} (respiration) of *C. acicula* dropped to a minimum value between 32 and 35°C, suggesting that they were in a stressful status. The O:N ratio ranged from 3.24 to 5.13, indicating that protein was the major catabolism substrate. Temperature exerted a stronger effect on the OCR and AER of *C. acicula*. Salinity has a more influence on CR. The preferable temperature for *C. acicula* ranges from 29 to 32°C, and the preferable salinity ranges from 28 to 33 ppt. Based on a comprehensive consideration, we presumed that the warmer seawater temperature around the thermal discharge area of Daya Bay nuclear power plant is a possible cause for the bloom of *C. acicula*.

Keywords: Creseis acicula, temperature, salinity, physiological performance, bloom

INTRODUCTION

Creseis acicula belongs to the Pteropoda order, Cavoliniidae family, and *Creseis* genus. This pteropod is the largest in the *Creseis* genus. *C. acicula* lives in the upper portion of the water column (less than 500 m), and is abundant in the Atlantic, Indian, and Pacific Oceans (Albergoni, 1975). The temporal dynamics and spatial distribution patterns of pteropods are well studied. *C. acicula* outbreaks have been reported in the coastal waters of India (Sakthivel and Haridas, 1974; Peter and Paulinose, 1978; Pillai and Rodrigo, 1984; Naomi, 1988), Japan (Nishimura, 1965; Morioka, 1980), Gulf of Mexico (Hutton, 1960), and in the Mediterranean Sea (Burgi and Devos, 1962;

Albergoni, 1975; Kokelj et al., 1994) between the 1960s and the 1990s, with the highest abundance recorded at about 500 inds m^{-3}. In Daya Bay, South China Sea, *C. acicula* generally appear from March to November each year, with average abundances ranging from 0.04 to 1.9 inds m^{-3} (Xu, 1989). Since the 1990s, there was no report of *C. acicula* bloom in the world. However, a massive aggregation occurred from June to July 2020 near the thermal discharge area of Daya Bay Nuclear Power Plant (DNPP), with the highest abundance reaching 4525–5595 inds m^{-3} (Dai et al., 2020; Zhong et al., 2021). Large amount of *C. acicula* gathered around the thermal discharge area of DNPP and seriously affected the normal operation of the plant.

The outbreak mechanism of *C. acicula* is very complex, like any other species (Wishner et al., 1995; Baliarsingh et al., 2020; Maas et al., 2020). The distribution of *C. acicula* is restricted by various physical and chemical environmental parameters, such as temperature, salinity, food, oxygen, and water depth (Herman, 1998; Dai et al., 2020; Zeng et al., 2021). The potential mechanisms for the bloom of *C. acicula* include optimum temperature and salinity (or the temperature and salinity are not at the most optimal levels for *C. acicula*, but they might be worse for the competitors or predators of *C. acicula*) and an adequate food supply for *C. acicula*. As Dai et al. (2020) suggested that the initiation of *C. acicula* bloom in Daya Bay well-matched a sharp increase of temperature and chlorophyll *a*, as well as an abrupt decrease of salinity attributed to a heavy rainfall happened before the bloom which lasted for more than 20 days. However, till now, there is very little information on the causes of *C. acicula* blooms from a physiological point of view. A further understanding of the physiological mechanisms regulating the blooms will contribute to our knowledge of the survival tolerance of this species.

The objective of the present study was to investigate the effects of temperature and salinity on the physiological responses of *C. acicula*, and to provide the useful information on the outbreak reasons of *C. acicula* around the thermal discharge area of DNPP.

MATERIALS AND METHODS

Study Area and Experimental *Creseis acicula* Collection

Experimental *C. acicula* samples were collected on July 6, 2020, in Daya Bay, which is located in the northern part of the South China Sea in Guangdong Province, Southern China (**Figure 1**). It is a semi-enclosed drowned valley bay with water depth ranging from 5 to 20 m and a water area of about 600 km^2. More than 50 islands locate inside the bay area. The DNPP base was built in 1994 on the southwestern shore of Daya Bay, which is located on Dapeng Peninsula, Dapeng New District, Shenzhen city, Guangdong Province (**Figure 1**). The bay is dominated by an irregular semidiurnal tide with a narrow tidal range, which affects the transport of thermal discharge (Jiang and Wang, 2020). On June 12, 2020, a large amount of *C. acicula* was spotted in the waters close to the southwestern shore of Daya Bay.

During sampling, the average seawater temperature was 30.92 ± 0.86°C and the average salinity was 32.36 ± 0.38 ppt. Experimental *C. acicula* were at their mature stage as ovulating

phenomenon was observed. They were collected using a bucket with water to avoid possible stress of capture. Specimens were placed in a 100-L container and were transported to the laboratory at the Marine Biology Experimental Base, located on the Daya Bay seafront, within 1 h of capture. Before the start of experiments, *C. acicula* samples were acclimated to laboratory conditions for 2 days. In Daya Bay, *C. acicula* mostly appears from March to November each year (Xu, 1989), when the average seawater temperature and salinity are 26.97 ± 2.85°C and 33.14 ± 1.10 ppt, respectively (unpublished data). Thus, during acclimation, pteropods were transferred into 40-L transparent glass bottles filled with sand-filtered seawater (to a density of ca. 1000 pteropods per bottle), at a temperature of 26°C and salinity of 33 ppt, bubbled with ambient air. *C. acicula* were fed once daily with a mixture of the chlorophyte *Chlorella vulgaris* and the diatom *Skeketonema costatum*, with an equal algal cell density of 40,000 cells mL^{-1} at 08:00.

Experimental Design

Oxygen consumption rate (OCR), ammonia excretion rate (AER), and calcification rate (CR) were measured using a closed-chamber method, as described in Ikeda et al. (2000). Brown respiration bottles (1-L vol.) were used for incubation experiments. Seven graded temperatures (i.e., 17, 20, 23, 26, 29, 32, and 35°C, respectively), and five graded salinities (i.e., 18, 23, 28, 33, and 38 ppt, respectively) were set to determine the effects of these two environmental factors on physiological responses. The incubation water temperature was gradually decreased or increased to each experimental value in a 5-h period by an automatic temperature controller. Water salinity was also gradually decreased or increased within a 5-h period to each experimental value by adding freshwater or artificial sea salt to natural seawater, respectively.

Pre-experiments were conducted to make sure that at the end of incubation, the oxygen concentration must be kept at a high level–at no less than 50% of the initial concentration–in order to avoid any possible hypoxia stress on the normal physiological activities of *C. acicula*. The incubation time and density of *C. acicula* in each respiration bottle were determined accordingly. The optimum number of *C. acicula* was determined as 100 individuals per bottle. The optimum incubation duration was 8 h. Healthy (actively moving) individuals with similar size (8–9 mm of shell length) were selected for the subsequent experiments. Prior to the experiments, *C. acicula* were not fed for 24 h to allow for gut clearance and avoid interference from post-prandial metabolism and excretion of feces. The experimental *C. acicula* were rinsed 3–4 times with filtered seawater (using Whatman GF/F filters), and then were transferred into respiration bottles. The respiration bottles were submerged and incubated in a water bath to maintain an identical ambient temperature for all of them.

Measurement of Physiological Parameters

Experimental *C. acicula* were incubated in respiration bottles (100 individuals per bottle), and were further assigned to the seven temperature treatments and the five salinity treatments.
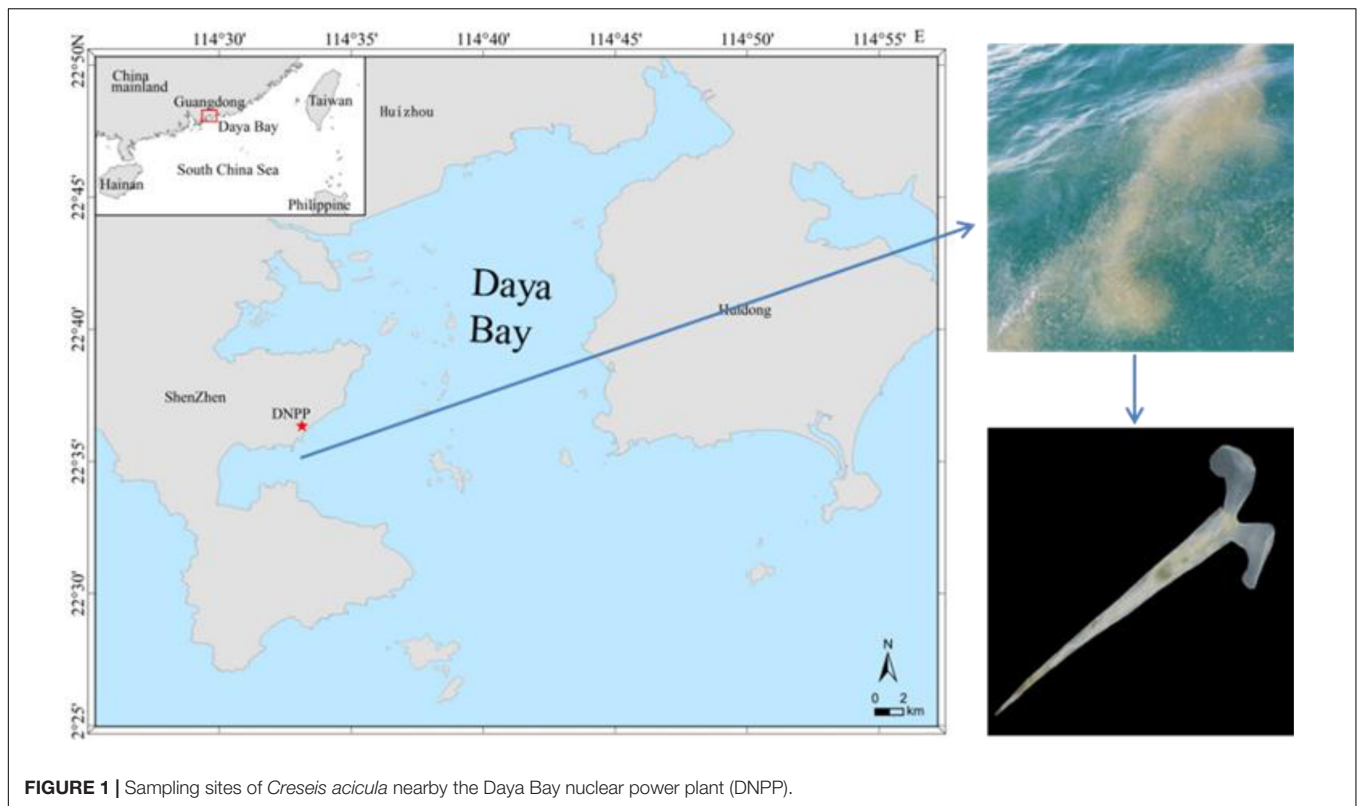
FIGURE 1 | Sampling sites of *Creseis acicula* nearby the Daya Bay nuclear power plant (DNPP).

For the temperature experiment, the bottles were filled with filtered seawater (using Whatman GF/F filters) and were left open for 24 h to obtain the air-sea equilibrium of the CO_2-carbonate system. Each temperature treatment has six bottles (three for treatment group and three for control). Bottles were capped and submerged in water with corresponding experimental water temperature. In each temperature treatment, three bottles filled with filtered seawater and without pteropods served as respective controls. For the salinity experiment, all the bottles were submerged in a water bath at a temperature of 26°C.

At the end of the incubation period, dissolved oxygen (DO) was measured with an optical DO meter (YSI Pro, Yellow Springs Instrument Company, Yellow Springs, OH, United States), and pH was measured using a pH meter (Thermo Scientific Orion 320P-01, Thermo Fisher Scientific, Waltham, MA, United States). Then, 100 mL of water samples was siphoned out to measure ammonia (through the oxidizing reaction of sodium hypobromite, GB/T 12763.4-2007, 2007), and total alkalinity (TA) (Gran titration with 0.1 M HCl using an alkalinity titrator, AS-ALK2 Total Alkalinity Titration System, Apollo, United States). For each bottle, these parameters were measured in triplicates. Calcium carbonate ($CaCO_3$) saturation for aragonite (Ωa) was obtained from water temperature, salinity, pH, and TA using the CO2_SYS_XLS calculation program (Pierrot et al., 2006). The wet weights of the pteropods were estimated through weighting the 100 residual individuals, similar with the experimental samples, after blotting dry, and this weighting repeated 6 times to acquire the mean wet weight of 100 pteropods.

The OCR ($\mu mol\ O_2\ g^{-1}\ h^{-1}$), AER ($\mu mol\ N\ g^{-1}\ h^{-1}$) and CR ($\mu mol\ g^{-1}\ h^{-1}$) of the *C. acicula* samples were calculated as follows:

$$OCR = (DO_0 - DO_t) \times V/W/t$$
$$AER = (A_t - A_0) \times V/W/t$$
$$CR = (TA_0 - TA_t) \times V/2/W/t$$

where DO_0, A_0, and TA_0 are the oxygen, ammonia, and total alkalinity concentrations ($\mu mol\ L^{-1}$) of the control bottle after incubation, respectively; DO_t, A_t, and TA_t are the oxygen, ammonia, and total alkalinity concentrations ($\mu mol\ L^{-1}$) of the treatment bottle after incubation, respectively; V is the bottle volume (L); W is the total wet weight of the pteropods in each bottle (g); and t is the duration of the experiment (h).

Thermal coefficients (Q_{10}) were calculated using the following the equation (Bayne and Newell, 1983):

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

where R_1 and R_2 are the corresponding metabolic rates (OCR or AER) at temperatures t_1 and t_2, respectively.

The O:N ratio indicated the ratio of proteins, lipids, and carbohydrates that were used as energy substrates by the organisms under different experimental conditions, and it was estimated for the tested pteropods in each bottle based on the OCR and AER, in atomic equivalents (Yu et al., 2018).

Statistical Analysis
Statistical analyses were conducted using SPSS 19.0 for Windows (IBM Corp., Armonk, NY, United States). The values of OCR,

AER, CR, Q_{10}, and O:N ratio in all experimental groups were analyzed using one-way ANOVA, followed by a comparison of means through the Tukey test. Prior to conducting statistical analyses, the normality and homogeneity of variance of all data were examined using the Shapiro–Wilk test and Levene's test, respectively. The effects of temperature and salinity on OCR, AER, CR, and O:N ratio were tested using stepwise multiple regression analysis. Statistical significance was set at $P < 0.05$.

RESULTS

At the end of the incubation, the survival rates of *C. acicula* were > 90% for the 17, 20, 23, 26, 29, and 32°C treatments, and the 28, 33, and 38 ppt treatments. The survival rate of *C. acicula* was ~50% for the 23 ppt treatment. Unfortunately, no *C. acicula* survived in the 35°C and 18 ppt treatments. The survival results suggest that *C. acicula* can tolerate a temperature range between 17 and 32°C and a salinity range between 23 and 38 ppt.

Oxygen Consumption
The OCR of *C. acicula* significantly increased with temperature and salinity values of 32°C and 28 ppt, respectively, and decreased thereafter [one-way ANOVA, temperature: $F_{(6,20)} = 80.112$, $P < 0.05$; salinity: $F_{(4,14)} = 5.906$, $P < 0.05$; **Figure 2**]. The rate differed significantly among all temperatures (Tukey test, $P < 0.05$), except between 29 and 35°C. OCR significantly increased at 28 ppt compared to the values observed at the lowest (18 ppt) and highest (38 ppt) salinities tested, respectively (Tukey test, $P < 0.05$). But there was no significant difference of OCR within the 23–33 ppt range (Tukey test, $P > 0.05$).

Stepwise multiple regression analysis explained 86.6% of the variation in the OCR of *C. acicula* [$F_{(1,35)} = 114.563$, R^2-adjusted = 0.866]. This regression analysis also showed that temperature was significantly correlated ($P < 0.05$) with the OCR, but salinity was not ($P > 0.05$). The coefficients of temperature and salinity were assumed as 0.980 and 0.051, respectively. The overall regression analysis results illustrate that the OCR of *C. acicula* was more influenced by temperature than by salinity.

Ammonia Excretion
AER increased significantly as temperature increased within the 17–35°C range [one-way ANOVA, $F_{(6,20)} = 48.311$, $P < 0.05$]. It increased by 70% at the highest temperature (35°C) in comparison with the value at the lowest temperature (17°C) (**Figure 3A**). Although AER decreased gradually with salinity up to 33 ppt, and then increased slightly at 38 ppt [one-way ANOVA, $F_{(4,14)} = 1.105$, $P < 0.05$], there was no significant difference among any of salinities (Tukey test, $P > 0.05$; **Figure 3B**).

Stepwise multiple regression analysis explained 85.9% of the variation in the AER of *C. acicula* [$F_{(1,35)} = 107.682$, R^2-adjusted = 0.859]. Both temperature and salinity were statistically significant ($P < 0.05$). The coefficients of temperature and salinity were assumed as 0.140 and −0.031, respectively, which showed that AER was also more influenced by temperature than by salinity.

Calcification
The CR of *C. acicula* firstly increased with increasing temperature and salinity, with the highest values at 26°C and 33 ppt, respectively, and decreased thereafter [one-way ANOVA, temperature: $F_{(6,20)} = 26.781$, $P < 0.05$; salinity: $F_{(4,14)} = 36.229$, $P < 0.05$; **Figure 4**]. The CR values at 20–29°C were significantly higher than those at 32–35°C (Tukey test, $P < 0.05$); at 28–38 ppt, CR values were significantly higher than those at 18–23 ppt (Tukey test, $P < 0.05$). Negative CR values were detected at 35°C, and 18 and 23 ppt. No significant difference in CR was found within the 20–29°C and 28–38 ppt ranges (Tukey test, $P > 0.05$).

Stepwise multiple regression analysis explained 66.2% of the variation in the CR of *C. acicula* [$F_{(1,35)} = 35.326$, R^2-adjusted = 0.662]. The regression analysis showed that salinity was significantly correlated with CR ($P < 0.05$), but temperature was not ($P > 0.05$). The coefficients of temperature and salinity were −0.150 and 0.563, respectively. In *C. acicula*, CR was more influenced by salinity than by temperature.

O:N Ratio
The O:N ratio of *C. acicula* ranged from 3.24 to 5.13; it increased significantly as temperature increased from 17 to 32°C, and then it significantly decreased at 35°C [one-way ANOVA, $F_{(6,20)} = 33.789$, $P < 0.05$; Tukey test, $P < 0.05$; **Figure 5A**]. And the ratio also showed a significant difference among the salinities [one-way ANOVA, $F_{(4,14)} = 5.947$, $P < 0.05$; **Figure 5B**]. It initially increased with increasing salinity, with the highest values detected at 28 and 33 ppt, and it decreased at 38 ppt. There was no significant difference within the 23–38 ppt range (Tukey test, $P > 0.05$), but the ratio at 18 ppt was significantly lower than that at 28 and 33 ppt (Tukey test, $P < 0.05$).
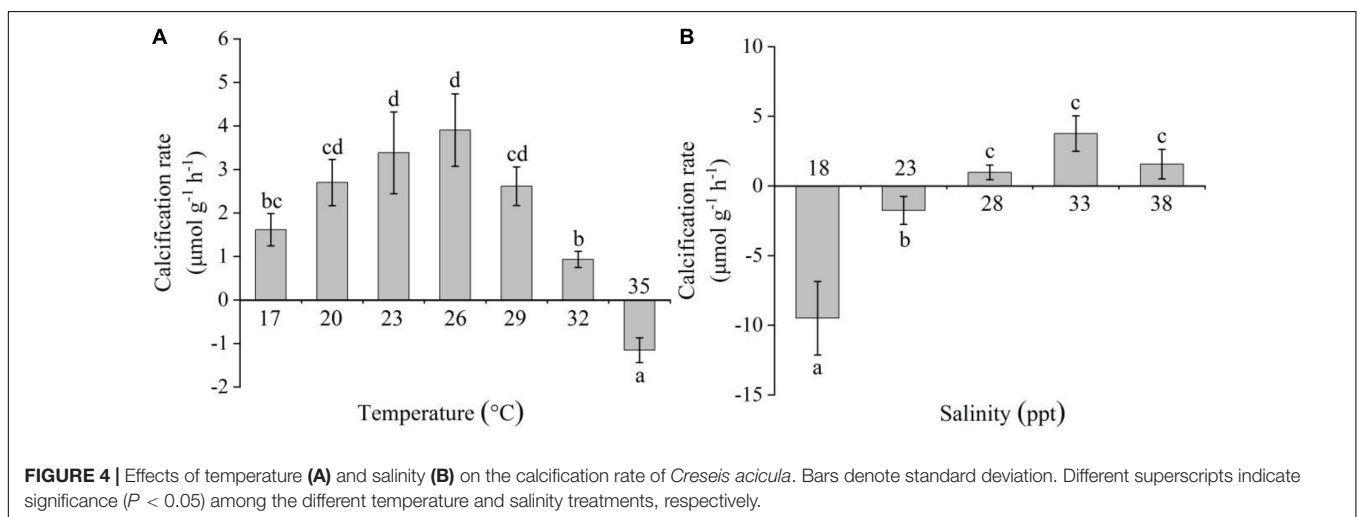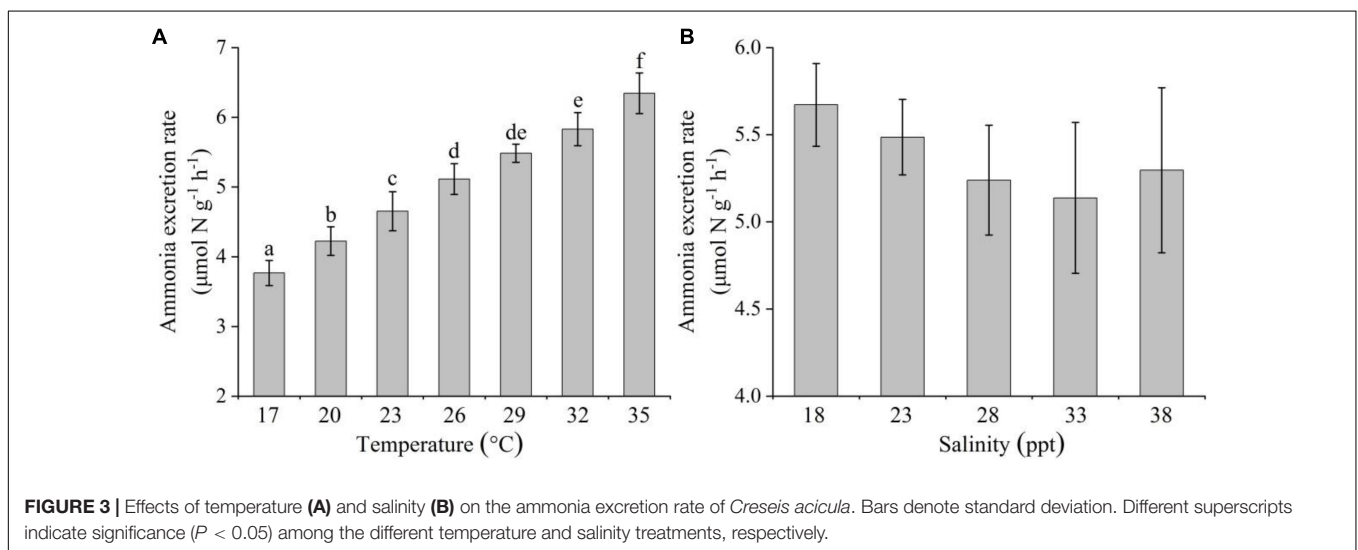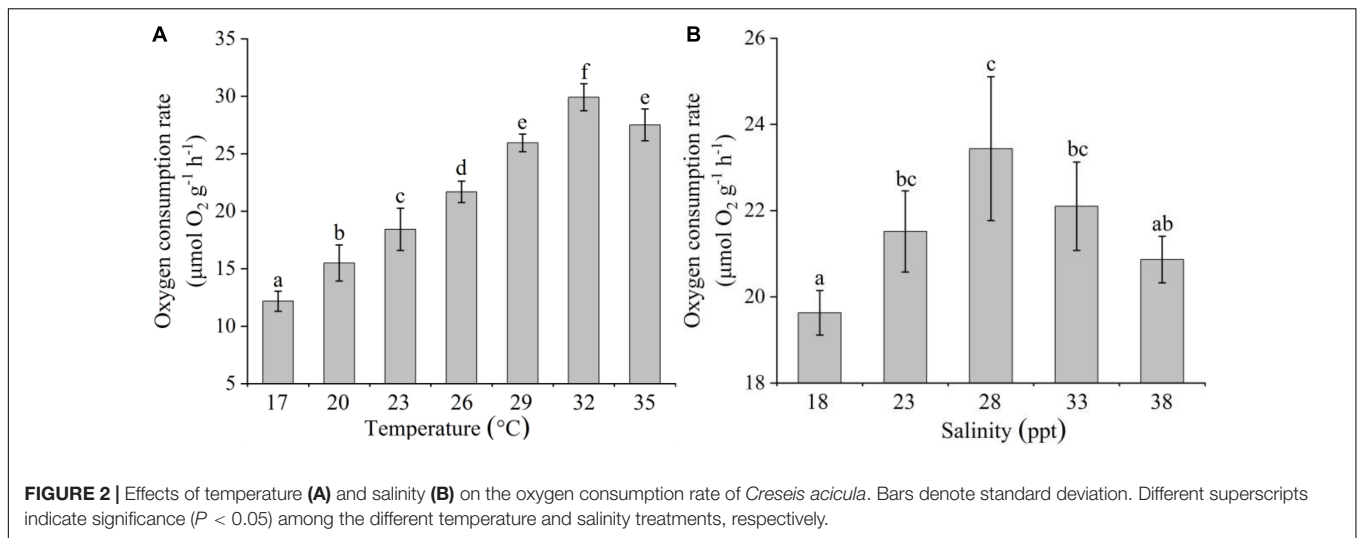
Stepwise multiple regression analysis explained 53.7% of the variation in the O:N ratio of *C. acicula* [$F_{(1,35)} = 21.336$, R^2-adjusted = 0.537]. The regression analysis showed that both temperature and salinity were significantly correlated with the O:N ratio ($P < 0.05$). The temperature and salinity coefficients were 0.083 and 0.028, respectively, indicating that temperature had a greater effect on O:N ratio than salinity.

Q_{10}
The Q_{10} values calculated for the different tested temperatures are shown in **Table 1**. Both the Q_{10} for respiration and excretion decreased with increasing temperature, except for the Q_{10} values for excretion within 32 and 35°C. The highest Q_{10} values for respiration and excretion were observed within 17 and 20°C, followed by that within 20 and 23°C. The lowest Q_{10} value for respiration was 0.79 within 32 and 35°C, and the lowest Q_{10} value for excretion value was 1.23 within 29 and 32°C. A narrow range of Q_{10} for excretion was observed for *C. acicula* when exposed to the different temperatures.

DISCUSSION

The results of the present study suggest that *C. acicula* has the ability to regulate its metabolisms, e.g., respiration, ammonia

FIGURE 2 | Effects of temperature **(A)** and salinity **(B)** on the oxygen consumption rate of *Creseis acicula*. Bars denote standard deviation. Different superscripts indicate significance ($P < 0.05$) among the different temperature and salinity treatments, respectively.

FIGURE 3 | Effects of temperature **(A)** and salinity **(B)** on the ammonia excretion rate of *Creseis acicula*. Bars denote standard deviation. Different superscripts indicate significance ($P < 0.05$) among the different temperature and salinity treatments, respectively.

FIGURE 4 | Effects of temperature **(A)** and salinity **(B)** on the calcification rate of *Creseis acicula*. Bars denote standard deviation. Different superscripts indicate significance ($P < 0.05$) among the different temperature and salinity treatments, respectively.

FIGURE 5 | Effects of temperature **(A)** and salinity **(B)** on the O:N ratio of *Creseis acicula*. Bars denote standard deviation. Different superscripts indicate significance ($P < 0.05$) among the different temperature and salinity treatments, respectively.

excretion, and calcification rates, within a relatively broad range of water temperature and salinity. This could be an advantage for its worldwide distribution. The results showed that the OCR of *C. acicula* increased by 145%, as the experimental temperature increased from 17 to 32°C, and its value decreased at 35°C. The respiration rates of pteropod species increased with increasing temperature (Seibel et al., 2007; Comeau et al., 2010; Maas et al., 2012; Bednaršek et al., 2016). However, the correlation between respiration and temperature was not linear in *C. acicula*. This is consistent with previous findings reported for copepod species, usually rising more steeply at the upper end and falling when the lethal temperature is approached (Krause et al., 2003). This indicated that *C. acicula* has a metabolic regulation ability. The decrease in OCR here reported suggests that when the water temperature reached 35°C, *C. acicula* entered a state of physiological stress and paid relatively higher energy cost. On the other hand, AER in *C. acicula* increased with increasing temperature, with the highest value observed at 35°C. It is well-known that ammonia is the major form of nitrogen waste–derived from protein metabolism–excreted by marine zooplankton (Butler et al., 1970; Corner and Davies, 1971). Therefore, the high ammonia production at 35°C indicates that *C. acicula* uses more protein as its energy source in higher water temperature than in lower water temperature conditions.

Q_{10} has been recognized as a common parameter that reflects the adjustments related to the enzymatic and physiological

requirements for energy when temperature increases within natural range (Kita et al., 1996; Manush et al., 2004; Kim et al., 2005). As for most animals, the normal Q_{10} value for pteropods is approximately 2–3 (Fry, 1971). In the present study, the Q_{10} for excretion did not clearly change with the increase in temperature. Whereas, the increase in oxygen consumption with rising temperature is fully accounted for by the basal metabolism (Hirche, 1987). Thus, in *C. acicula* the Q_{10} for respiration ranged from 2.23 to 1.61 at the 17–32°C range, which indicated that this pteropod is well adapted to these temperatures. However, when the temperature increased to 32–35°C, the Q_{10} for respiration was lowered to 0.79, indicating that *C. acicula* is no longer sensitive to temperature variation when temperature exceeded the optimum value.

The principle of osmotic adjustment holds that when osmotic pressure is at a minimum, the metabolic level is the lowest; when salinity is higher or lower than the isotonic point, more energy is consumed to maintain homeostasis, and the metabolic level increases (Cao and Wang, 2015). In this study, the OCR of *C. acicula* increased as the salinity increased from 18 to 28 ppt. A turning point was observed at 28 ppt, beyond which the OCR decreased as salinity increased from 28 to 38 ppt. Although AER in *C. acicula* did not significantly vary with salinity, it firstly decreased to the lowest level at 33 ppt, and then increased at 38 ppt, showing an opposite pattern to that observed for OCR. This supports the theory that the energetic cost associated with ionic and osmotic regulation is minimal within the normal salinity range tolerated by a species at species life stages (Morgan and Iwama, 1991). And at the same time, neither OCR nor AER at 28 ppt significantly differed from the corresponding values both at 23 and 33 ppt, indicating that *C. acicula* could well adapt to salinity range from 23 to 33 ppt. The decrease in OCR at salinities of 18 and 38 ppt is also reflected in the observed retraction of the pteropod body inside its shell–which is similar to valve closure in bivalves–that occurs when salinity either drops or increases excessively (Berger and Kharazova, 1997).

The O:N ratio can be considered as representative of the overall metabolic balance of an organism. It is usually used

TABLE 1 | Q_{10} values for respiration and excretion in *Creseis acicula* exposed to different temperatures.

Temperature range °C	Q_{10} (respiration)	Q_{10} (excretion)
17–20	2.23	1.50
20–23	2.12	1.44
23–26	1.88	1.40
26–29	1.83	1.28
29–32	1.61	1.23
32–35	0.79	1.36

as an index of lipid versus protein catabolism that reflects the effects of environmental stressors on zooplankton energy reserves (Mayzaud and Conover, 1988). Typically, an O:N ratio lower than 7 indicates a protein-only catabolism; values between 7 and 17, indicating a protein-oriented catabolism; and values >17, indicating a lipid/carbohydrate catabolism (Mayzaud and Conover, 1988; Ikeda et al., 2000). In the present study, the O:N ratio values calculated for *C. acicula* fall into a narrow range (from 3.24 to 5.13). Previous studies also reported a low O:N ratio for some adult pteropod species, such as *Clio pyramidata* (4.4) and *Limacina helicina antarctica* (2–9) (Thibodeau et al., 2020). Thus, pteropod species, including *C. acicula* in this study, use protein as the major metabolism substrate.

CaCO$_3$ occurs in several solid forms, including aragonite and calcite, and between these two, the former is less stable than the latter (Fransson et al., 2016). *C. acicula* is sensitive to chemical changes in seawater due to its highly soluble aragonite shells (Tunçer et al., 2021). The CaCO$_3$ saturation (Ω) is used as a chemical indicator for the dissolution potential of this compound (Fransson et al., 2016). Increasing temperature leads to increased Ω (Chierici et al., 2011), while freshwater supply causes a decrease in this parameter (Sejr et al., 2011; Fransson et al., 2015). Severe shell dissolution of aragonite-forming organisms takes place when $\Omega a < 1.4$ (Bednaršek and Ohman, 2015). In this study, the CR of *C. acicula* firstly increased to the highest level at 26°C and 33 ppt, but then decreased at 29–35°C and 38 ppt; CR values were negative at high temperature (35°C) and at low salinities (18–23 ppt). The Ωa increased from 17 to 35°C and from 18 to 38 ppt, with all Ωa values being > 1.4, except at 26 ppt after incubation (**Table 2**). Thus, it is easy to understand that the CR was negative at low salinities (18 and 23 ppt) and the salinity had a stronger effect on the CR than temperature. While, the negative CR values observed at 35°C may have been caused by the presence of physiological stress due to higher temperature–which increases the risk of dissolution and thinning of the aragonitic shells in *C. acicula* (Fransson et al., 2016)–rather than by the variation of Ωa. For example, higher energetic costs may derive from necessary repair calcification processes in response to shell dissolution (Lischka and Riebesell, 2012; Blachowiak-Samołyk et al., 2015; Vader et al., 2015). In this study, for the negative CR, *C. acicula* could not meet the needs for increased energetic costs, and responded with metabolic depression to save energy and increase its chance of survival. This is consistent with the findings of previous studies (Bibby et al., 2007; Rosa and Seibel, 2008; Navarro et al., 2013).

Studies of various marine species suggest that a number of large swarms or aggregations are due to biological forces (i.e., development time, bloom timing, species interactions) (Wishner et al., 1995; Ji et al., 2009; Baliarsingh et al., 2020). In this study, the experimental *C. acicula* were at mature stage with ovulating. After this study, we also observed the reproductive development of *C. acicula*. But the metamorphosis to juveniles with shells was absent after 20 days of veliger stage. Most studies confirm that the early stages of pteropod development are the most vulnerable (Kurihara, 2008; Comeau et al., 2010; Lischka et al., 2011), as the organisms do not have fully formed shells (Kobayashi, 1974; Fabry et al., 2008; Howes et al., 2014; Thabet et al., 2015). Meanwhile this study showed that the protein was the major catabolism substrate of *C. acicula*. Due to a strong depletion of lipids suffered during the spawning phase in the summer, active lipid storage in eggs may occur in pteropods (Gannefors et al., 2005). And female pteropods generally die shortly after spawning (Gannefors et al., 2005), which may explain the extinction of *C. acicula* after 1 month of the bloom. Thus, this massive aggregation of *C. acicula* may explain to be a reproductive bloom.

CONCLUSION

Our results indicate that seawater temperature has a stronger influence on OCR and AER of *C. acicula*, compared to salinity. On the other hand, CR was more affected by salinity than by temperature. The preferable temperature for *C. acicula* ranged from 29 to 32°C and the preferable salinity ranged from 28 to 33 ppt. The warmer water (30.92 ± 0.86°C) around the thermal discharge area of DNPP may favor the physiological performances for reproduction. In addition, it is well-known that water temperature shows a regular seasonal cycle in Daya Bay (Wu et al., 2016). The *C. acicula* bloom occurred only in 2020. This impenetrable phenomenon may be mainly related to the tidal current which drives the horizontal migration of *C. acicula* from the inside to the outside of the bay during the ebb tide period (Zeng et al., 2021). Thus, it is contingency for this bloom (Zhong et al., 2021). However, it must be pointed out that the bloom formatting mechanism of *C. acicula* is very complex and almost be combination of many factors. Further studies are in need for a fully understanding on the bloom forming mechanism of *C. acicula*.

DATA AVAILABILITY STATEMENT

The original contributions presented in the study are included in the article/supplementary material, further inquiries can be directed to the corresponding author.

TABLE 2 | Saturation states of the water related to aragonite minerals (Ω_a) before and after the physiological experiment with different temperatures (T) and salinities (S).

T (°C)	S (ppt)	Ω_a Before	Ω_a After
17	33	3.27	3.04
20	33	3.38	2.83
23	33	3.53	2.68
26	33	3.75	2.56
29	33	3.91	2.59
32	33	4.07	2.46
35	33	4.21	3.08
26	18	1.87	1.29
26	23	2.38	1.61
26	28	3.02	2.03
26	33	3.71	2.40
26	38	3.82	2.72

AUTHOR CONTRIBUTIONS

TH and HH conceived the study. TH and ZQ designed the experiments, analyzed the data, and wrote the manuscript. RS and QL conducted the research and collected the data. MD and HH provided materials and interpreted the data. All authors contributed to the article and approved the submitted version.

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