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ORIGINAL ARTICLE

Establishing larval feeding regimens for the decorator crab *Camposcia retusa*: Effects of live prey types and density on larval survival and development

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Abstract

Decorator crab *Camposcia retusa* is a popular marine ornamental species; however, it has never been bred previously. To establish a feeding regime for *C. retusa* larvae, which include two zoeal and a megalopal stage, three experiments were conducted. In all experiments, ≥60% of unfed 1st zoeal (Z_1) larvae survived to the next stage, combined with the orange guts observed in larvae fed *Artemia*, suggesting Z_1 larvae are facultative lecithotrophic. Experiment 1 evaluated the suitability of ss-type rotifer *Brachionus rotundiformis* as prey. Z_1 larvae were fed rotifer at a density from 0 to 90 ind./ml. There was no significant difference in Z_1 survival among treatments (56.7–68.3%, $p > 0.05$); therefore, ss-type rotifer is considered an unsuitable prey for the larvae. Experiment 2 examined the suitability and optimal density of *Artemia* nauplii, and co-feeding copepod *Pavocalanus crassirostris* with *Artemia*, for larval rearing. The larvae fed 10 *Artemia*/ml had the highest survival to megalopae (91.3 ± 3.1%, $p < 0.05$). However, high mortality occurred in megalopae, resulting in poor survival to the 1st crab stage (1.3%–12.5%) in all treatments ($p > 0.05$). Meanwhile, co-feeding copepods with *Artemia* showed significantly inferior survival and development to megalopae when compared to that of 10 *Artemia*/ml treatment. Experiment 3 evaluated the effects of *Artemia* enrichment on larval performance. The results suggested that *Artemia* enrichment did not improve larval survival or development. Based on our results, *Artemia* nauplii fed at 10 ind./ml throughout larval development appears to be appropriate for *C. retusa*.

KEYWORDS
Artemia density, *Artemia* enrichment, copepod co-feeding, marine ornamental crab, rotifers

1 | INTRODUCTION

The global trade of marine ornamental organisms experienced rapid expansion over the past two decades (Bartley, 2000; Calado et al., 2003; Palmtag, 2017). According to recent estimations, over 46 million marine ornamental organisms with a value exceeding 300 million US dollars are traded annually (King, 2019; Palmtag, 2017). The continual expansion of the marine aquarium industry

stimulates the demands of various marine ornamental species, including crustaceans (Calado et al., 2003; Cohen et al., 2013). However, currently, it is estimated 90%–95% of marine ornamentals are wild-captured, with the majority sourced from tropical coral reefs (King, 2019; Olivotto et al., 2016; Wabnitz et al., 2003). Such heavy reliance on wild-collected specimens combined with ill-managed fisheries in some developing counties as the major suppliers is likely to have negative impacts on the biodiversity of the fragile coral reef ecosystem (Olivotto et al., 2016). Marine ornamental aquaculture could alleviate the collection pressure on the reefs and, therefore, has been considered as an option that could balance the economic benefit while offers sustainability to the marine ornamental industry (King, 2019; Murray & Watson, 2014). Unfortunately, compared with finfish, relatively limited attention has so far been paid to the development of larval culture techniques for marine ornamental decapods (Calado et al., 2003). Moreover, current research efforts on marine ornamental decapods captive breeding are biased towards ornamental shrimps. As for ornamental crabs, the deficiency of information on larval culture is clear since only two species (i.e. *Mithraculus forceps* and *Mithraculus sulptus*) have been studied in detail for their larval rearing (Penha-Lopes et al., 2005; Rhyne et al., 2005).

Establishing an effective larval feeding regime is fundamental to the development of captive breeding techniques for any aquaculture species (Daly et al., 2009; Ruscoe et al., 2004). The type of live prey and their suitable feeding density are often two key considerations in the establishment of such a feeding regime (Calado et al., 2008; Tsuji et al., 2015). Rotifers *Brachionus* spp. and *Artemia* are two traditional live prey routinely used in brachyuran crab larval rearing (Dan, Ashidate, et al., 2016; Holme et al., 2009; Oliver et al., 2017; Ruscoe et al., 2004; Støttrup & McEvoy, 2003; Zeng & Li, 1999). However, rotifers and *Artemia* are both known to lack highly unsaturated fatty acids (HUFA), which are crucial to marine larval growth and development (Olivotto et al., 2017; Støttrup & McEvoy, 2003; Suprayudi et al., 2004b). As the result, enrichment has been commonly practised to improve their nutritional values (Ferreira et al., 2008; Figueiredo et al., 2009; Støttrup & McEvoy, 2003). For example, *Artemia* enrichment was reported to significantly improve larval performance in various fish and crustacean species (Avella et al., 2007; Beder et al., 2018; Jinbo et al., 2013; Suprayudi et al., 2004a, 2004b). For crabs, *Artemia* enrichment was reported to significantly improve larval survival of mud crab *Scylla serrata* (Suprayudi et al., 2004b) and accelerated larval development of horsehair crab *Erimacrus isenbeckii* (Jinbo et al., 2013). Therefore, enriching *Artemia* is generally recommended in the hatchery practice for marine crabs (Figueiredo et al., 2009; Suprayudi et al., 2004b). However, there are also reports suggested fatty acid enrichment of *Artemia* did not significantly improve larval performance in other crab species, including edible species of common spider crab *Maja brachydactyla* (Andrés et al., 2007), blue swimmer crab *Portunus armatus* (Basford et al., 2021), mud crab *S. serrata* (Williams et al., 1999) and marine ornamental crabs *Mithraculus sculptus* and *Mithraculus forceps* (Rhyne et al., 2005). Furthermore, excess HUFA intake was identified as a cause induced moulting death syndrome (MDS) in larvae of portunid crabs, *Scylla paramamosain*, *P. trituberculatus* and *S. serrata* (Dan, Sui, et al., 2016; Hamasaki et al., 2002a, 2002b).

Copepods have received increasing attention as live feed for marine larval rearing in recent years (Støttrup & McEvoy, 2003). Compared with rotifers and *Artemia*, copepods are superior in nutritional value and digestibility (Ajiboye et al., 2011; Corner & O'Hara, 1986; Dhont et al., 2013; Støttrup & McEvoy, 2003); however, due to the difficulty and very low culture productivity of intensive culture of planktonic copepods (Alajmi & Zeng, 2014; Camus & Zeng, 2009), the utilization of copepods in the hatcheries is still limited (Drillet et al., 2011; van der Meeren et al., 2014). In fact, studies on copepod use in larval culture were mainly carried out for fish species, which generally obtained positive results (Barroso et al., 2013; Payne & Rippingale, 2000; Zeng et al., 2018). Similar studies on crustacean larvae are very few (Farhadian et al., 2009; Tang et al., 2020; Waiho et al., 2018). Among these limited studies, generally positive results were reported. For instance, the survival and growth of the black tiger shrimp *Penaeus monodon* postlarvae were significantly higher when co-fed cyclopoid copepod *Apocyclops dengizicus* with *Artemia* (Farhadian et al., 2009). Similarly, the megalopae of the Chinese mitten crab *Eriocheir sinensis* showed a significantly higher metamorphosis rate were fed wild-harvested frozen copepods with *Centropages dorsispinatus* as the dominant species (Tang et al., 2020).

The decorator crab *Camposcia retusa* is a popular marine ornamental crab species but has not been reported successfully bred previously (Calado et al., 2003). As the consequence, all individuals traded in the global aquarium market are currently sourced from the wild. The overall objective of the present study was to establish a reliable larval feeding regime for *C. retusa*. A series of experiments were conducted to investigate the suitability and appropriate feeding density of ss-type rotifer *Brachionus rotundiformis* and *Artemia* nauplii as prey for larval rearing of *C. retusa*, as well as the effects of copepod co-feeding with *Artemia* and *Artemia* enrichment on *C. retusa* larval performance.

2 | MATERIALS AND METHODS

2.1 | Broodstock maintenance

Decorator crab *C. retusa* broodstocks were purchased from Cairns Marine (Cairns Marine), a commercial marine ornamental collector and whole seller. After being air-freighted to James Cook University, Townsville, each pair of the broodstock was kept in 50-L tanks connected to a recirculation system (renewal rate at ca. 90 L/h). Half terracotta pots and rocks were placed in the tanks as shelters. All crabs were fed ad libitum with chopped prawns and mussels, as well as thawed blood worms twice daily. Throughout the experiments, the broodstock tanks were maintained at temperature 26–28°C, salinity 34–36, photoperiod L:D = 14 h:10 h, pH: 7.9–8.2, NH_4^+/NH_3 and $NO_2^- < 0.25$ ppm and $NO_3^- < 5.0$ ppm.

2.2 | Live prey culture and preparation

Three types of live prey were used in the present study: ss-type rotifer *Brachionus rotundiformis*, brine shrimp *Artemia* (INVE Thailand) and calanoid copepod *Pavocalanus crassirostris*.

The rotifer, *B. rotundiformis*, were cultured in 100-L conical tanks and fed a commercial concentrated microalgae *Nannochloropsis* (RotiGrow® Nanno, Reed Mariculture) daily. Rotifers were harvested daily with density estimated by averaging three 1-ml samples taken from the harvested rotifer stock. *Artemia* (INVE Thailand) was hatched daily. Newly hatched *Artemia* nauplii were collected the next morning for either feeding larvae immediately or subsequent enrichment. A commercial enrichment emulsion (Selco S.presso®, INVE Aquaculture) was used to enrich *Artemia*. The enrichment procedure followed the manufacturer's instruction, including the technical card on enrichment protocol (TC-EN-SPRESSO-190509) by the producer. Briefly, the S.presso was firstly emulsified in freshwater for 3 min; it was then added at a dosage of 0.5 g/L each time in two times with an *Artemia* density up to 400 nauplii per ml for enrichment. The enrichment lasted for a period between 18 and 22 h. *Artemia* density estimation was similarly done as for rotifers.

Copepods, *P. crassirostris*, were cultured in a series of 250 L tanks using the method established by this laboratory (Alajmi et al., 2015; Alajmi & Zeng, 2014). Briefly, the copepods were fed a 1:1 mixed algal diet of *Isochrysis* sp. and *Chaetoceros muelleri* and harvested daily during Experiment 2 to feed the larvae. When harvesting, the culture water was firstly drained through a 150-μm mesh and then a 25-μm mesh. The 150-μm mesh retained copepodites and adults, while the 25-μm mesh collected nauplii. Only the copepodites and adults were used for feeding crab larvae, while the nauplii collected were returned to the culture tanks. The copepod density estimation was similar to rotifers.

2.3 | Larval feeding experiments

2.3.1 | General procedures

Under the aforementioned broodstock maintenance condition, egg incubation took 21–25 days. Once larval hatching was noticed in the morning, the positively phototactic larvae were first attracted to a strong light source and then gently scooped out with a beaker before being allocated randomly to replicates of different treatments of an experiment. According to our previous study, the larval development of *C. retusa* consists of two zoeal and a megalopal stage (Xu et al., 2019).

For all experiments, larvae were reared in 600-ml glass beakers with seawater filled to 500 ml. Each beaker stocked 20 newly hatched larvae, which formed a replicate. Throughout all experiments, the water temperature was maintained at 27 ± 0.5°C, salinity 35.3 ± 0.5 and photoperiod set at L:D = 14 h:10 h. Daily 100% water exchange was conducted by moving live larvae using a broad-mouth pipette to a new beaker containing fresh seawater and identical feed. Meanwhile, larval mortality and development stages (based on larval stage description by the present authors; Xu et al., 2019) were recorded. Once larval developed into the megalopal stage, to prevent cannibalism, any newly metamorphosed megalopae found during daily water exchange were transferred to a separate beaker containing identical live prey for rearing. An experiment was terminated when all larvae had either metamorphosed into juvenile crabs or died.

2.3.2 | Experimental design

2.3.2.1 | Experiment 1: Rotifer feeding trial

Aimed at evaluating the suitability of ss-type rotifer *B. rotundiformis* as prey for *C. retusa* larval rearing, this experiment consisted of four triplicated treatments in which the newly hatched larvae (Z_1) were fed rotifers at 30, 60 and 90 ind./ml respectively.

2.3.2.2 | Experiment 2: Artemia *nauplii feeding density and copepod co-feeding trial*

As the results of Experiment 1 showed that rotifer is not a suitable prey for *C. retusa* larvae, this experiment was designed to test the suitability of *Artemia* nauplii as prey for *C. retusa* larvae and its appropriate feeding density; in addition, effects of co-feeding *Artemia* with copepods *P. crassirostris* were evaluated. The newly hatched larvae of *C. retusa* were subjected to five treatments: the unfed control, *Artemia* nauplii provided at three densities of 5, 10 and 15 *Artemia*/ml; and a co-feeding treatment of 5 *Artemia* + 5 copepods/ml. There were four replicates per treatment for this experiment.

2.3.2.3 | Experiment 3: Effects of Artemia *enrichment trial*

This experi-ment was to determine whether fatty acid enrichment of *Artemia* improved larval performance. In this experiment, five treatments were set up: unfed control; larvae fed either newly hatched *Artemia* nauplii or enriched metanauplii at 5 and 10 ind./ml respectively. Each treatment had four replicates.

2.4 | Data analysis

Data are expressed as mean ± standard error (SE). 'Larval surviving time (LST) of Z_1' and 'LST of Z_2' were calculated for all treatments of the rotifer feeding trial (Experiment 1) and the unfed controls of the other two experiments. LST of Z_1 and LST of Z_2 were defined as the average surviving time (days) from hatching to the death of all Z_1 or Z_2 larvae in a treatment respectively. LST of Z_1 (Z_2) was calculated using the formula:

$$\text{Larval surviving time (days) of } Z_1(Z_2) = \frac{\left(\sum_{t=1}^{n} t \times N_t\right)}{\sum_{t=1}^{n} N_t},$$

where N_t is the number of dead Z_1 (Z_2) larvae recorded on day t of an experiment; and n represents the days that dead Z_1 (Z_2) larvae were found.

Since the results of the current study showed that the Z_1 larvae of *C. retusa* are facultative lecithotrophic, the survival of Z_1 larvae and mean surviving time of unfed larvae can be used as an indicator for larval quality. To compare larval quality between three batches used in different experiments in this study, the Z_1 survival and mean surviving time of unfed larvae from these experiments were analysed using one-way ANOVA, followed by Tukey's HSD test.

A Kaplan–Meier test was run to compare the survival trend between the unfed control and different rotifer feeding density treatments in Experiment 1. Differences between survival curves were detected by a pairwise comparison using the log-rank test from the package 'survival' of the R language (Therneau, 2020).

Larval survival and average developmental duration of different treatments in Experiments 1 and 2 were analysed using one-way ANOVA followed by Tukey's HSD post hoc test. A two-way ANOVA was performed to analysis data of Experiment 3. The normality of the data and homogeneity of variance were tested prior to the ANOVA analysis. In addition, to compare differences of Z_1 survival between the unfed control and each feeding treatment in Experiment 3, a general linear model was applied. The percentage survival data were transformed using arcsine square root transformation to meet the assumptions of the analysis methods. For all experiments, unfed control was not included for statistical analysis from Z_2 onwards due to no larvae of the controls survived beyond Z_2. A statistical probability of $p < 0.05$ was accepted as significant. All statistics were performed using R language (R 4.0.3; R Core Team, 2020).

3 | RESULTS

3.1 | Facultative lecithotrophic of newly hatched larvae and larval batch quality comparison

Survival of newly hatched Zoea 1 (Z_1) larvae to the next stage (Zoea 2 or Z_2) in the unfed controls of all three experiments was high (≥60%), confirming lecithotrophic of the larvae. When larval survival of the unfed controls from the three experiments is compared, Experiment 2 (85.5 ± 2.9%) had significantly higher survival than the other two experiments (65.0 ± 5.0% and 60.0 ± 2.0% for Experiment 1 and 3 respectively; Table 1). In addition, although 'larval surviving time' (LST) of Z_1 larvae from the unfed controls of the three experiments was similar (3.2–3.5 days, Table 1, $p = 0.771$), LST of Z_2 larvae of the Experiment 2 control was the longest (4.6 ± 0.3 days) and significantly longer than that of Experiment 3 control (3.8 ± 0.1 days, $p = 0.036$). The mean Z_1 duration of the larvae successfully moulted to Z_2 in the three experiments ranged from 1.5 to 1.8 days, and the larvae of Experiment 2 again was the fastest in their development (1.5 ± 0.1 days) although no significant difference was detected among three batches of larvae (Table 1, $p = 0.483$).

3.2 | Experiment 1: Rotifer feeding trial

The daily survival of *C. retusa* larvae of different rotifer density treatments showed a similar trend, and no significant difference was detected among them (Figure 1, Kaplan–Meier test, $p = 0.193$). Larval survival to the Z_2 was relatively high (65.0%–68.3%) in all treatments, including the unfed control, and no significant difference was detected between treatments (Table 2, $p = 0.502$). Likewise, Z_1 duration was similar between treatments (Table 2, $p = 0.058$). Despite high larval survival to Z_2, Z_2 larvae suffered a total mortality in all treatments. The LST of the Z_2 larvae ranged from 3.7 to 4.4 days from different treatments, but no significant difference was detected between any of them (Table 2, $p = 0.244$).

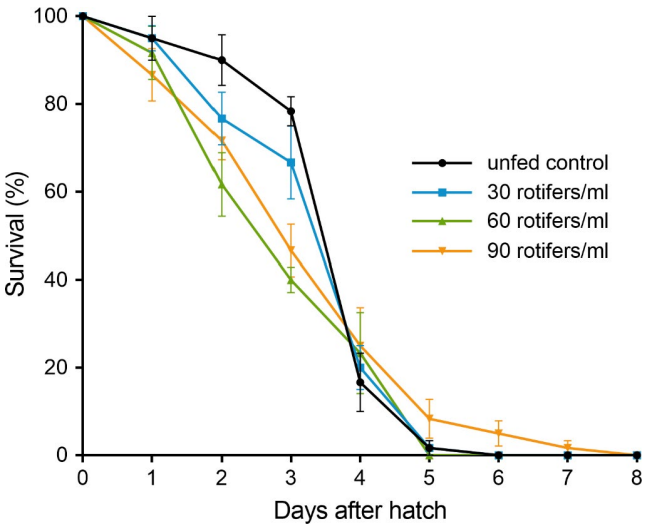

FIGURE 1 Daily larval survival of decorator crab *Camposcia retusa* fed different densities of rotifers. The error bars indicate standard errors

	Mean surviving time of unfed larvae (days)			
	Zoea 1	Zoea 2	Survival to Zoea 2	Zoea 1 stage duration (days)
Exp. 1	3.3 ± 0.4	4.1 ± 0.2^{ab}	65.0 ± 5.0^{b}	1.8 ± 0.2
Exp. 2	3.5 ± 0.3	4.6 ± 0.3^{a}	85.5 ± 2.9^{a}	1.5 ± 0.1
Exp. 3	3.2 ± 0.3	3.8 ± 0.1^{b}	60.0 ± 2.0^{b}	1.7 ± 0.2

Note: Data are presented as mean ± standard error. Different subscript letters within a same column indicate significant differences ($p < 0.05$).

TABLE 1 Comparison of quality of different batches of newly hatched larvae of *Camposcia retusa* from three experiments

TABLE 2 Survival and development of *Camposcia retusa* larvae fed rotifers at different densities

Rotifer density (ind./ml)	0	30	60	90
Survival to Zoea 2 (%)	65.0 ± 5.0	68.3 ± 8.3	56.7 ± 1.7	58.3 ± 6.7
Zoea 1 duration (days)	1.8 ± 0.2	1.7 ± 0.2	1.8 ± 0.2	1.9 ± 0.1
Survival to megalopa (%)	0	0	0	0
Mean surviving time of Zoea 2 (days)*	4.1 ± 0.2	3.9 ± 0.2	3.7 ± 0.2	4.4 ± 0.3

Note: Data are presented as mean ± standard error.

*These surviving times are from hatching to die as Zoea 2 larvae.

3.3 | Experiment 2: *Artemia* nauplii feeding density and copepod co-feeding trial

Daily larval survival was similar across all treatments during the first 2 days; however, survival of the unfed control declined rapidly from day 3 onwards with a total mortality on day 7, while larval survival of all fed treatments was higher than 20% with the highest survival of 60.0% found the 10 *Artemia*/ml feeding treatment (Figure 2). Indeed, between day 5 and 10, larvae survival of the 10 *Artemia*/ml treatment was consistently significantly higher than other treatments ($p < 0.05$). However, from day 10 onwards, larval mortality of the10 *Artemia*/ml treatment also increased sharply, which eventually declined to a level only marginally higher than other treatments on day 12 when the experiment was terminated ($p > 0.05$; Figure 2 and Table 3).

When survival is evaluated as the percentage of larvae successful moulted to the next stages, the survival of Z_1 larvae to Z_2 stage was very high (>85.5%) across all treatments with the highest survival of 93.8 ± 2.4% found in the treatment that *Artemia* nauplii were given at 10 *Artemia*/ml, but no significant difference was detected between any treatments, including the unfed control (Table 3). Z_2 survival to the subsequent megalopal stage was also high for all treatments (>70%) except the unfed control, which suffered a total mortality. Among fed treatments, Z_2 survival of the 10 and 15 *Artemia*/ml feeding treatments was significantly higher than those of the 5 *Artemia*/ml and 5 *Artemia* + 5 copepod/ml treatments ($p < 0.05$; Table 3).

In terms of overall zoeal survival, it was also high (>60%) across all fed treatments, and the highest survival achieved was again from the 10 *Artemia*/ml feeding treatment (91.3 ± 2.7%), which was significantly higher than all other treatments (Table 3). However, despite high zoeal survival, survival of megalopae was poor across all treatments (<20%), resulting in low overall larval survival to the first crab stage (C_1). The best larval survival from Z_1 to C_1 was from the 10 *Artemia*/ml treatment (11.3 ± 3.8%), which was not significantly different from that of 15 *Artemia*/ml (7.5 ± 4.8%) and 5 *Artemia* + 5 copepods/ml (3.8 ± 2.4%) co-feeding treatment, but significantly higher than 5 *Artemia*/ml treatment (1.3 ± 1.3%). Throughout the experiment, no significant difference was found in larval survival between the 5 *Artemia*/ml and 5 *Artemia* + 5 copepods/ml treatments (Table 3).

In terms of larval development, Z_1 duration was not significantly different among all treatments (Table 3). However, for the overall zoeal development (Z_1 to M), the two higher *Artemia* feeding density treatments (10 and 15 *Artemia*/ml) were significantly shorter when compared to 5 *Artemia*/ml and 5 *Artemia* + 5 copepod/ml treatments ($p < 0.05$; Table 3). For the overall larval development duration (Z_1 to C_1), due to only a single larva survival to C_1 in the 5 *Artemia*/ml treatment, the treatment was excluded from the statistical analysis. Of the remaining three fed treatments, the overall larval duration was again significantly shorter in the 10 and 15 *Artemia*/ml treatments when compared to that of 5 *Artemia* + 5 copepod/ml treatment (Table 3).

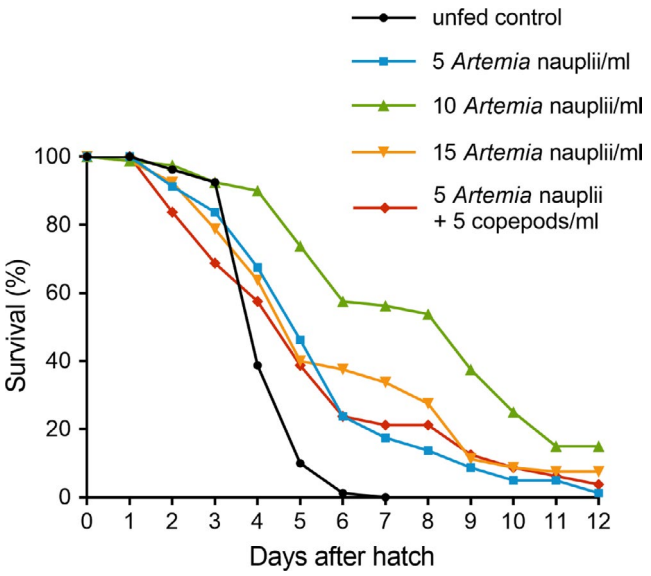

FIGURE 2 Daily larval survival of decorator crab *Camposcia retusa* fed different densities of *Artemia* nauplii and co-fed *Artemia* nauplii with copepod *Pavocalanus crassirostris*. For clarity of the figure, the standard error bars are omitted

3.4 | Experiment 3: Effects of *Artemia* enrichment trial

For the first 3 days, daily larval survival showed a very similar pattern among all treatments; however, similar to the previous experiments, survival in the unfed control plummeted and a 100% mortality occurred on day 6 (Figure 3). Of the fed treatments, between day 4 and 6, larval survival dropped sharply in all treatments except the

TABLE 3 Survival and development of *Camposcia retusa* larvae fed different densities of *Artemia* nauplii and co-fed *Artemia* nauplii with copepod *Pavocalanus crassirostris*

Treatment		Unfed	5 *Artemia* nauplii/ml	10 *Artemia* nauplii/ml	15 *Artemia* nauplii/ml	5 *Artemia* nauplii + 5 copepods/ml
Survival (%)	Z_1 to Z_2	85.5 ± 2.9	88.8 ± 1.3	93.8 ± 2.4	87.5 ± 1.4	87.5 ± 3.2
	Z_2 to M	0	71.8 ± 2.3^{a}	97.4 ± 2.6^{b}	85.9 ± 4.9^{b}	70.2 ± 2.5^{a}
	Z_1 to M	0	63.8 ± 2.4^{bc}	91.3 ± 3.1^{a}	75.0 ± 3.5^{b}	61.3 ± 1.3^{c}
	M to C_1	–	2.1 ± 2.1^{a}	12.7 ± 4.5^{b}	10.1 ± 6.8^{ab}	5.9 ± 3.7^{ab}
	Z_1 to C_1	0	1.3 ± 1.3^{a}	11.3 ± 3.8^{b}	7.5 ± 4.8^{ab}	3.8 ± 2.4^{ab}
Development duration (days)	Z_1 to Z_2	1.5 ± 0.2	1.2 ± 0.0	1.4 ± 0.1	1.3 ± 0.0	1.3 ± 0.1
	Z_1 to M	–	4.3 ± 0.1^{a}	3.8 ± 0.1^{b}	3.5 ± 0.1^{b}	4.7 ± 0.1^{c}
	Z_1 to C_1	–	11*	8.8 ± 0.3^{a}	8.3 ± 0.3^{a}	10.3 ± 0.3^{b}

Note: Data are presented as mean ± standard error. Z_1: first stage zoea; Z_2: second stage zoea; M: megalopa; C_1: first stage juvenile. Means with different superscript letters within a same row indicate significant differences (one-way ANOVA; $p < 0.05$).

*Statistical analysis did not include 5 *Artemia*/ml treatment due to only one larva survived to C_1 of the treatment.

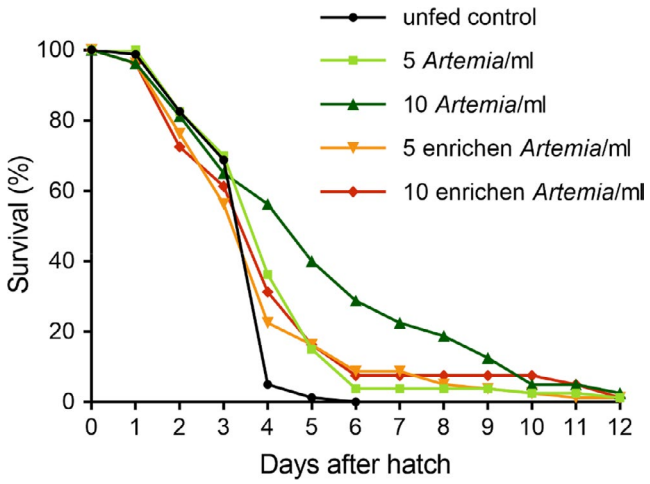

FIGURE 3 Daily larval survival of decorator crab *Camposcia retusa* fed *Artemia* at two densities (5 and 10 ind./ml) in the forms of unenriched nauplii and enriched metanauplii respectively. For clarity of the figure, the standard error bars are omitted

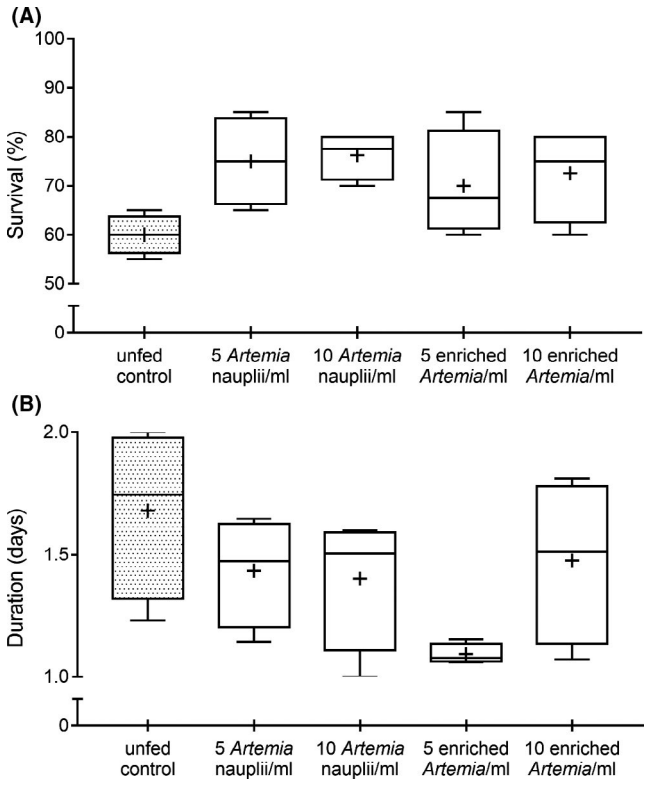

FIGURE 4 Survival (A) and development duration (B) of the first stage zoeal larvae of *Camposcia retusa* from the unfed control and the treatments in which larvae were fed *Artemia* at two densities (5 and 10 ind./ml) in the forms of unenriched nauplii and enriched metanauplii respectively. Data are presented as general linear model: A line within the box marks the median value; a cross within the box marks the mean value, the box spans the 25% and 75% while the bar represents the minimum and maximum value of a treatment. No significant difference was detected between unfed control and feeding treatments

treatment of *Artemia* nauplii fed at 10 ind./ml, resulting in significantly higher larval survival of the treatment than all other treatments between day 4 and 9 ($p < 0.05$). However, larval mortality of the treatment increased subsequently, and at the end of the experiment, larval survival was not significantly different among all fed treatments (Figure 3).

In terms of larval survival based on instar, Z_1 survival of the unfed control was the lowest but was not significantly different from all *Artemia* feeding treatments (Figure 4A). Similar to the result in Experiment 2, among fed treatments, the best survival to Z_2 was found in the treatments in which larvae were fed *Artemia* at 10 ind./ml, either in the form of unenriched nauplii (76.3 ± 2.4%) or enriched metanauplii (72.5 ± 4.8%; Figure 5A), although the difference was not significant compared with lower *Artemia* feeding density treatments at 5 ind./ml ($F_{1,12} = 0.179$; $p = 0.680$). However, from Z_2 onward, the differences in larval survival became significant: highly significant effects of both *Artemia* density and enrichment on Z_2 survival were detected ($p < 0.01$) although no significant interaction of the two factors was found (Table 4). The unenriched *Artemia* nauplii fed at 10 ind./ml yielded both the highest Z_2 survival (65.1 ± 6.3%) and

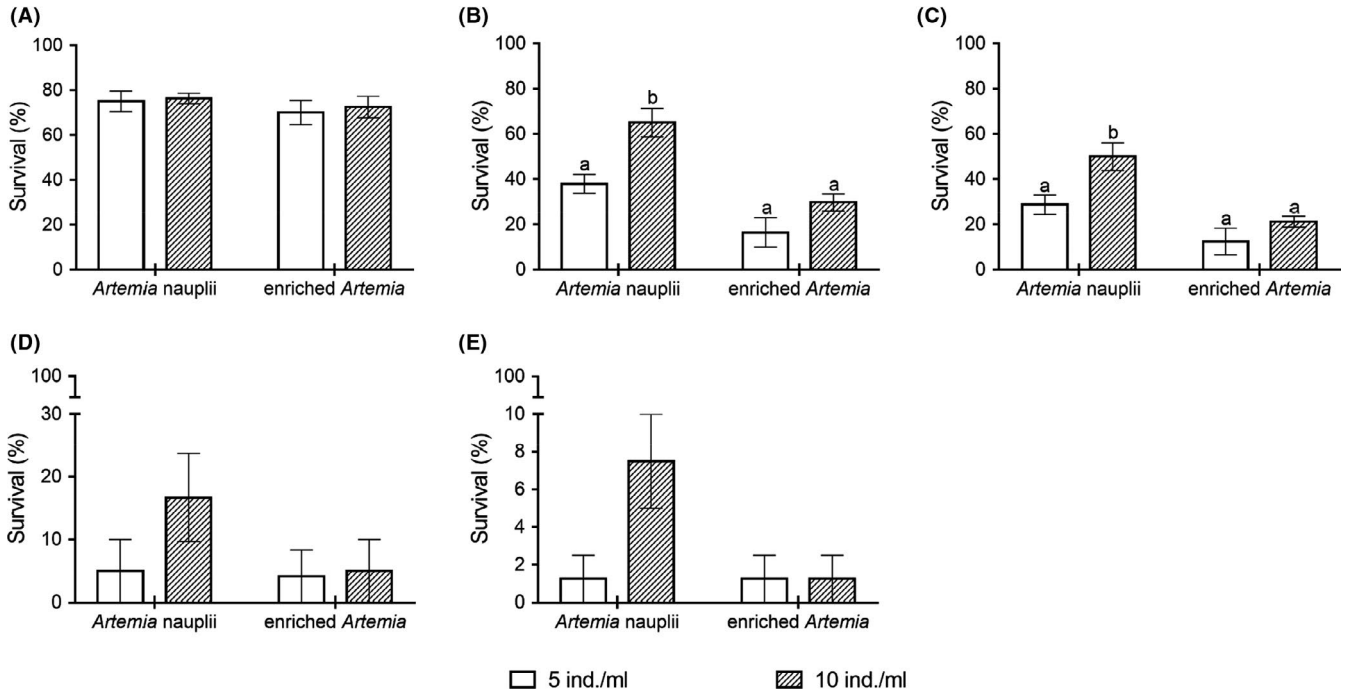

FIGURE 5 Effects of the *Artemia* enrichment on the survival of *Camposcia retusa* larvae. (A) From Zoea 1 (Z_1) to Zoea 2 (Z_2); (B) from Z_2 to megalopal stage (M); (C) from Z_1 to M; (D) from M to 1st crab stage (C_1); (E) from Z_1 to C_1. Different letters on the tops of bars indicate significant differences (two-way ANOVA; $p < 0.05$)

TABLE 4 Results of two-way ANOVA on the survival and development of *Camposcia retusa* larvae fed at two densities of *Artemia* (5 and 10 ind./ml) in the forms of unenriched nauplii and enriched metanauplii respectively

Treatment		A: enrichment			B: *Artemia* density			A × B			Error
Source of variation		df	*F*	*p*	df	*F*	*p*	df	*F*	*p*	df
Survival (%)	Z_1 to Z_2	1	0.974	0.343	1	0.179	0.680	1	0.202	0.890	12
	Z_2 to M	1	28.323	<0.001	1	14.346	0.003	1	1.711	0.215	12
	Z_1 to M	1	20.903	0.001	1	9.290	0.010	1	1.613	0.228	12
	M to C_1	1	1.348	0.268	1	1.348	0.268	1	1.013	0.334	12
	Z_1 to C_1	1	3.953	0.070	1	3.953	0.070	1	3.953	0.070	12
Duration (days)	Z_1 to Z_2	1	1.189	0.297	1	2.048	0.178	1	2.860	0.117	12
	Z_1 to M	1	0.024	0.880	1	0.029	0.869	1	0.028	0.869	12

Note: Z_1: first stage zoea; Z_2: second stage zoea; M: megalopa; C_1: 1st juvenile crab. Significant differences (two-way ANOVA: $p < 0.05$) are underlined. ANOVA was not performed for the development duration from Z_1 to C_1 due to insufficient data collected as the result of low survival in several treatments.

the overall zoeal survival (i.e. Z_1 to megalopae; 50.0 ± 6.1%), and both survivals were significantly higher than all other treatments (Figure 5B,C). However, similar to Experiment 2, high mortality occurred during megalopal stage in all treatments, resulting in the best survival to C_1 at 7.5 ± 2.5% from larvae fed 10 ind./ml unenriched *Artemia* treatment; two-way ANOVA detected no significant difference in overall larval survival from Z_1 to C_1 related to either *Artemia* density, enrichment or their interaction (Table 4).

In terms of larval development, two-way ANOVA detected no significant effect of either *Artemia* density or enrichment, nor the interaction of the two factors on Z_1 and Z_2 development durations ($p > 0.05$; Table 4; Figures 5B and 6A,B). For overall larval development from Z_1 to C_1 (Figure 6C), no statistics were performed due to only 1 datum was obtained in several treatments as the result of low survival.

4 | DISCUSSION

The results of the three larval experiments showed that when not fed at all, newly hatched Z_1 larvae of *C. retusa* could still obtain a survival of between 60.0% and 83.8% to the Z_2 stage, confirming

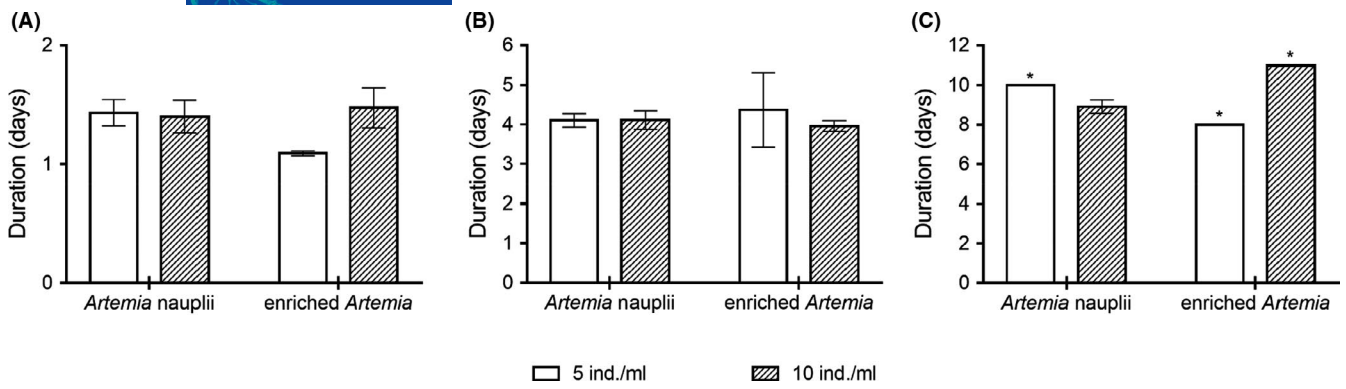

FIGURE 6 Effects of the *Artemia* enrichment and feeding density on the larval development of *Camposcia retusa*. (A) Zoea 1 (Z_1) duration; (B) Zoeal duration (Z_1 to megalopal stage); (C) Overall larval duration (Z_1 to 1st crab stage). *Only 1 datum obtained from each of these treatments

that the Z_1 larvae are lecithotrophic. There are in fact two types of lecithotrophic, obligatory and facultative lecithotrophy. Obligatory lecithotrophy is defined as larvae are unable to feed and solely rely on yolk reserve to develop into the next stage, while facultative lecithotrophy is defined as larvae are capable of successful development to the next stage when food is absent, but will feed if food is available (Anger, 2001; Zeng et al., 2020). Based on such definitions, *C. retusa* Z_1 larvae should be considered as facultative lecithotrophy because in both Experiment 2 and 3, it was noticed that in all *Artemia* feeding treatments, the guts of Z_1 larvae turned orange after a while. Additionally, for both Experiments, Z_1 survival of the unfed control was always lower than any *Artemia* feeding treatments. Furthermore, for larval stages that have been confirmed as primary obligatory lecithotrophy from various decapod species, including *Macrobrachium acanthurus* (Rocha et al., 2017), *Macrobrachium jelskii* (Rocha et al., 2016), *Lithodes santotta* (McLaughlin et al., 2001), *Sesarma windsor* (González-Gordillo et al., 2010) and *Metopaulias depressus* (González-Gordillo et al., 2010), the mouthparts have been reported as rudimentary, that is, with simplified mandible, rudimentary maxillule and setal-reduced maxilla. On the contrary, the mouthparts of Z_1 larvae of *C. retusa* were much better developed (Xu et al., 2019, Figure 1D–F), enabling them to feed if prey is available.

Facultative lecithotrophy is generally considered as an adaptive mechanism evolved to cope with low or unpredictable food availability in the environment where larval development takes place (Anger, 2001; Tapella et al., 2012; Zeng et al., 2020). It is hence more commonly found in environments characterized with poor or unpredictable food availability, such as high-latitude marine waters or freshwater (Anger, 2001). The current finding that *C. retusa* Z_1 larvae are facultative lecithotrophy is therefore interesting, since *C. retusa* is considered a tropical species (WoRMS, 2021). Nevertheless, even environments that are normally considered as high productivity may also experience temporal or spatial variations in plankton availability, leading to a transitory or localized food limitation (Constable et al., 2003; Mackas et al., 1985). A development mode with a high level of flexibility, such as facultative lecithotrophy, clearly should enhance the chance of larval survival in the nature (Anger, 2001; Giménez & Anger, 2005).

By comparing survival to Z_2 of unfed newly hatched larvae, and average surviving time of deceased Z_1 larvae among three batches of larvae used in different experiments, significant differences in both parameters were confirmed. Since the larvae from the unfed controls of the three experiments were subjected to nearly identical rearing conditions, the results indicate significant variations in larval quality of the batches. Larval quality variation among different batches is a well-known phenomenon in aquaculture, which can significantly affect larval rearing outcomes (Calado & Leal, 2015; Mann et al., 1999). The current results suggested that larvae used for Experiment 2 were likely of better quality than the other two batches used in Experiment 1 and 3, and the larvae used in Experiment 3 are probably of the lowest quality of the three. The difference in larval quality between batches is further confirmed by the major difference in larval survival when those larvae reared under a same feeding regime in two experiments are compared. For example, when *C. retusa* larvae fed *Artemia* nauplii at 10 ind./ml, survivorship to megalopa reached 91.3 ± 3.1% in Experiment 2, but it was only 50.0 ± 6.1% in Experiment 3 (Table 3 and Figure 4).

While as the result of facultative lecithotrophy, high percentages (>60%) of Z_1 larvae in the unfed controls of all three experiments successfully moulted to Z_2, none of them survived to the next stage as megalopa. This result showed that from the Z_2 onwards, food availability, as well as their quality and quantity become crucial to the survival of the larvae. In Experiment 1, larvae were fed rotifers at a density ranging from 30 to 90 ind./ml; none of the Z_2 larvae in any of the treatments survived to megalopae, with total mortality occurred within 8 days regardless of rotifer feeding density. In addition, Z_1 survival of the fed treatments (56.7%–68.3%) and the unfed control (65.0%) was very similar (Table 2). Despite rotifers have been shown to be an appropriate diet for newly hatched larvae of a range of portunid and spider crabs (Baylon, 2009; Kogane et al., 2007; Nghia et al., 2007; Suprayudi et al., 2002; Zmora et al., 2005), our results suggest that ss-type rotifers are not a suitable diet for *C. retusa* larvae, which likely due to too small size of the rotifers. In this study, the super-small type or ss-type rotifer *Brachionus rotundiformis* was used, which has a size range of 90–160 μm; such a size range is likely

too small for the *C. retusa* larvae to either capture or despite being consumed, energy budget was poor so that could not sustain successful development to the next stage. Similarly, in southern rock lobster, *Jasus edwardsii*, phyllosoma larvae showed prolonged development when live prey was too small (Ritar et al., 2003). Indeed, the relationship between prey size and larvae is a critical factor to consider in larval feeding, the disproportion of the size between food particles and larval feeding appendages may incapacitate the larvae to effectively capture the prey or despite larvae can capture and consumed the prey, they would eventually die due to unable to acquire a sufficient energy to sustain normal development (Guarizo et al., 2020; Ritar et al., 2003; Ruscoe et al., 2004; Sui et al., 2008).

Since copepods are known to have superior nutritional value than rotifers (Støttrup & McEvoy, 2003), a treatment using copepods co-fed with *Artemia* nauplii was tested in Experiment 2. Although copepods have been reported to be superior to rotifers and *Artemia* for fish hatchery (Barroso et al., 2013; Payne & Rippingale, 2000; Zeng et al., 2018), few previous research reported using copepods as sole prey for decapod larval culture, probably due to their very low culture productivity (i.e. typically <10 ind./ml for planktonic copepods vs >1000 ind./ml for ss-rotifers). On the contrary, co-feeding copepods with other traditional prey in decapod larval rearing have been trailed and reportedly enhanced survival and growth in several decapod species, including mud crab *Scylla olivacea* (Jantrarotai et al., 2004), Chinese mitten crab *E. sinensis* (Tang et al., 2020) and black tiger prawn *Penaeus monodon* (Farhadian et al., 2009). However, in the present study, co-feeding 5 copepods/ml with 5 *Artemia*/ml not only did not produce any clear beneficial effects, but both larval survival and development were significantly inferior when compared to those fed 10 *Artemia*/ml. In fact, larval survival and development of the copepod co-feeding treatment were similar to the treatment that *Artemia* nauplii were fed at 5 *Artemia*/ml (Table 3), suggesting that copepod *P. crassirostris* feeding hardly offered any additional benefits to *C. retusa* larvae. There were two possible explanations for the ineffectiveness of copepod co-feeding, firstly the length of copepodite and adult *P. crassirostris* ranging between 195 and 445 μm (Alajmi et al., 2015); thus, most of them are smaller than *Artemia* nauplii (400–500 μm). As mentioned previously, the disproportion of the size of the prey and the mouthpart of larvae may render it difficult for the larvae to capture the prey (Guarizo et al., 2020). Secondly, the fast and zigzag spurting swimming behaviour characterized by calanoid copepods may make it difficult for the larvae to successfully capture and consume them (Lumasag et al., 2007).

In contrast to rotifers and copepods, the results of Experiment 2 showed that in all treatments in which *Artemia* nauplii were fed to the larvae, larval survival to megalopae was higher than 60%. Thus, it proved that *Artemia* is an appropriate prey for zoeal larval rearing of *C. retusa*. In particular, larvae fed *Artemia* nauplii at 10 *Artemia*/ml have significantly better survival and development to the megalopae than those fed at a lower density of 5 *Artemia*/ml, suggesting *Artemia* feeding density is also important, which can be explained by the fact that decapod larvae are considered 'inactive predators' and their food consumption often relied on the chance of encounters with the prey (Jeffs & O'Rorke, 2020; Tsuji et al., 2015; Zeng & Li, 1999). Indeed, previous studies have shown a positive relationship between ingestion rate and prey density until a saturation level is reached in various decapod larvae, including crabs *E. sinensis* (Sui et al., 2009), *Hyas araneus* (Anger & Dietrich, 1984), *Ranina ranina* (Minagawa & Murano, 1993) and shrimps *Lysmata wurdemanni* (Zhang et al., 1998), *Penaeus kerathurus* (Yúfera et al., 1984) and *Penaeus monodon* (Loya-Javellana, 1989). The importance of prey density is probably particularly relevant to early larvae who typically have weak swimming ability (Lumasag et al., 2007). Interestingly, in the same experiment, as *Artemia* density further increased to 15 ind./ml, larval survival decreased significantly. This probably can be explained by excess number of *Artemia* degraded water quality (Sui et al., 2009; Zhang et al., 1998). Dan, Ashidate, et al. (2016) also reported that an excessive high *Artemia* density could induce morphology abnormality in larvae of the last stage zoeal larvae of swimming crab *Portunus trituberculatus*, leading to mortalities during metamorphose. Therefore, based on the results of the current study, *Artemia* feeding density of 10 *Artemia*/ml appears to be most appropriate for *C. retusa* larvae.

Although larval survival to megalopae was relatively high in Experiment 2, mass mortality still occurred during the megalopal stage. In particular, for the 10 *Artemia*/ml treatment that had maintained a relatively high zoeal survival, mortality increased sharply during the period when megalopae metamorphosed to the first crabs (i.e. days 8–10; Figure 2). Since malnutrition could be a major cause of mass mortality of megalopae observed, Experiment 3 was designed and conducted to test whether *Artemia* enrichment may improve megalopal survival. *Artemia* enrichment has been reported to enhance larval survival and development of various decapod species (Dan, Oshiro, et al., 2016; Dan, Sui, et al., 2016; Suprayudi et al., 2004b). For example, in swimming crab *P. trituberculatus*, it was shown that larvae fed microalgae *Nannochloropsis* enriched *Artemia* achieved substantially higher megalopal survival (40.7%) than those fed unenriched *Artemia* (1.9%–3.5%; Dan, Oshiro, et al., 2016). Likewise, mud crab *S. serrata* larvae fed unenriched *Artemia* were reported to exhibit reduced survival and prolonged development to C_1 stage (Suprayudi et al., 2004b). However, in the present study, *Artemia* enrichment not only failed to improve megalopal survival and development, but led to significant lower zoeal survival when compared to the same density treatments in which larvae were fed unenriched *Artemia* nauplii. In fact, zoeal survival almost halved when larvae were fed enriched *Artemia* as compared to those fed unenriched nauplii at both density of 5 and 10 ind./ml. Therefore, it appears that *C. retusa* larvae should be reared on unenriched *Artemia* nauplii instead of enriched *Artemia*. Interestingly, a recent paper by Basford et al. (2021) also reported *Artemia* enriched with the same medium used in this study (Selco S.presso) did not improve zoeal survival and development of the blue swimmer crab *Portunus armatus*. Another paper from the same primary author reported a clear effect of HUFA enrichment using S.presso with fatty acid analysis (Basford et al., 2020), which showed that DHA content increased from 1.76 mg/g lipid in unenriched *Artemia* to 71.10 mg/g lipid in enriched *Artemia*, and similarly, EPA content increased from 13.32

to 29.76 mg/g lipid after enrichment. In fact, an earlier study also indicated that *Artemia* enriched with Frippak booster (a commercial microencapsulate enrichment diet, Frippak, England) did not significantly improve megalopal survival of *S. serrata* (Williams et al., 1999). In the cases of marine ornamental crabs, Rhyne et al. (2005) also reported that *Artemia* enrichment failed to improve larval survival, growth and development of *M. sculptus* and *M. forceps*.

It is worth noting that in this study, *Artemia* enrichment actually led to significant inferior zoeal survival, which was not reported by previous studies. It has been reported that in *S. serrata*, excessive dietary DHA (docosahexaenoic acid) accelerated morphogenesis of the last zoeal stage larvae, leading to the development of abnormal enlarged chelipeds inside the exoskeleton that obstructed the subsequent metamorphosis moult, resulted in mass mortality during the metamorphose (Dan & Hamasaki, 2011; Hamasaki et al., 2002a, 2002b). In this study, *Artemia* was enriched with Selco S.presso, a commonly used oil emulsion that had a high DHA/EPA (eicosapentaenoic acid) ratio (>7); hence, it is possible that the inferior zoeal survival of larvae fed enriched *Artemia* may relate to accelerated morphogenesis occurred in Z_2 larvae. Alternatively, the lower larval survival might be linked to relatively lower protein/energy content of enriched *Artemia* metanauplii as compared to nauplii. The newly hatched *Artemia* nauplii are lecithotrophic; therefore, prior to their development to the next metanaupliar stage when filter-feeding starts, which also enable enrichment, the nutrition/energy content of the nauplii is continuously utilized for maintenance and, therefore, will reduce substantially when they moult to metanuaplii (Navarro et al., 1999). Indeed, for the GSL *Artemia* strain used in this study, it was reported that metanauplii had a 34% reduction in individual dry weight and a 37% reduction in energy content compared with newly hatched nauplii (Vanhaecke et al., 1983). Since commonly used oil emulsions are aimed at fatty acid enrichment, they often do not supplement with proteins or other nutrients. In fact, it has been reported that enriching GSL *Artemia* with an oil emulsion (DHA Selco, INVE) similar to the one used in this study led to a 19% reduction in proteins compared with newly hatched nauplii (Evjemo et al., 2001). Proteins are known as a key nutrient for crustaceans, especially during larval development when rapid tissue synthesis occurs (Anderson & De Silva, 2011; Anger, 2001). Hence, it is possible that in the present study, when *Artemia* metanauplii were enriched with S.presso, which is known to be rich in lipids but with a limited protein content, an unbalanced protein/lipid ratio may result, which led to inferior survival and development in those zoeal larvae fed the enriched *Artemia*.

Multiple factors have been reported to cause mass mortality of megalopae during larval rearing of various crab species, which include moult death syndrome, cannibalism, lack of appropriate settlement cues and substrates, and inappropriate physical culture environment (Beder et al., 2018; Dan, Ashidate, et al., 2016; Dan, Sui, et al., 2016; Hamasaki et al., 2002b, 2007; Jinbo et al., 2013). For example, in this study, it was observed that up to 51% of dead megalopae missing limbs or body parts, suggesting cannibalism may play a major role in the megalopal mortality. Hence, steps taken to reduce cannibalism during megalopae rearing, such as reduced stocking density and providing shelters, may help improve megalopae survival. Additionally, the inappropriate cultural environment, such as the lack of suitable substrate and settlement cues, could also negatively affect megalopae metamorphosis (Forward et al., 2001), which has been confirmed by further studies (Xu, 2021).

5 | CONCLUSION

Through a series of three experiments, this study investigated suitable larval feeding regime for captive breeding of decorator crab *C. retusa*, a popular marine ornamental crustacean. This is the first report on successful rearing of the species from newly hatched larvae to the first juvenile stage, and survival up to 91.3% to megalopal stage and 11.3% to the first juvenile stage, was achieved respectively. The results suggested that newly hatched *C. retusa* Z_1 larvae are facultative lecithotrophic, who are capable of developing to the next stage independent of food but can also perform exogenous feeding when food is available. It also reveals that unlike many crab species, ss-type rotifer is not an appropriate diet for *C. retusa* zoeal larvae as it led to total mortality at Z_2 stage. In contrast, *Artemia* nauplii were found to be a good diet for the larvae while the feeding density of *Artemia* is also important. An *Artemia* feeding density of 10 ind./ml was identified as performing the best. Meanwhile, both co-feeding *Artemia* with copepods and *Artemia* enrichment were not found to improve larval survival and development, therefore, unnecessary.

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CONFLICT OF INTEREST

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.

AUTHOR CONTRIBUTIONS

Experiment design, conducting experiments, data collection and analysis, writing manuscript: Tian Xu. Supervision, experiment design, providing experimental materials, and revising manuscript: Chaoshu Zeng.

DATA AVAILABILITY STATEMENT

All relevant data are within the paper.

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