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Effects of temperature on fertilization, hatching, larval growth, ingestion, metabolism, and metamorphosis of the purple sea urchins, *Heliocidaris crassispina*

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Abstract
It is crucial to understand the role of temperature in rearing sea urchin larvae for large-scale production of sea urchin seeds. The development of purple sea urchins, *Heliocidaris crassispina*, such as fertilization, hatching, embryonic development, larval growth, ingestion, metabolism, and metamorphosis, was investigated at four temperatures: 20, 24, 28, and 32 °C. In the four temperatures, fertilization and hatching of sea urchins first increased with temperature and then decreased. The optimal condition was found at a temperature of 28 °C, where the fertilization and hatching rates exceeded 90%, and embryonic development was highly synchronized. This condition did not differ from the results observed at a temperature of 24 °C ($P > 0.05$). The fertilization and hatching rates were lower at 20 °C and 32 °C, among which 32 °C had the fastest embryo development but the second highest mortality. In contrast, 20 °C had the slowest embryo development and the most increased mortality. The optimal growth temperature for larvae is 28 °C, at which their growth and development rate are the fastest. However, at 24 °C, it is the second highest. At 20 °C, the growth rate is the lowest, with sluggish physiological responses and the lowest digestion and metabolism capacity. The metamorphosis rate did not differ between 28 and 32 °C ($P < 0.05$), with 50.0% and 61.1%, respectively, while remaining below 4% at 20 °C. This indicates that temperature significantly impacts the early development of *Heliocidaris crassispina* sea urchins, whose larvae may be sensitive to low temperatures but have higher temperature tolerance.

Keywords Sea urchin · *Heliocidaris crassispina* · Temperature · Larva

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Introduction

Heliocidaris crassispina (A. Agassiz, 1864, formerly *Anthocidaris crassispina*) is distributed in the Sea of Japan to the South China Sea and is commonly found in Southern China, including Zhejiang, Fujian, Guangdong, and Hainan (Liu and Chang 2015; Wang et al. 2012; Yoo 1982). This species is essential for biology, ecotoxicology (Lu and Wu 2005; Vaschenko et al. 1999), and aquaculture (Ding et al. 2007; Liu and Chang 2015). In China, sea urchin roe has long been considered a delicacy due to its high content of vitamins, highly unsaturated fatty acids (HUFAs), and trace elements (Liu and Chang 2015). *H. crassispina* is an important benthic herbivore that dominates many algal communities in intertidal zones and shallow subtidal areas along the southern coast of China (Chiu 1984; Clark 1982; Freeman 2003; Thompson 1982). Many studies have reported that the breeding season of *H. crassispina* varies at different locations within their distribution range from the Sea of Japan to the South China Sea (Agatsuma 2013; Liu and Chang 2015). The populations at these locations have similar breeding seasons, with gametogenesis beginning in late autumn, after most individuals mature during summer, followed by spawning from late spring to early autumn. There is a latitudinal pattern regarding the length of the spawning season. Urriago et al. (2016) indicated that the delay in the onset of spawning for *H. crassispina* in Hong Kong waters corresponds to a shorter period of ocean warming from south to north. Compared to colder water areas such as those studied by Fujisawa and Shigei (1990) in Japan, spawning in Hong Kong begins earlier and lasts longer. Interestingly, Urriago et al. (2016) reported that latitude variations in the spawning season of *H. crassispina* may be due to optimal seawater temperatures required for the survival of larvae and juveniles. According to the 2023 China Fishery Statistical Yearbook, the annual production of *H. crassispina* in 2022 was recorded at 100.7 t, with an estimated market value ranging between 180 and 220 CNY per kilogram of *H. crassispina* gonads.

With the increase of carbon dioxide in the atmosphere, the Earth and ocean are warming. Ocean warming, through direct heat absorption and changes in ocean circulation, is a significant source of pressure on marine ecosystems (Brierley and Kingsford 2009; Byrne et al. 2017; Huggett et al. 2006; Mueter and Litzow 2008; Poloczanska et al. 2007; Przeslawski et al. 2008). Therefore, environmental conditions play a crucial role in developing invertebrate larvae. Temperature is one of the most critical environmental factors for optimal development of ectothermic animals in the ocean, as it influences chemical reactions and physiological processes (Sanford 2002). Meanwhile, temperature is a significant environmental condition affecting marine aquaculture (Hernández et al. 2007).

The impact of temperature on physiology can vary during critical life history stages, such as fertilization and early development, which can influence the distribution of adult insects (Andronikov 1975). Andronikov (1975) suggested that the heat resistance of gametes in marine invertebrates is largely fixed within each species, with the environment's temperature throughout the year determining the timing of spawning and limiting the geographical distribution of such species. Fujisawa (1989) and Fujisawa and Shigei (1990) further demonstrated that sea urchin embryo temperature sensitivity is consistent with seawater temperatures during the spawning season. Given future predictions surpassing optimal developmental temperatures for species (IPCC 2014), ocean warming may impact species by pushing them beyond their tolerance thresholds by altering metabolic costs under new conditions. It may also change patterns of development and

reproduction, leading to population declines and mass extinctions of numerous species (Hoffmann and Sgro 2011). However, other studies suggested that ocean warming promotes the expansion of commercially important sea urchin species towards higher latitudes (Feng et al. 2019).

The impact of temperature on the planktonic stage of marine invertebrates has been extensively studied, including embryonic development rate (Rupp 1973), developmental constraints (Sewell and Young 1999), morphological changes (Wangensteen et al. 2013), larval development and morphology (Stumpp et al. 2011) as well as recruitment dynamics (Mos et al. 2011). These abundant data indicate that temperature is a major environmental factor influencing marine invertebrate development. Many studies have suggested that development is negatively affected under extreme temperatures, resulting in developmental abnormality, metabolic abnormality, high mortality rate, and delayed metamorphosis (Byrne et al. 2011; Diaz-Perez and Carpizo-Ituarte 2011; Fujisawa 1989; Minuti et al. 2021; Sewell and Young 1999; Sin et al. 2019). Abnormal development and low survival rate may lead to potential population decline at local or regional scales due to reduced population growth affecting population structure and dynamics (Pörtner 2002).

In recent years, the aquaculture production of purple sea urchins has been continuously declining, possibly due to a lack of seed production. At the same time, sea urchins in coastal waters worldwide have suffered from stock declines due to overfishing over the past several decades (Lawrence 2013). Given the significant importance of *H. crassispina* in the aquaculture industry, there is an urgent need to develop captive breeding programs for induced reproduction, larval rearing, and seed production. Moreover, no comprehensive research has been conducted to determine temperature levels in aquaculture for *H. crassispina* regarding fertilization, embryo and larval development, ingestion behavior, oxygen consumption, and recruitment to scale up the production of *H. crassispina* seedlings for commercial purposes. Therefore, we aim to study the temperature tolerance ability of *H. crassispina* under controlled conditions and determine optimal levels for fertilization efficiency and development stages, including embryos and larvae, along with their ingestion behavior patterns and oxygen consumption.

Materials and methods

Broodstock and gamete collection

The mature and healthy adults of *H. crassispina* were collected from the Dongshan Sea area (117.5N, 23.7E) in Zhangzhou City, Fujian Province, and temporarily reared in the aquatic experiment field of Jimei University in March 2020.

The selected broodstock of *H. crassispina* (males and females, each five) exhibited complete appearance, with a test diameter ranging from 5 to 6 cm and a weight between 90 and 110 g. According to the size of the sea urchin parents, 1–2 ml of 0.5 M KCl solution was injected into their body cavity through the peristomial membrane for artificial induction of spawning. Following injection, each sea urchin was placed on a dry petri dish with its oral surface facing upwards to facilitate the spawning process. Sperm and eggs were collected separately during this time, ensuring no premature fertilization occurred before conducting experiments. During gametes collection, the maturation of eggs were assessed using an inverted microscope while examining motility by diluting a small quantity of sperm with filtered seawater (FSW) at a concentration of approximately 0.01 μm.

Experiment design

The effects of temperature on the processes of fertilization, hatching, embryonic development, larval growth, ingestion, metabolism, and metamorphosis in *H. crassispina* were investigated at four distinct temperature levels (20 °C, 24 °C, 28 °C, and 32 °C) using constant temperature incubator (Ningbo Caifu Technology Co., Ltd. in Zhejiang Province, PGX-280A-12HM).

Sample cultivation across various developmental stages

Embryo development

Add 500 ml of FSW to a 1-L beaker, resulting in three replicates (three beakers) per temperature treatment. Sperm and eggs are added to a beaker in a ratio of 5:1, respectively, and the beaker is gently shaken to ensure complete contact between gametes. At 1 h post-fertilization (hpf), > 100 eggs were randomly selected to count development and fertilization rates. At 2.5 and 27 hpf, > 100 embryos were randomly selected and observed under an inverted microscope to measure embryo development and calculate hatchability. During this period, 2/5 of the total FSW was replaced every 8 h to ensure the stability of the experimental water environment.

The first cleavage occurrence indicates successful fertilization because the fertilization envelope can form under acidifying ocean conditions (Bögner 2016; Bögner et al. 2014), while upward floating ability signifies hatching for larvae. Individuals remaining at the bottom after 27 hpf are deemed non-viable. The calculation formulas for indicators comprise the following.

$$\text{Fertilization rate } (\%) = (\text{number of fertilized eggs}/\text{total number}) \times 100\%$$

$$\text{Hatching rate } (\%) = (\text{number hatched}/\text{number fertilized}) \times 100\%$$

Larval development

A developmental experiment was conducted in 12-CCC using larval stages, including 4-arm, 6-arm larvae, and different stages of 8-arm larvae. The 4-arm larvae at 1.5 days post-fertilization (dpf) were placed individually in separate wells of 12-CCC containing 5 ml of FSW. Each temperature treatment group is equipped with four 12-CCCs, totaling 48 independent individual replicates. Based on the study of Shigai et al. (2020), the diatom *Chaetoceros gracilis* was set to be fed twice a day to the larvae at each stage, as shown in Table 1. To maintain a stable experimental environment, 2/5 volume of the total FSW was replaced each time, three times a day. Larval development requires measurements and

Table 1 Microalgal diet of cell concentration (cells/ml) used at the different stages of larval development

Larval experiment	4-arm larvae	6-arm larvae	8-arm larvae
Larval development	20,000	30,000	40,000
Larval ingestion	80,000		80,000
Larval metamorphosis			40,000

statistics of the larval stage, rod length, and stomach size, including post-oral arm (POA) length (Sarifudin et al. 2016), anterolateral arm (ALA) length, stomach length (SL), and stomach width (SW) (Zhao et al. 2018) under inverted microscopic observation at 48-h intervals (Fig. 1).

Based on the findings of Smith et al. (2008), we have categorized the 8-arm larvae of *H. crassispina* into four distinct developmental stages. In the initial stage, stage I 8-arm larvae, an ALA protrudes outward, and the number of arms in the larvae reaches eight (corresponding to Smith's stage II). Approximately 1 day later, the larvae exhibit anterior and posterior epaulets (Smith et al. 2008), transitioning into stage II 8-arm larvae (corresponding to Smith's stage III, vestibular invagination). One day, subsequently, they advance to stage III 8-arm larvae with the emergence of a single rudiment on the left side of their stomach (corresponding to Smith's stage IV rudiment initiation). The enlargement and compression of this rudiment within the original stomach indicate that they have progressed into stage IV 8-arm larvae (corresponding to Smith's stage VI, advanced rudiment stage). The calculation formula for indicator comprises the following.

$$\text{Stomach area } (\mu m^2) = (\text{stomach length}/2) \times (\text{stomach width}/2) \times \pi$$

Larval ingestion and metabolism

The 4-arm and stage I 8-arm larvae were starved in both experiments for 3 h. A randomly selected portion of larvae was then transferred to 12-CCC for 12 h of incubation, 5 ml of FSW per well containing one larva. Each temperature treatment group is equipped with one 12-CCC, totaling 12 independent individual replicates. The larvae were fed with the diatom *C. gracilis* following the ingestion doses present in Table 1 (Shigai et al. 2020). Initial and post-12 h diatom concentrations were measured for each well using an automatic

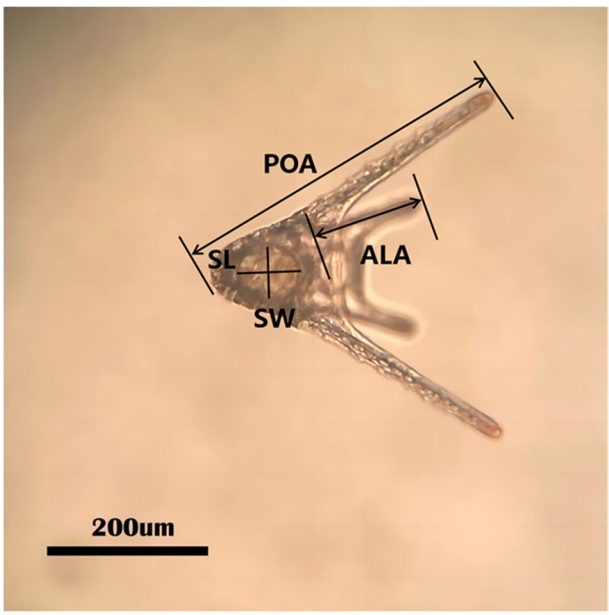

Fig. 1 The larval measurements of the early stages of *Heliocidaris crassispina* were conducted under an inverted microscope, including the stomach length (SL), stomach width (SW), post-oral arm (POA) length, and anterolateral arm (ALA) length

algal counter (Countstar IA1000). The well without larvae was used as control group to correct the change in *C. gracilis* concentration. The calculation formula for indicator comprises the following.

$$\text{Ingestion rate (cell/ind} \cdot \text{h)} = (\text{initial concentration} - \text{final concentration})/(\text{larval number} \times \text{time})$$

Metabolic rate is determined by measuring oxygen consumption rate to reflect the basal metabolism of larvae. One thousand larvae starved for 2 h were added into a 135.5-ml brown bottle, sealed immediately, and dissolved oxygen was determined by iodometry after 4 h. Iodometry is a commonly used method to determine dissolved oxygen in water. It is a method of deciding dissolved oxygen content by converting it into a tangible substance based on the reaction between dissolved oxygen and iodine. The control group ($n=3$) was carried out similarly, except no larvae were placed in the bottle.

Using oxygen consumption data, the thermal coefficient (Q_{10}) was calculated using the equation:

$$Q = \left(K_2/K_1\right)^{10/t_2-t_1}$$

K_1 and K_2 are the metabolic rates at temperatures t_1 and t_2, respectively (Spanopoulos-Hernández et al. 2005).

Competent larval metamorphosis

The experiments were performed in 6-CCC containing 10 ml of FSW per well into polyvinyl chloride (2 cm × 2 cm) containing benthic diatoms (mainly *Cocconeis* spp. and *Navicula* spp.), followed by incubation of 10 stage IV 8-arm larvae per well for 72 h. Three replicates were used for each temperature treatment group. Additionally, the larvae were provided with *C. gracilis* diatoms as per the ingestion doses specified in Table 1 (Shigai et al. 2020). The calculation formula for indicator comprises the following.

$$\text{Metamorphosis rate (\%)} = (\text{juveniles sea urchin number} / \text{total number of larvae added at the beginning of the experiment}) \times 100\%$$

Statistical analyses

The raw data were initially organized using Excel 2018 software, where the fertilization rate, hatching rate, and metamorphosis rate variables were transformed using inverse sine functions. The normal distribution of the data and chi-squaredness of variance were validated using IBM SPSS™ Statistics 22 (IBM Corporation, Armonk, NY, USA) for the Kolmogorov–Smirnov and Levene tests, respectively. The POA length, ALA length, stomach area (SA), ingestion rate, metabolic rate, and metamorphosis rate underwent one-way ANOVA followed by Duncan's multiple comparison analysis. Non-parametric analyses of Ridit and non-parametric multiple comparisons were applied to synchronous developmental studies of embryos and larvae. A significance level of $P<0.05$ was used for all comments. Data visualization was performed using GraphPad Prism (version 8.0.1).

Results

Fertilization and hatching rate

The fertilization and hatching rates demonstrate a temperature-dependent pattern (Fig. 2). Within the temperature range of 20 to 32 °C, both rates initially exhibit an upward trend with increasing temperature, followed by a subsequent decline. Notably, the highest observed fertilization rate (96.1 ± 1.8%) and hatching rate (90.3 ± 3.9%) were recorded at 28 °C ($F_{(3,8)}$ = 28.1, P < 0.05; $F_{(3,8)}$ = 18.9, P < 0.05). Furthermore, the fertilization rate at 28 °C displayed significant superiority over that at temperatures of 20 (54.8 ± 9.4%), 24 (80.0 ± 4.0%), and 32 °C (51.3 ± 9.5%) (P < 0.05). In contrast, the hatching rate at 28 °C was significantly higher than that at temperatures of both 20 (60.6 ± 4.5%) and 32 °C (57.4 ± 9.6%) (P < 0.05) but did not differ significantly from that observed at a temperature of 24 °C (79.9 ± 5.5%) (P > 0.05).

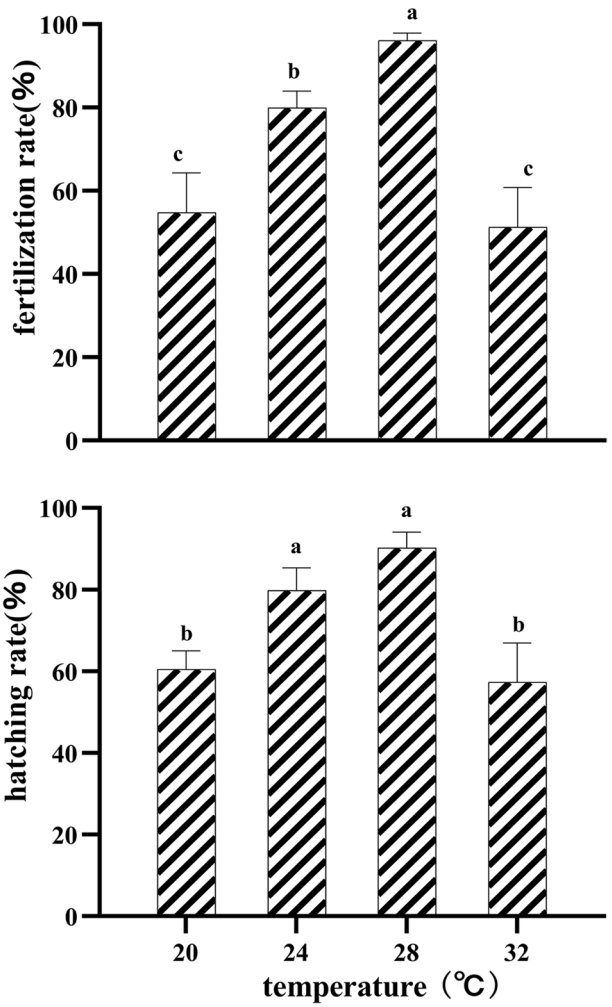

Fig. 2 Effects of temperature on the fertilization and hatching rate of *Heliocidaris crassispina* (mean ± SD, n = 3). Different superscript means significant difference (P < 0.05)

Embryonic development

The effects of different temperatures on the synchrony of *H. crassispina* embryonic development were shown in Fig. 3. At 20 °C, embryos developed the slowest, significantly slower than the other three groups ($P<0.05$), with 74.6% of embryos developing to the 4-cell stage only at 2.5 hpf, only 60.6% of them developing to prism larvae at 27 hpf, and the rest remained unhatched. Relative to 20 °C, embryos at 24 °C were able to create to the 4-cell stage (56.5%) at 1 hpf, to the multicellular stage (86.3%) at 2.5 hpf, and to hatch into prism (47.8%) and 2-arm larvae (32.1%) at 27 h, whose mortality was reduced to 20.1%. At 28 °C, embryos developed rapidly and synchronously. After 1 hpf, 92.6% of embryos were produced to the 4-cell stage, and at 2.5 hpf, all embryos developed to the multicellular stage; at 27 hpf, 68.9% of 2-arm larvae appeared. Although the embryo developed fastest at 32 °C, 12.7% of 4-arm larvae appeared at 27 hpf, but the hatching rate was only 57.4%

Larval development

After 1.5 dpf, the POA, ALA length, and SA of 4-arm larvae were found to be 356 ± 23.3 μm, 242 ± 19.6 μm, and 3781 ± 315.5 μm^2, respectively.

The impact of temperature on the POA development of 4-arm to 8-arm larvae of *H. crassispina* was investigated ($F_{(3,188)}=256.1$, $P<0.001$; $F_{(3,188)}=160.7$, $P<0.001$; $F_{(3,188)}=39.91$, $P<0.001$) (Fig. 4). During the incubation period ranging from 3.5 to 7.5 dpf, the growth of larval POA exhibited an initial positive correlation with temperature followed by a subsequent decline. After 7.5 dpf, the larvae's POA length was 780 ± 40.3 μm at 28 °C. Meanwhile, the growth of POA in the group treated at 28 °C was significantly higher than that at 20 and 32 °C ($P<0.05$), while no significant difference was observed at 24 °C ($P>0.05$).

The impact of temperature on the ALA development of 4-arm to 8-arm larvae of *H. crassispina* was investigated ($F_{(3,188)}=120.9$, $P<0.001$; $F_{(3,188)}=153.1$, $P<0.001$; $F_{(3,188)}=41.6$, $P<0.001$) (Fig. 4). During the incubation period ranging from 3.5 to 7.5 days post-fertilization, the growth of larval ALA exhibited an initial positive

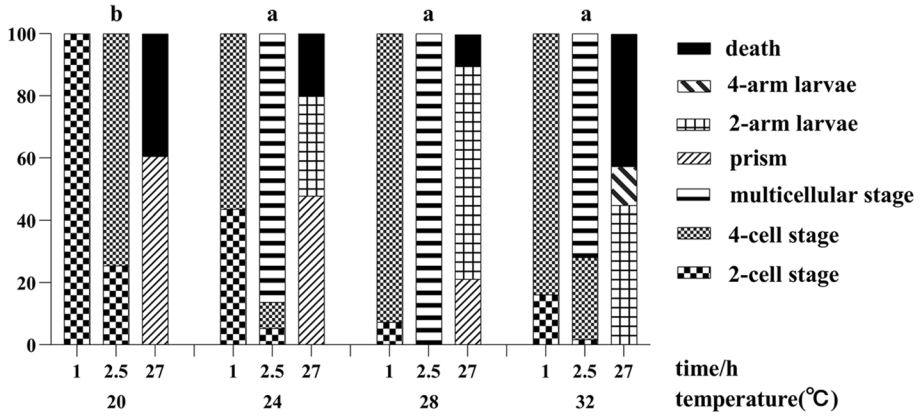

Fig. 3 Effect of temperature on the developmental synchronization of *Heliocidaris crassispina* embryos. Different superscript means significant difference ($P<0.05$)

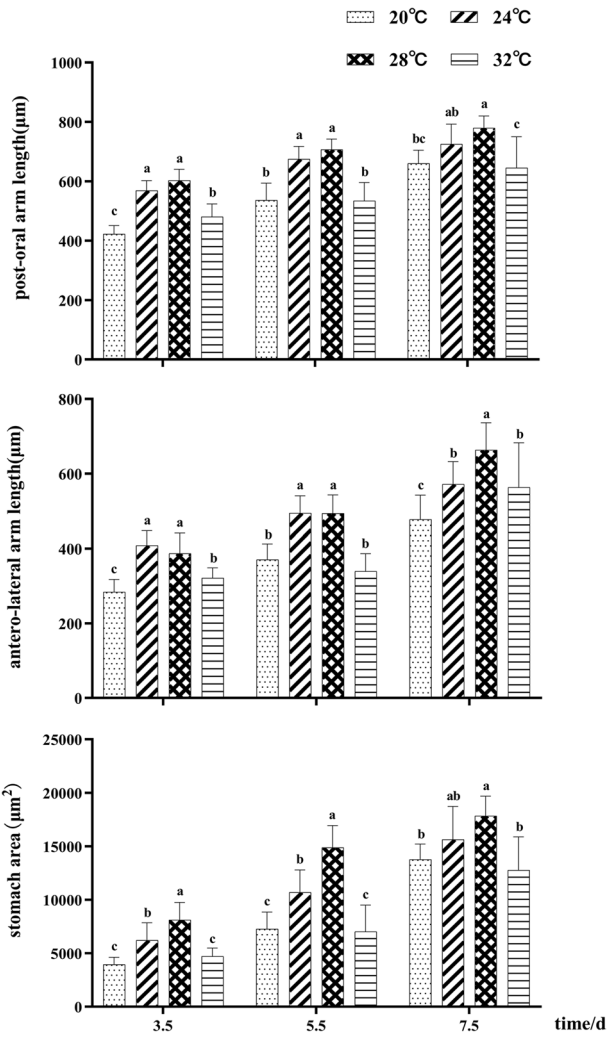

Fig. 4 Effects of temperature on the post-oral arm, anterolateral arm, and stomach area development of 4-arm to 8-arm larvae of *Heliocidaris crassispina* (mean ± SD, $n = 48$). Different superscript means significant difference ($P < 0.05$)

correlation with temperature followed by a subsequent decline. After 7.5 dpf, the ALA length of larvae was found to be 663.7 ± 72.6 μm at 28 °C. Meanwhile, the growth of ALA in the group treated at 28 °C was significantly higher than that at 20 and 32 °C ($P < 0.05$). However, there was no significant difference between the growth of ALA in the group treated at 24 °C and that in the group treated at 32 °C ($P > 0.05$).

The impact of temperature on the stomach development of 4-arm to 8-arm larvae of *H. crassispina* was investigated ($F_{(3,188)} = 104.9$, $P < 0.001$; $F_{(3,188)} = 153.1$, $P < 0.001$; $F_{(3,188)} = 39.5$, $P < 0.001$) (Fig. 4) During the incubation period ranging from 3.5 to 7.5 days post-fertilization, the growth of larval stomach exhibited an initial positive correlation with temperature followed by a subsequent decline. After 7.5 dpf, the SA of larvae was found to be 17,844.8 ± 1846.6 μm^2. Meanwhile, the stomach growth in the group treated at 28 °C was significantly higher than that at 20 and 32 °C ($P < 0.05$). However, there was no significant difference between the growth of SA in the group treated at 24 °C and that in the group treated at 32 °C ($P > 0.05$).

The synchronicity of larval development is significantly influenced by temperature variations ($P < 0.05$) (Fig. 5). The fastest and most synchronized larval development was observed at 28 °C, followed by 24, 32, and 20 °C, and 28 °C was significantly higher than the other temperatures ($P < 0.05$). At 28 °C, 91.7% of the larvae had developed to stage I 8-arm larvae by 5.5 dpf, all larvae had developed to 8-arm larvae by 7.5 dpf, 16.7% had developed to stage III 8-arm larvae, and 83.3% had developed to stage IV 8-arm larvae by 13.5 dpf. At 24 °C, 80.6% of the larvae entered the 8-arm larvae stage at 9.5 dpf, and 16.7% of the larvae entered stage IV 8-arm larvae after 13.5 dpf, which was significantly higher than that at 20 and 32 °C ($P < 0.05$). At 20 °C, the larvae were the least developed and remained in stage I 8-arm larvae or 4-arm or 6-arm larvae stage at 13.5 dpf, significantly lower than those at 32 °C ($P < 0.05$). At 32 °C, 27.8% of larvae developed to stage IV 8-arm larvae at 13.5 dpf, but the overall survival rate was only 77.8%, and 11.1% of larvae were still in the 4-arm larvae stage.

Larval ingestion rate

There was a significant effect of temperature on the ingestion capacity of 4-arm and 8-arm larvae ($F_{(3,12)} = 20.8$, $P < 0.001$; $F_{(3,12)} = 19.8$, $P < 0.001$) (Fig. 6), with ingestion rate of 4-arm larvae significantly higher at 28 °C than 20 or 32 °C ($P < 0.05$), and with ingestion rate of 8-arm larvae significantly higher at 28 °C than 24 or 20 °C ($P < 0.05$). The most ingestion rate at 4-arm and 8-arm also was at 28 °C (2753.5 ± 532.8 and 3313.2 ± 382.1 cell/ind/h, respectively), then followed by 24, 32, and 20 °C (2479.5 ± 274.6, 1637.9 ± 663.3, and 1453.1 ± 350.6 cell/ind/h, respectively) for 4-arm larvae, and 32, 24, and 20 °C (2891.4 ± 589.9, 2589.6 ± 613.3, and 1851.0 ± 230.7 cell/ind/h, respectively) for 8-arm larvae, respectively.

Larval oxygen consumption

There was a significant effect of temperature on oxygen consumption of 8-arm larvae ($F_{(3,8)} = 60.4$, $P < 0.001$) (Fig. 6), with larval oxygen consumption increasing with the increase of temperature ($P < 0.05$). The oxygen consumption of 8-arm larvae at 32 °C and

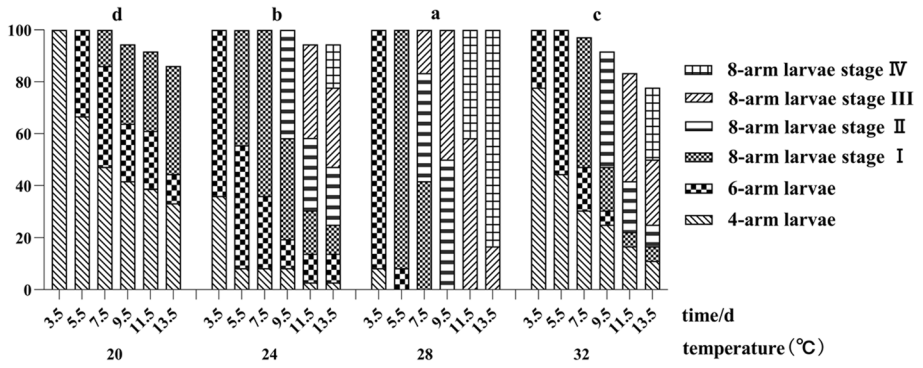

Fig. 5 Effect of temperature on the synchronous development of 4-arm to 8-arm larvae of *Heliocidaris crassispina*. Different superscript means significant difference ($P < 0.05$)

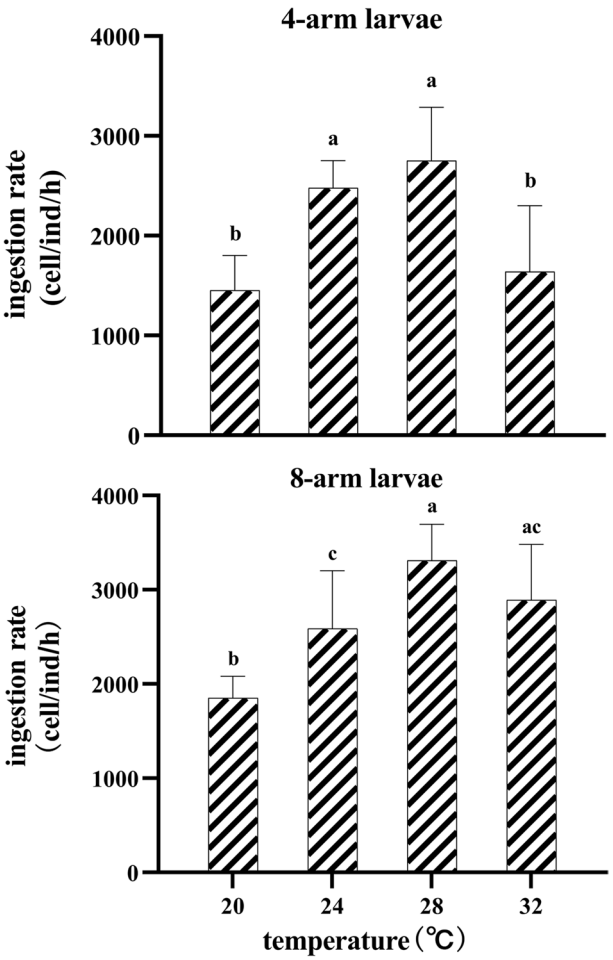

Fig. 6 Effects of temperature on the ingestion rate of *Heliocidaris crassispina* 4-arm and 8-arm larvae (mean ± SD, $n = 12$). Different superscript means significant difference ($P < 0.05$)

28 °C were significantly different ($P < 0.05$) from the other three temperatures, with the highest oxygen consumption of 8-arm larvae at 32 °C. It can be seen that only Q_{10} values of 20 ~ 24 °C were more significant than 4, and the minimum value of 24 ~ 28 °C was 3.098 (Table 2).

Larval metamorphosis

Metamorphic individuals appeared first in the 28 and 32 °C treatments after 24 h of the experiment and also in the 24 °C treatments after 48 h. In contrast, metamorphic individuals appeared in the 20 °C treatments only after 60 h ($F_{(3,8)} = 28.1$, $P < 0.001$). The metamorphosis rate of stage IV 8-arm larvae after 72 h was highest at 32 °C, followed by 28 °C, with 61.1% and 50.0%, respectively, with non-significant differences ($P > 0.05$), but significantly higher than in the 20 and 24 °C treatments ($P < 0.05$). In contrast, the metamorphosis rate of stage IV 8-arm larvae at 24 °C was 27.2%, but much higher than the 3.9% juvenile metamorphosis rate at 20 °C ($P < 0.05$).

Table 2 Thermal coefficient (Q_{10}) in larvae of *Heliocidaris crassispina* for different temperature ranges

Q_{10}	32 °C	28 °C	24 °C
20 °C	3.899	3.868	4.828
24 °C	3.504	3.098	
28 °C	3.962		

Discussion

The fertilization and hatching of *H. crassispina* sea urchins have a high-temperature tolerance. Our experimental results demonstrate that purple sea urchins exhibit successful fertilization and hatching within a temperature range of 20 to 32 °C, with both rates surpassing 50%. This observation is deemed highly successful and aligns with the findings reported by Mak and Chan (2018) and Margarita Mejia-Gutierrez et al. (2019). While purple sea urchins *H. crassispina* have high critical temperatures for fertilization and hatching (39 °C and 31 °C, respectively), high temperature severely affects early development of *H. crassispina*, which in turn reduces the population size of *H. crassispina* (Mak and Chan (2018)). Within the temperature ranges of 20 to 32 °C, the highest fertilization and hatching rates were observed at 28 °C, accompanied by the most synchronous development of embryos. At 24 °C, embryo synchrony was slightly lower than at 28 °C but maintained relatively high fertilization and hatching rates. The synchronized embryo development suggests their suitability for growth within an optimal temperature range (Rahman et al. 2009). Embryos develop fastest at 32 °C and slowest at 20 °C, but both extremes had higher mortality rates, basically similar to the conditions of New Zealand sea urchins *Centrostephanus rodgersii* (Pecorino et al. 2013). These findings are similar to previous studies on other marine invertebrates that show shown a greater thermotolerance to fertilization, likely due to the presence of maternal factors and cellular mechanisms that provide protection during early development (Byrne et al. 2011; Farmanfarmaian and Giese 1963; Margarita Mejia-Gutierrez et al. 2019; Quiniou et al. 1999; Sewell and Young 1999), as well as increased frequency of stunted/abnormal development as temperatures rise (Byrne et al. 2009, 2011; Farmanfarmaian and Giese 1963; Fujisawa 1989; Margarita Mejia-Gutierrez et al. 2019; Rupp 1973; Sewell and Young 1999).

Relative to high temperatures, the embryo development of *H. crassispina* exhibited more sensitivity to low temperature at 20 °C. This result is basically consistent with the research report of Fujisawa and Shigei (1990). Roccheri et al. (1986) suggested that the survival of sea urchin embryos after heating depends on the presence of heat shock proteins (Hofmann and Todgham 2010; Tomanek 2008). Therefore, the wide thermal range in which *H. crassispina* typically produces its first cleavage may be due to the maternal supply of heat shock proteins in the eggs (Hamdoun and Epel 2007), explaining why cleavage can proceed typically above ambient temperatures (1 ~ 3 °C) while no intervention by stabilizing proteins is necessary under colder conditions. Low temperatures slow development primarily by reducing the rate of biochemical and physiological processes (Hoffmann 1983; Sokolova and Portner 2003) and may also lead to developmental defects (Schirone and Gross 1968). Therefore, this is the reason why only 60.6% of prism larvae could be observed in the 20 °C temperature-treated group after 27 hpf, whereas 39.4% of the embryos failed to hatch successfully.

Andronikov (1975) reported that environmental temperature is a limiting factor during early development, including the eggs, zygotes, and cleavage. Therefore, the highest

temperature limit for normal development of eggs and embryos is only 1–3 °C higher than that is encountered under natural conditions. Further studies by Reitzel et al. (2004) showed that temperature plays a vital role in determining the spawning time of marine invertebrates, and animals tend to spawn during periods when their offspring develop faster throughout the year.

The optimal temperature for the growth of *H. crassispina* larvae is 24 ~ 28 °C, with the most suitable temperature being 28 °C. This result is consistent with the study of Mak and Chan (2018). It closely matches the average surface water temperature of Fujian Xiamen during the breeding season (May ~ October), which is 26.6 ± 2.6 °C (Fujian Provincial Department of Ocean and Fisheries, data available at https://data.fujian.gov.cn, mean ± SD). This study found that 1–3 °C higher than the environmental average temperature would lead to faster development and growth in larvae. After fertilization for 14.5 days, 83.33% stage IV 8-arm larvae were cultured at a temperature of 28 °C. Similar phenomena have also been observed in other sea urchins (*Tripneustes gratilla*, *Toxopneustes roseus*, and *Diadema setosum*) (Margarita Mejia-Gutierrez et al. 2019; Sarifudin et al. 2016; Sheppard Brennand et al. 2010). However, an increase of 6 °C above the average water temperature has serious adverse effects on larval development, resulting in a mortality rate of up to 22.2%, while a temperature of 20 °C leads to a slow, severe development of larvae. Temperatures that limit fertilization and embryonic development in sea urchins early in life may not limit later larval development (Andronikov 1975). However, the embryos of other species (*Echinometra mathaei*, *Hemicentrotus pulcherrimus*, *Asterias amurensis*, and *Holothuria spinifera*) have a high tolerance towards heat, so it has been suggested that widely distributed species may survive warming oceans through dispersal of adaptable genotypes (Foo et al. 2012).

Previous studies have indicated that *H. crassispina* has a wide dimensional distribution range, spanning from the Sea of Japan (38°N) to Hainan Island (19°N) (Wang et al. 2012; Yoo 1982). Consequently, embryos and larvae of this species may have a relatively broad tolerance range for temperature increases. As a warm-water species, the optimal temperature range of *H. crassispina* is between 25.0 and 30.8 °C (Liu and Chang 2015). *H. crassispina* is commonly found in intertidal zones and shallow subtidal areas along the southern coast of China (Chiu 1984; Clark 1982; Thompson 1982), which may explain their offspring's higher heat tolerance due to exposure to extensive diurnal temperature fluctuations experienced by adults. For species such as *H. crassispina* with a wide latitudinal distribution, the concept of physiological races of populations exhibiting metabolic temperature compensation to different thermal regimes implies significant plasticity in response to environmental change (Byrne et al. 2011; Palmer 1994; Sokolova and Portner 2001). It can be anticipated that *H. crassispina* has potential developmental plasticity through reproductive thermotolerance similar to what has been demonstrated in their congener urchins *H. tuberculata* (O'Connor and Mulley 1977). Recent research also indicates that *H. erythrogramma* may possess an evolutionary (genetic) capacity to adapt to thermal changes. Additionally, a study on the thermal limits of intertidal limpets reported the existence of heat-tolerant genotypes across different parts within their activity range (Kuo and Sanford 2009).

It is a typical example that increasing temperature enhances the developmental progress of *H. crassispina* larvae (Byrne et al. 2011; Hardy and Byrne 2014; Minuti et al. 2022). In this study, the *H. crassispina* larvae showed the fastest growth and development rate at 28 °C (Figs. 4 and 5) regarding POA, ALA, and SA. Moderate warming can act as a metabolic stimulant to improve growth and development rates (Hoegh-Guldberg and Pearse 1995; Hofmann and Todgham 2010; Stanwell-Smith and Peck 1998). Particularly, longer post-oral arms can be transformed into enhanced ingestion and swimming abilities (Emlet

1983; Grünbaum and Strathmann 2003), which increases opportunities for predator evasion (Allen 2008) and shorten the duration of vulnerable planktonic life stages (Lamare and Barker 1999). The elongated arm rods in echinoderms are crucial for predation, swimming, and protection against predators, and successful ingestion is related to arm length (Allen 2008; Soars et al. 2009). Low temperatures significantly alter larval morphology by narrowing body size and shortening arm rod lengths (Wangensteen et al. 2013) (Figs. 7 and 8).

In this study, the metabolic rate of stage IV 8-arm larvae increased with increasing temperature, with the highest metabolic rate at 32 °C. This may be due to sudden temperature stress leading to increased metabolic rate. Metabolic activity in poikilothermic animals depends on the degree, speed, and frequency of temperature changes (Eldridge et al. 2015). Generally, as temperature increases, metabolic rates also increase because biochemical reactions and biological activity rates increase with temperature (Sin et al. 2019). The Q_{10} is a measure of the ability of aquatic animals to regulate metabolism after a temperature change. For the metabolic rate of aquatic animals, the Q_{10} values are generally close to 2, indicating that for every 10 °C changes in temperature, there is a doubling of metabolic rate (Spanopoulos-Hernández et al. 2005). The Q_{10} has been considered a common value that reflects adjustments related to energy demand by enzymes and physiology when temperatures rise within natural ranges (Vernberg 1983). The Q_{10} value for oxygen consumption metabolism in sea urchin *Mesocentrotus nudus* is greater than 2 (Sin et al. 2019). In this study, at temperatures between 28 and 24 °C, the Q_{10} value for VO_2 was found to be 3.098, which indicates stable metabolism for 8-arm larvae within this range. At the same time, other temperature provinces showed an increase in the Q_{10} values, suggesting enhanced larval metabolism. However, under environmental pressure, the eventual increase in metabolic rates can threaten sustained development as it reduces growth range and reallocates energy needed for growth towards maintaining metabolism, thereby delaying development (Stumpp et al. 2011). It has been reported that under pressure, microbial energy shifts from functions such as ingestion or movement towards mobilization of lipid energy reserves to enhance respiratory rates at higher temperatures

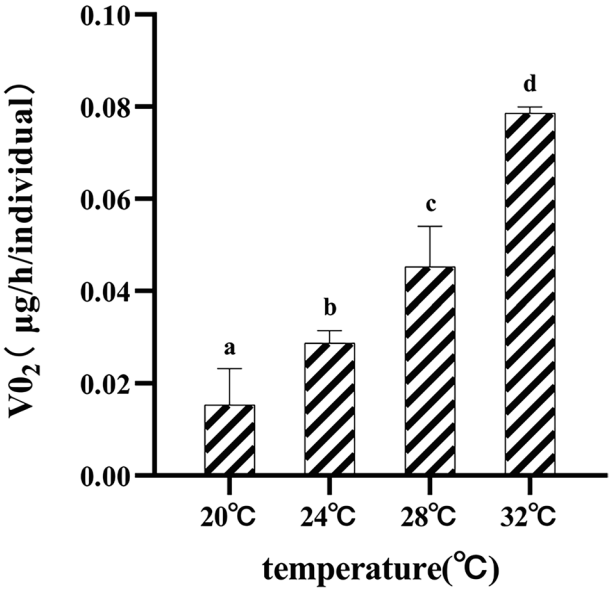

Fig. 7 Effects of temperature on the oxygen consumption of *Heliocidaris crassispina* 8-arm larvae (mean ± SD, $n = 3$). Different superscript means significant difference ($P < 0.05$)

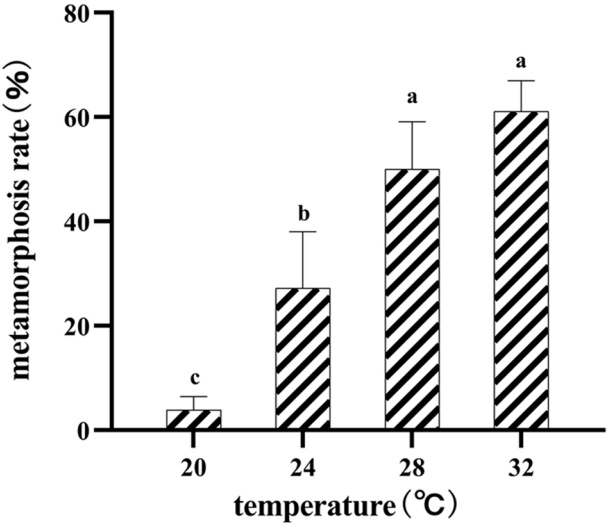

Fig. 8 Effects of temperature on the metamorphosis of stage IV 8-arm larvae of *Heliocidaris crassispina* (mean ± SD, $n = 3$). Different superscript means significant difference ($P < 0.05$)

(Artigaud et al. 2015). Under extreme pressures and costly energies, survival is impacted by remaining reserves and food availability (Anthony et al. 2009).

In this study, the rate at which *H. crassispina* undergoes metamorphosis was observed to increase with higher temperatures. It may be overestimated that the metamorphic rate is highest at 32 °C, as the survival rate was considered within 72 h post-metamorphosis. A critical stage in sea urchin aquaculture involves the transition of planktonic larvae into benthic juveniles and their subsequent survival, as the settling rate and metamorphosis rate of these larvae can exhibit significant variation, ranging from 0 to 90% (Buitrago et al. 2005; Cameron and Hinegardner 1974; Dworjanyn and Pirozzi 2008; Gosselin and Jangoux 1996; Grosjean et al. 1998; Huggett et al. 2006; Lawrence et al. 2019; Pearce and Scheibling 1991; Rahim et al. 2004). Similarly, mortality rates were also high during the first few weeks, such as 94% for *Paracentrotus lividus* (Grosjean et al. 1998) and 90% for *Tripneustes gratilla* (Shimabukuro 1991) and *Strongylocentrotus intermedius* (Lawrence et al. 2019). Therefore, 28 °C remains the optimal temperature for post-metamorphic development and survival of *H. crassispina*. Temperature can affect settlement when larvae are exposed to extreme environments with parameters higher than their normal habitat range (Lawrence and Agatsuma 2007). It is worth noting that lower metamorphosis rates at low temperatures are similar to studies on *T. gratilla*, which also resides in tropical/subtropical regions (Mos et al. 2011).

This is the first time that the effect of temperature on the metamorphosis of *H. crassispina* has been tested, but it supports the evidence that temperature is an essential determinant of metamorphosis and post-settlement survival in marine invertebrates (Feng et al. 2010; Maldonado and Young 1996; Mos et al. 2011; Whalan et al. 2008).

Conclusion

The experimental research results indicate that 28 °C is optimal for fertilization, embryo development, larval rearing, and metamorphosis of purple sea urchins, *H. crassispina*. Meanwhile, as China's main large-scale algae cultivation base, Fujian Province has a

natural advantage in cultivating seedlings of *H. crassispina* on a large scale. Based on these findings, the natural seawater temperature should be the primary factor for selecting land-based factory farming locations and large-scale seedling cultivation with coastal shellfish farms (such as sea cucumbers and abalones). Therefore, it is necessary to have the best temperature conditions and suitable range.

Meanwhile, these research findings suggest that *H. crassispina* may inhabit areas relatively close to the upper thermal threshold for successful development. Future climate-driven ocean warming could threaten this species' persistence at the southern margin of Chinese waters, potentially leading to range contraction or fragmentation. It can be concluded that the populations studied in this research are located in warmer regions within the species distribution range. Therefore, if global seawater temperatures rise, it could result in the disappearance of these populations living on their heat tolerance edge. However, for other populations situated at temperate latitudes, it may provide favorable conditions for fertilization and survival of embryos and larvae.

This is the first experiment to investigate the effects of temperature range on fertilization, embryonic development, larval growth, ingestion, metabolism, and metamorphosis of *H. crassispina*. The findings of the present study not only contribute to understanding the critical limit of temperature but also to finding the appropriate temperature level for fertilization, optimal growth and development of embryos and larvae, and metamorphosis, ultimately contributing to the future mass production of *H. crassispina* juveniles for aquaculture or wild population reseeding.

Author contribution JY: validation, formal analysis, writing—original draft. LZ: conceptualization, project administration. SH: resources, supervision. GW: conceptualization, methodology, funding acquisition, writing—review and editing.

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Data availability All of the data and material is owned by the corresponding author.

Declarations

Ethics approval The manuscript is conducted within the ethical manner advised by the targeted journal.

Competing interests The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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