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BRIEF COMMUNICATION

Larviculture of the purple sea urchin *Heliocidaris crassispina* in artificial and natural seawater

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Abstract
Artificial seawater has been utilised as an alternative culture medium in the larval rearing of various marine organisms. However, the viability and the effects of artificial seawater on the early-life stages of echinoderms have not been extensively studied. In this study, we examined the impacts of artificial seawater on the larval development, physiology, metamorphosis, and settlement of the purple sea urchin *Heliocidaris crassispina*, which is an ecologically and economically important seafood native in Hong Kong waters. Our results showed that larvae cultured in artificial seawater exhibited a 31% reduction in growth rates with more than 60% increase in respiration rates and more than 80% increase in frequency of larval deformities. Larval settlement was not delayed but only 0.5% settled. Larval mortality was significantly high in artificial seawater with less than 1% compared to 14% survival in natural seawater. The differences in salt composition and carbonate chemistry between artificial and natural seawater may have detrimental effects on the sensitive developmental stages of *H. crassispina*. Nevertheless, given the increasing pollution risks affecting natural seawater quality and reliability, continued research into developing optimised artificial seawater formulations with improved ecological compatibility remains a crucial avenue for sustainable *H. crassispina* seedling production.

Keywords Larval development · Settlement · Metamorphosis · Echinoderm · Hong Kong

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Introduction

The purple sea urchin *Heliocidaris crassispina* is widely distributed in the shallow waters of the Western Pacific, occurring from the Sea of Japan to the South China Sea (Freeman 2003; Feng et al. 2019). This species is an important benthic herbivore and ecosystem engineer that naturally inhabits many algal communities on the intertidal and subtidal rocky shores (Chiu 1984; Yatsuya and Nakahara 2004). The gonads of sea urchins, known as roe or *uni* (in Japanese), are considered a delicacy due to their tasty flavour and high nutritional value (Liu and Chang 2015) with significant demand for them from the Asian, North American, and European markets (Unuma et al. 2015; Chu et al. 2023). As a result, sea urchins have become one of the most commercially harvested fisheries (Urriago et al. 2021; Chu et al. 2023).

In recent years, the aquaculture production of sea urchins has experienced a continuous decline (Yu et al. 2024). This decline may be attributed to various factors, including a lack of seed production, overfishing, and ineffective resource management policies (Ding et al. 2007; Lawrence 2013; Stefánsson et al. 2017). Despite the reduced supply, the demand for these sea urchins remains high. Given the significance of *H. crassispina* in the aquaculture industry, there is an urgent need to develop captive breeding programmes. Such initiatives would not only help meet the increasing market demand but also contribute to the enhancement of declining wild populations (Cen et al. 2024).

Coastal marine organisms including sea urchins are exposed to multiple anthropogenic stressors. The increasing pollution levels in coastal areas (Morton and Blackmore 2001; Vikas and Dwarakish 2015) and climate-induced ocean warming and acidification could compromise larval urchin survival, development, and physiological performance (Kurihara and Shirayama 2004; Stumpp et al. 2011; Lenz et al. 2019). The decline in seawater quality thereby could further impact the seed production of sea urchins.

For many years, artificial seawater has been used as culture media for several species when high-quality natural seawater is unavailable or to avoid the confounding effects of unknown pollutants and microorganisms (Landau and D'Agostino 1978; Berges and Franklin 2001; Pechenik et al. 2019). The use of artificial seawater can also reduce the cost and logistics, particularly for facilities far from the coast (Challener et al. 2013). Pires (2014) and Pechenik et al. (2019) successfully cultured the slipper limpet *Crepidula fornicata* in artificial seawater fed with *Isochrysis galbana* maintained at 20–23 °C and with every other day water changes. The crustacean *Mysidopsis almyra* maintained at 25 °C and 20‰ (Reitsema and Neff 1980) and the tunicate *Botrylloides diegensis* maintained at 22 °C, 35‰, and 8.2 pH (Wawrzyniak et al. 2021) were both successfully cultured long term in a recirculating artificial seawater system. The larvae of the green sea urchin *Lytechinus variegatus* commonly found in the tropical waters of the western Atlantic Ocean were cultured in artificial seawater fed with microencapsulated capsules showed high rates of survival and metamorphosis (George et al. 2004). These studies demonstrate that the use of artificial seawater may be a reasonable substitute for natural seawater.

The reproductive biology and larval culture of *H. crassispina* are relatively well established (Liu and Chang 2015; Urriago et al. 2016; Chu et al. 2023). However, the use of artificial seawater for scaling up the production of *H. crassispina* for commercial and conservation purposes has not yet been tested. In this study, we aimed to compare the larval development and settlement of *H. crassispina* cultured under artificial and natural seawater and to evaluate the viability and impacts of artificial seawater on the performance and larval traits of the early-life stages of *H. crassispina*.

Materials and methods

Sea urchin collection and larval culture

Adult sea urchins of *H. crassispina* were collected at 1 m depth in Tai Tau Island, Sai Kung, Hong Kong (22° 22′ 26.33″ N, 114° 19′ 36.37″ E). The physico-chemical parameters at the collection site fluctuate with the season (Pecquet et al. 2017). However, seawater parameters measured below the surface during the collection were 24.1 °C, 32.0 ‰, and 8.15 pH_{NBS}. Immediately after collection, sea urchins were transported in seawater with small ice packs to the laboratory at The Hong Kong Polytechnic University. The sea urchins were acclimated for a month in an indoor recirculating aquaculture system in artificial seawater following field conditions (800 L; 24.3 ± 1.0 °C, 32.5 ± 0.6 ‰; 8.3 ± 0.04 pH_{NBS}). Individual sea urchins were fed three times a week with the Kombu alga *Saccharina japonica* at 5–6 g wet weight per sea urchin.

The sea urchins were induced to spawn by injection of 0.5–1 mL of 0.5 M potassium chloride solution into the peristomial membrane. Eggs (from two females) were washed twice with filtered seawater in a 1000-mL beaker. Sperm from two males was collected dry and kept on ice until use. Sperm motility and density were checked under the microscope. A 10 µL of dry sperm suspension was diluted in 500 mL of filtered natural seawater. Five milliliters of diluted sperm concentration was added to fertilise the egg suspension. Fertilisation success was confirmed by the presence of fertilisation envelopes 5 min after fertilisation (Fig. 1a). Excess sperm was removed 1 h later by changing the seawater twice after confirming a fertilisation rate above 95%. The developing embryos (2-h post-fertilisation) until metamorphosis were cultured in 500-mL beakers containing artificial and natural seawater treatments at a density of 4 larvae mL^{-1} (Chu et al. 2023). Both treatments with four replications and with gentle aeration to keep the larvae and microalgae (as food) suspended. Feeding started on day 2 post-fertilisation. Larvae were fed daily with the microalga *Chaetoceros gracilis* (Ding et al. 2007) cultured in the F2 medium (Guillard and Ryther 1962). The feeding density of the larvae was 5000 cells mL^{-1}. As the larvae developed, feeding was increased to 10,000 cells mL^{-1} on day 4 and 30,000 cells mL^{-1} on day 6 onwards. Algal densities were checked and counted daily using a hemocytometer under a microscope.

Artificial seawater was prepared 24 h before the experiment and before each water change by mixing the commercial Instant Ocean Reef Crystals Reef Salt (Blacksburg VA, USA) with distilled water and adjusted to 32‰. The newly prepared artificial seawater (100 L) was aerated overnight to fully mix the salt. Both the artificial and natural seawater (32‰) were filtered through 0.5 µm pores before use. The embryos and subsequent larvae were reared at a salinity of 32‰ and pH_{NBS} of 8.2 (Table 1), which were monitored and measured every 2 days until settlement (~30 days). The culture beakers were placed in a recirculating water bath to maintain the water temperature at 24 °C (Table 1). The water in all the beakers was completely changed every 2 days. The water was siphoned out and filtered with a 50-µm mesh size sieve collecting the larvae. To ensure the collected larvae would not dry out, the sieve was placed on top of a petri dish with artificial or natural seawater. The emptied beakers were cleaned and filled with newly filtered artificial or natural seawater, and the larvae were transferred back to the beaker. Alkalinity was also measured in both artificial and natural seawater (Table 1). Water samples were filtered through 0.22 µm pores and titrated against 0.1M

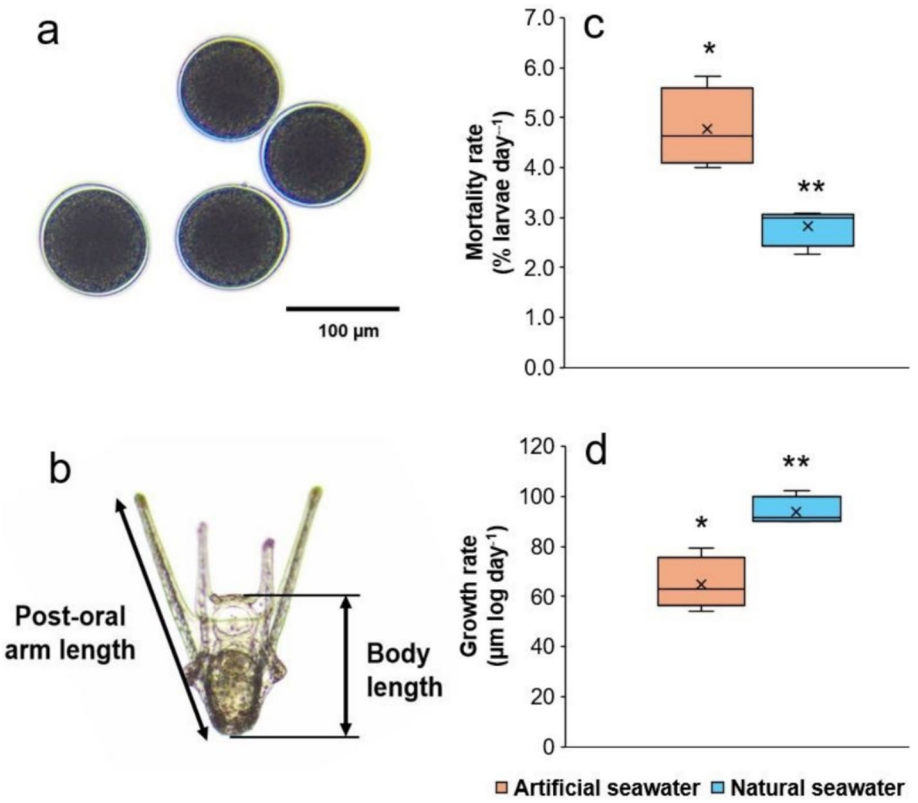

Fig. 1 **a** Fertilised eggs with fertilisation envelopes; **b** larval growth estimated by the increase in body length and post-oral arm length; **c** results of mortality rate, and **d** growth rate observed from the larvae of *Heliocidaris crassispina* reared in artificial and natural seawater ($n = 4$). Box plots with different asterisk numbers are significantly different from each other at $p < 0.05$ (see Table 2)

Table 1 Physico-chemical parameters measured in the artificial and natural seawater. All values are expressed as mean ± SD (salinity, temperature, and pH; $n = 136$ measurements, alkalinity, and calcite saturation; $n = 5$). Values with different letters are significantly different from each other ($p < 0.05$)

Treatments	Salinity (‰)	Temperature (°C)	pH (NBS scale)	Alkalinity (µmol kg^{-1})	Calcite saturation
Artificial seawater	32.00 ± 0.00^{a}	24.01 ± 1.04^{a}	8.23 ± 0.04^{a}	3253.95 ± 110.27^{a}	9.79 ± 0.33^{a}
Natural seawater	31.96 ± 0.32^{a}	24.02 ± 1.08^{a}	8.21 ± 0.05^{a}	2043.67 ± 57.68^{b}	5.76 ± 0.14^{b}

hydrochloric acid using a 916 Ti-Touch potentiometric titrator with an 800 Dosino dosing system (Metrohm, Switzerland). Total alkalinity was determined from the titration results based on the Gran function as described by Dickson et al. (2007). The measured values of total alkalinity, salinity, temperature, and pH were used to calculate the saturation states of calcite (Table 1) using the software CO_2 System v2.1 (Pierrot et al. 2015).

Biological measurements

Each larval culture replicate was sampled daily (1 mL in triplicates) from day 1 to day 30 to assess larval density. Larvae in the samples were immediately counted. For each culture, survival was calculated for each day (0–30 days) as the proportion of larval density divided by the number of larvae ever counted in the corresponding culture. Mortality rates were then computed from the coefficient of significant linear regression between survival and time (Dorey et al. 2018).

Another 10 mL subsample was taken every day until day 12 and every 2 days until day 24 for larval size measurements and growth. At least 10 larvae per sampling point and culture treatment were photographed under a microscope (BL-180 with E3/SPM, Cossim, China). Larval body length and post-oral arm length (Fig. 1b) were measured with the software ImageJ (Schneider et al. 2012). Body growth rates were calculated for each culture as the regression coefficient of the logarithmic relationship between measured body length and time (Dorey et al. 2018). The proportion of larvae with deformities and abnormal development at each sampling point was also recorded (His et al. 1999).

Larval respiration was measured on day 10 and day 15 from the artificial and natural seawater cultures ($n = 4$). Larvae were hand-picked under the microscope and thoroughly rinsed with corresponding artificial and natural seawater. Eight to ten larvae per tube per culture were incubated into 1.5-mL Eppendorf tubes filled with corresponding seawater at 24 °C in a water bath. Respiration due to potential bacterial contamination was evaluated in tubes without larvae as blank controls ($n = 4$ per culture treatment). Oxygen concentration in the seawater was measured every 5 s for 10 min with a PreSens OXY-10 ST oxygen microsensor (Regensburg, Germany). Oxygen consumption rates were calculated with a linear regression between oxygen concentration in each tube and time (Dorey et al. 2018). The estimated rate was then corrected by the number of larvae per tube, the average measured body length (BL) of the larvae in each culture, and respiration in blank controls. The oxygen consumption was expressed as $p$mol O_2 hr^{-1} μm BL^{-1}.

The total number of spontaneously settled and metamorphosed larvae on day 30 was counted in all cultures. The remaining surviving larvae and newly settled larvae were further monitored until the juvenile stage.

Data analysis

All biological parameters measured in this study were checked for normality (Shapiro-Wilk test) and homogeneity (Levene's test). One-way ANOVA was used to test differences between culture treatments (artificial and natural seawater) in the alkalinity, calcite saturation state, larval mortality, growth rate, and settlement. Repeated measures ANOVA was used to test differences between culture treatments and time (days) in post-oral arm length, larval abnormality, and respiration rates. A post hoc Tukey's test was performed when significant differences were detected. Salinity, temperature and pH were analysed with the non-parametric Mann-Whitney U test. All statistical tests were performed at a 5% significance level with Statistica 13 (TIBCO Software, Palo Alto, CA).

Results

Larval mortality and growth rates

Larval mortality rates in artificial seawater (4.8 ± 0.8% larvae day^{-1}) were significantly higher (ANOVA, $F_{1, 6} = 19.617$, $p = 0.004$; Table 2) compared to natural seawater with 2.8 ± 0.4% larvae day^{-1} (Fig. 1c). After 2 weeks of larval culture, the total larvae in artificial seawater reduced to 12 ± 6% with a larval density of 0.7 ± 0.3 larvae mL^{-1} while the total larvae in natural seawater was 52 ± 17% with a larval density of 2 ± 1 larvae mL^{-1}. By day 28, less than 1% of larvae survived in artificial seawater compared to 14 ± 8% remaining larvae in natural seawater.

The larval body size of *H. crassispina* from both culture treatments increased over time; however, the growth rate was significantly higher in natural seawater (ANOVA, $F_{1, 6} = 23.307$, $p = 0.003$). Larvae in artificial seawater had a growth rate of 65.0 ± 10.5 µm log day^{-1} and larvae in natural seawater had a growth rate of 94.0 ± 5.7 µm log day^{-1} (Fig. 1d). Before the settlement phase, average body length of the 8-arm larvae in artificial seawater was 311.8 ± 31.9 µm and larvae in natural seawater had a body length of 422.0 ± 10.1 µm.

Growth in post-oral arm length and larval abnormality

The culture media (artificial and natural seawater) significantly affected the post-oral arm length in the larvae (RM-ANOVA, $F_{1, 12} = 63.380$, $p < 0.001$) with significant changes in the arm length over time (RM-ANOVA, $F_{2, 12} = 23.722$, $p < 0.001$). A significant interaction effect between the culture treatments and days was also observed (RM-ANOVA, $F_{2, 12} = 15.587$, $p = 0.001$). Larval post-oral arms in the artificial seawater were shorter and significantly decreased over time (Tukey's test, $p < 0.05$). On day 5, the average post-oral arm length was 526.4 ± 31.6 µm, and on day 15, decreased to 247.4 ± 25.6 µm (Fig. 2a). Meanwhile, post-oral arms in the natural seawater were longer but no significant changes in

Table 2 Results of the one-way analysis of variance (ANOVA) and repeated measures ANOVA (RM-ANOVA) for the effects of culture medium (artificial and natural seawater) and monitoring days on the larval traits of *Heliocidaris crassispina*. The asterisk indicates significance at $p < 0.05$

Tests	Parameters	Fixed effect	df	*F*-ratio	*P*-value
One-way ANOVA	*Larval mortality*	Culture medium	1	19.62	0.004*
	Growth rate	Culture medium	1	23.31	0.003*
	Settlement	Culture medium	1	9.42	0.022*
RM-ANOVA	*Post-oral arm length*	Culture medium	1	63.38	<0.001*
		Days	2	23.72	<0.001*
		Culture medium × days	2	15.59	<0.001*
	Larval abnormality	Culture medium	1	313.30	<0.0001*
		Days	2	176.64	<0.0001*
		Culture medium × days	2	89.99	<0.0001*
	Respiration rate	Culture medium	1	8.11	0.029*
		Days	1	3.25	0.121
		Culture medium × days	1	0.62	0.459

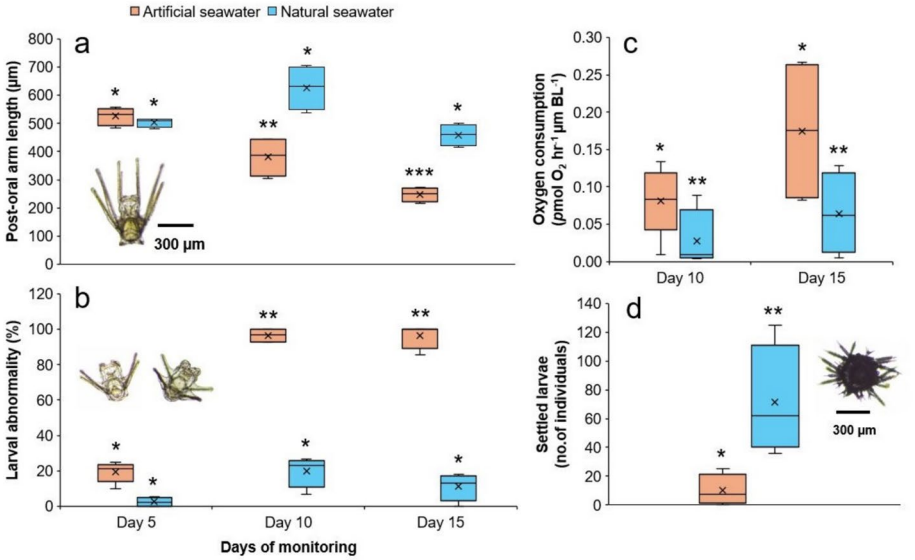

Fig. 2 **a** Results of post-oral arm length, **b** percent larval abnormality, **c** respiration rate, and **d** settlement of *Heliocidaris crassispina* reared in artificial and natural seawater ($n = 4$). Box plots with different asterisk numbers are significantly different from each other at $p < 0.05$ (see Table 2)

length were observed over time (Tukey's test, $p > 0.05$). The average post-oral arm length on day 5 was 503.3 ± 15.4 µm, and on day 15, a decrease in size was observed with 459.4 ± 38.1 µm, but this was not significant (Fig. 2a).

Artificial seawater also significantly influenced the frequency of larval abnormality (RM-ANOVA, $F_{1, 12} = 313.303$, $p < 0.0001$), and this abnormality significantly increased over time (RM-ANOVA, $F_{2, 12} = 176.639$, $p < 0.0001$). The interaction between the culture treatments and days also significantly affected the frequency of larval abnormality (RM-ANOVA, $F_{2, 12} = 89.996$, $p < 0.0001$). The frequency of larval abnormality in artificial seawater significantly increased (Tukey's test, $p < 0.05$) from 19.3 ± 5.7% on day 5 to 96.4 ± 7.1% on day 15 (Fig. 2b). In natural seawater, a slight increase in larval abnormality was also observed with 2.5 ± 2.8% on day 5 and 11.1 ± 7.8% on day 15. However, this variation was not significant (Fig. 2b). The high frequency (>90%) of larval abnormality observed in artificial seawater on days 10 and 15 included shortened arm length and body size (Fig. 2b). In contrast, 84.6 ± 9.1% of the larvae between days 10 and 15 in natural seawater developed normally (Fig. 2a).

Respiration rates and larval settlement

Larval oxygen consumption in artificial seawater was significantly higher compared to the larvae in natural seawater (RM-ANOVA, $F_{1, 6} = 8.105$, $p = 0.029$). There was an increasing trend in oxygen consumption between day 10 and day 15; however, this increase was not significant (RM-ANOVA, $F_{1, 6} = 3.250$, $p = 0.121$). No significant interactive effects between culture treatments and time were observed (RM-ANOVA, $F_{1, 6} = 0.623$, $p = 0.460$). On day 10, the oxygen consumption in artificial seawater was 0.08 ± 0.05 *p*mol

O_2 hr^{-1} μm BL^{-1}, significantly higher compared to natural seawater with 0.03 ± 0.04 *p*mol O_2 hr^{-1} μm BL^{-1} (Fig. 2c). Oxygen consumption nearly doubled in both treatments on day 15, with 0.18 ± 0.10 *p*mol O_2 hr^{-1} μm BL^{-1} in artificial seawater and 0.06 ± 0.05 *p*mol O_2 hr^{-1} μm BL^{-1} in natural seawater (Fig. 2c).

Larval settlement and metamorphosis on day 30 were significantly lower in artificial seawater compared to natural seawater (ANOVA, $F_{1,6} = 9.417$, $p = 0.022$). The total number of individuals spontaneously settled and metamorphosed in artificial seawater was 10 ± 11 individuals and 71 ± 38 individuals in natural seawater (Fig. 2d). Continued monitoring showed that all settled and metamorphosed larvae in artificial seawater did not survive thereafter. However, the settled and metamorphosed larvae in natural seawater further developed into young juveniles and completed the larval developmental cycle (Fig. 3).

Early ontogenic development of *H. crassispina* in natural seawater

After successful fertilisation (Fig. 1a and Fig. 3a), the larvae developed into a prism shape within 24 h. At this stage, the mouth and stomach began to form, along with the appearance of two side arms (Fig. 3b). After 48 h, the larvae transformed into a four-armed pluteus and the development of the mouth and stomach which indicated the onset of larval feeding. After 1 week of feeding, the larvae developed into a six-armed pluteus (Fig. 3e). As the larvae developed, both their size and swimming speed also increased. On day 10, the larvae developed into an eight-armed pluteus, characterised by well-developed post-oral arms

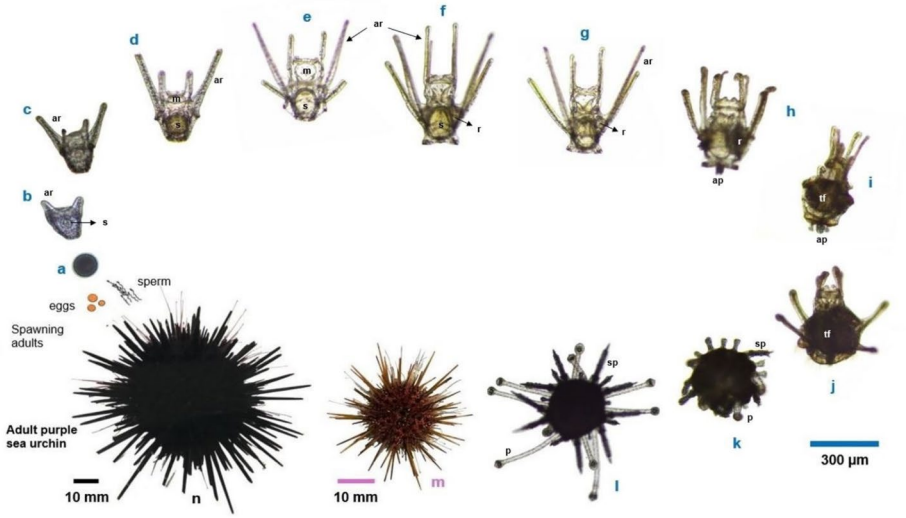

Fig. 3 Larval development and settlement stages of *Heliocidaris crassispina* in Hong Kong cultured in natural seawater: (a) newly fertilised zygote; (b) D1, 2-arm prism; (c) D2, 4-arm pluteus; (d) D4, 4-arm pluteus; (e) D7, 6-arm pluteus; (f) D10, 8-arm stage with elongated and well developed post-oral arms and development of the rudiment; (g) D12, 8-arm stage; (h) D17, 8-arm stage with enlargement of the rudiment; (i) D20, competent larva; (j) D23, late-stage larva starts to metamorphose; (k) D26, settled and undergoing metamorphosis; (l) D30, settled juvenile with developed spines and pedicellariae; (m) 10 months old sea urchin juvenile; and (n) adult sea urchin. ap, apical pedicellaria; ar, arm; m, mouth; p, pedicellaria; r, rudiment; s, stomach; sp, spine; tf, trophoectoderm. Coloured letters and scale bar references: a–l = 300 μm (blue bar); m = 10 mm (pink bar); n = 10 mm (black bar)

and a developing rudiment (Fig. 3f). After 14 days, the rudiment enlarged, and the apical pedicellaria began to appear (Fig. 3h). By day 20, the larvae reached a competent stage, exhibiting complete rudiment growth with arms arranged parallel to each other (Fig. 3i). This pre-metamorphic stage was followed by the reabsorption of the arms (Guete-Salazar et al. 2021), leading to the formation of a rounded body shape (Fig. 3j). At this stage, larval swimming activity was reduced and mostly sinked to the bottom to begin metamorphosis. Around day 26, the larvae developed a rounded skeleton with several spines and pedicellariae (Fig. 3k). At this stage, the larvae were fully settled and undergoing metamorphosis. After approximately 30 days, the larvae transformed into young juveniles with developed spines and pedicellariae (Fig. 3l).

Discussion

Artificial seawater has been successfully used as a substitute for natural seawater in the culture of various marine organisms, although its effectiveness can vary by species and group. In this study, we evaluated the viability of artificial seawater for rearing larvae of the purple sea urchin *H. crassispina*. Our findings indicated that larvae cultured in artificial seawater exhibited poor development with high morphological deformities and respiration rates. These factors likely contributed to lower settlement and survival compared to larvae reared in natural seawater. Overall, the results suggest that the artificial seawater used in this study may not be suitable for the larval rearing of *H. crassispina.*

Larval development of *H. crassispina* in artificial and natural seawater

Larvae cultured in artificial seawater displayed increased oxygen consumption which could suggest an increase in energy demand (Stumpp et al. 2011), as maintaining cellular homeostasis in larval sea urchins is energetically costly (Pan et al. 2015). A significant amount of energy may be required to support ion transport and cellular functions in the larvae during their rearing in artificial seawater. Furthermore, calcification is also a high-energy-demand process (Palmer 1992). This altered allocation of energy could reduce the energy available for growth and calcification (Stumpp et al. 2011; Stumpp et al. 2012), potentially explaining the observed decrease in larval growth, high frequency of larval deformities, and mortality. Additionally, the energetic trade-off has further consequences, as the rates of settlement and metamorphosis were notably lower. Settled juveniles in the artificial seawater also did not survive as energy shortages could likely have a negative carryover effect in later stages (Dupont et al. 2013).

Similarly, larvae of the sea star *Asterias forbesi*, when cultured in artificial seawater (Instant Ocean), demonstrated reduced growth rates (Pechenik et al. 2019). In contrast, the green sea urchin *Lytechinus variegatus* successfully reached metamorphosis when cultured in artificial seawater (Kent Marine, Acworth, GA) and fed with microencapsulated food (George et al. 2004). The differences in outcomes may be attributed to the various types of salt used, diet type and food ration, and differences in culture conditions. Moreover, the differing responses of larvae to artificial seawater may also be species-specific and vary among different echinoderm species and life stages. Adult purple sea urchins that have been long-term conditioned in recirculating artificial seawater can thrive and regenerate from injuries (Maboloc and Fang 2023). However, *H. crassispina* larvae in this study did not perform better in artificial seawater. The varying responses along with the limited

comparative studies available (Pechenik et al. 2019) highlight the need for further investigations into the impacts and assess the larval performance in artificial seawater among echinoderm species.

The larvae of *H. crassispina* cultured in natural seawater and fed with *C. gracilis* exhibited bigger larval sizes and higher survival rates compared to the larvae in artificial seawater. Competent larvae and spontaneous settlement in the natural seawater began on day 20. This observation aligns with the findings reported by Ding et al. (2007) where *H. crassispina* larvae became competent after 21–24 days of culture in the laboratory. In our study, we observed complete metamorphosis occurring by day 30 (Fig. 3l). However, this larval development period was longer compared to the observations made in Taiwan (Chu et al. 2023). In Taiwan, *H. crassispina* larvae settled and metamorphosed into the juvenile phase after just 12 days of feeding. This discrepancy in the duration of larval development may be attributed to differences in microalgal diet and temperature conditions during culture. Chu et al. (2023) reported that feeding *H. crassispina* with *C. muelleri* significantly improved larval growth and survival and can promote metamorphosis. Additionally, diatom-based and microbial films have been shown to facilitate metamorphosis in *H. crassispina* (Rahim et al. 2004). However, these biofilms did not develop and was not observed in all our cultures due to regular cleaning. The lack of biofilms may have also contributed to the extended development time of the larvae in our study. Temperature is also known to have a significant impact on the early development and increasing temperature enhances developmental rate in *H. crassispina* (Yu et al. 2024). In this study, the temperature was maintained at 24 °C, which corresponds to Hong Kong's average year-round temperature (Pecquet et al. 2017), while the culture condition conducted in Taiwan was set at 26 °C (Chu et al. 2023). The optimal temperature for the growth of *H. crassispina* larvae has been reported to be 28 °C, where their growth and development rates are the fastest (Yu et al. 2024). Overall, these studies demonstrate that the use of natural seawater together with the appropriate temperature level and microalgal diet is important to achieve high survival and optimal growth and metamorphosis in the production of *H. crassispina* seedlings.

Impacts of artificial seawater on larval development

The negative responses observed in the larvae of *H. crassispina* to artificial seawater could be attributed to possible variations in the salt composition compared to natural seawater (Atkinson and Bingman 1998; Berges and Franklin 2001). Inadequate levels of any major elements and ions in the salt mix could adversely affect the development of embryos and larvae (Cawthorne et al. 1983; Pechenik et al. 2019). For instance, differences in strontium concentration between artificial and natural seawater resulted in reduced calcification rates in corals growing in artificial seawater (Gattuso et al. 1998). Moreover, higher concentrations of trace metals can be toxic to organisms across different trophic levels and life stages. Atkinson and Bingman (1998) reported that artificial seawater derived from commonly used commercial salts contained higher concentrations of trace metals compared to natural seawater. The salt composition of the artificial seawater used in this study was not analysed and the potential toxicity of trace elements during the larval cycle of *H. crassispina* remains largely unexplored (Dorey et al. 2018). However, the observed smaller larval sizes and skeletal deformities could also indicate toxicological effects from the elevated levels of trace metals (El Idrissi et al. 2022) and other unreported factors present in the artificial seawater used in this study.

As calcifiers, sea urchins depend on calcium and carbonate ions for the formation of their skeletons. Calcium ions have been shown to promote calcification, larval settlement, and metamorphosis in various marine invertebrate larvae (Ilan et al. 1993; Clare 1996; Amador-Cano et al. 2006; Yang et al. 2015). However, higher concentrations of calcium ions have been found to inhibit larval skeletal growth in the sea urchin *Hemicentrotus pulcherrimus* (Okazaki 1956) and showed toxic effects on larval survival and growth in the coral *Pocillopora damicornis* (Yang et al. 2022). Survival and growth in the freshwater prawn *Macrobrachium resenbergii* also declined significantly in high calcium levels (Adhikari et al. 2007). Changes in alkalinity can also significantly impact larval development and the normal physiological processes of various organisms (Maoxiao et al. 2018). Sea urchin larvae possess receptors that are sensitive to fluctuations in ion concentrations (Cameron et al. 1989). Increased alkalinity in artificial seawater may lead to higher ion concentrations, which could disrupt the ability of larval receptors to initiate development and metamorphosis. Similarly, Gonzalez-Vera and Brown (2017) found that the growth rate and survival of post-larval *M. resenbergii* were negatively affected under conditions of elevated alkalinity. In fish culture, high alkalinity can induce stress in fish (Sun et al. 2020). Yao et al. (2010) also reported that high alkalinity conditions caused morphological abnormalities, halted embryonic development, and led to hatching failures in the medaka fish, *Oryzias latipes*.

In this study, both calcite saturation and alkalinity in the artificial seawater were significantly higher than those in natural seawater (Table 1). Similarly, Campbell et al. (2014) reported elevated alkalinity levels in the artificial seawater (Tropic Marin) compared to the average natural seawater. This increase in calcite saturation and alkalinity in artificial seawater may also have a synergistic effect that contributed to the negative responses observed in the larvae of *H. crassispina*. However, additional studies are needed to better understand the consequences of high calcite saturation and alkalinity in the larval development of echinoderms and their impacts in larval rearing production.

Conclusions

Our results demonstrated that the artificial seawater used in this study was not suitable in the larval culture of *H. crassispina*. Larvae exhibited slower growth rates (65.0 ± 10.5 μm log day^{-1}), higher frequency of larval abnormalities (96.4 ± 7.1%), and increased metabolic rates (0.18 ± 0.10 *p*mol O_2 hr^{-1} μm BL^{-1}). Larval settlement (10 ± 11 individuals) and survival (<1%) were also significantly reduced. In contrast, larvae cultured in natural seawater developed normally with higher growth rates (94.0 ± 5.7 μm log day^{-1}) and higher survival (14 ± 8%), with complete settlement and metamorphosis (71 ± 38 individuals) occurring within a month. The differences in salt composition and carbonate chemistry between artificial and natural seawater may likely affect the developing larvae. However, in light of deteriorating natural seawater quality from pollution pressures, developing ecologically compatible artificial seawater alternatives presents a vital research priority for sustainable *H. crassispina* larval culture.

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Data availability No datasets were generated or analysed during the current study.

Declarations

Competing interests The authors declare no competing interests.

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