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Development of an artificial propagation and breeding method for the beehorn snail (*Clithon diadema*)

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ABSTRACT

Beehorn snail (*Clithon diadema*), known for its vibrant colors, is threatened with extinction due to excessive field collection. Therefore, this study investigated the effects of different diets and salinity (0‰–35‰) on the gonadal development, capsule productivity, embryonic development, and larval survival and growth of *C. diadema*. Adult snails (shell length: 9.09 ± 0.87 mm) were fed diatoms, including pennate marine diatom (*Nitzschia grossestriata*), boat-shaped diatom algae (*Navicula sp.*) and *Cylindrotheca sp.*, along with giant tiger prawn (*Penaeus monodon*) commercial feed, sea lettuce (*Ulva lactuca*), or *Cladophora fascicularis*, while snail larvae (shell length: 154.85 ± 6.03 μm) were fed *Isochrysis galbana* or *Chaetoceros muelleri*. The diatom group outperformed commercial feed and *U. lactuca* groups. The highest capsule productivity, yielding 79–82 capsules in 4 weeks, occurred at 15‰-20‰ salinity for diatom-fed adults. Embryos thrived at 0‰ but didn't hatch; hatching required salinities above 0‰, highlighting saline necessity. In addition, acute salinity changes (0‰ to 30‰) can deform embryo. Embryonic development at 15‰–20‰ salinity lasted 13.56–14.33 days with 100% larval hatching. However, larvae hatched at >20‰ salinity exhibit improved the percentage of survival without feeding (longer median lethal times). *I. galbana*-fed larvae survived 68 days with a 75% rate, outlasting *C. muelleri*-fed larvae which perished within 28 days. *I. galbana*-fed larvae, raised for 68 days at 25‰ salinity, showed 100% survival, 44.44% metamorphosis, and a 3.1 times growth increase. Metamorphosis rates were highest at 25‰ salinity with *I. galbana*, exceeding the 20‰ group by 3.2 times. Mucus of *C. diadema* boosts larval metamorphosis to 50.00%, 9 times higher than without inducers. Based on previous results, we developed an artificial propagation and breeding method for *C. diadema*. Artificially propagating *C. diadema* in large quantities could reduce the need for wild harvesting, thereby mitigating negative ecological impacts from excessive collection.

1. Introduction

The Neritidae family (neritids or nerites) comprise over 300 species. They are found in freshwater, brackish, and seawater environments in tropical and temperate regions. Nerites have multiple uses, including as food, adornments, and ornamental pets. In Southeast Asia and the West Pacific, nerites are common shellfish that serve as a valuable source of protein. In Vietnam, the market price for lineate nerite (*Nerita balteata*) ranges from US$15–20 per kilogram (Carpenter and Niem, 1998; Đại, V. T, 2013; Hardjito et al., 2014). Because of their various colors and patterns, the shells of polished nerite (*Nerita polita*) are used to make necklaces, bracelets, and other adornments that are sold in Hawai'i (Kalei, 2018). Most species belonging to the Neritidae family are traded in the ornamental pet industry (Ng et al., 2016). Nerites are herbivorous creatures; they are often introduced into aquariums to help clean epiphytic algae from tank well and plants.

Most Neritidae species are amphidromous (Kano et al., 2006; Crandall et al., 2010), having a life cycle that involves both freshwater and seawater stages. Their life cycle can be divided into five stages. (1) Adult snails lay capsules on hard substrates, such as stones, other snails' shells, and wood in freshwater. (2) When the capsules hatch, they release larvae that are swept downstream to the ocean. (3) Although the larvae typically remain near the river mouth, some may travel long distances with ocean currents. Over the course of several weeks, these larvae feed on diatoms and other phytoplankton in the ocean. (4) Larvae respond to cues and metamorphose into crawling juveniles in estuaries or brackish areas of rivers. (5) Finally, young snails crawl upstream into the freshwater environment (Kano, 2006).

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Clithon diadema (Récluz, 1841) is a snail species characterized by spines on its yellow and black shell. It is found in the Philippines, Malaysia, and Indonesia. According to MolluscaBase and Kano et al. (2006), *C. diadema* can be found in both freshwater and brackish water environments. In numerous studies conducted to date, *C. diadema* have been collected from freshwater habitats in the provinces of Java, Banten, and Sulawesi in Indonesia (Mujiono, 2016; Dedy and Purnama, 2022; Purnama et al., 2022). Few studies have focused on the taxonomy and embryonic development of nerites, and little is known regarding the biology of *C. diadema*. Therefore, in this study, we explored the reproduction and larval development of *C. diadema*.

Larval morphology and structure undergo changes during development. These changes include the formation of features such as the velum, protoconch, cephalic tentacle, and propodium. The cephalic tentacle is equipped with numerous chemoreceptive sensory organs that enable larvae to detect a suitable environment for settling and undergoing metamorphosis (Cob et al., 2010; Cahill and Koury, 2016). Certain inducers can stimulate larval metamorphosis, thereby increasing the rate metamorphosis (Pechenik, 1990; Joyce and Vogeler, 2018). Due to the inadequate availability of wild *C. diadema* to meet the demands of the aquarium consumption market and the limited information on *C. diadema* propagation and breeding, the excessive collection and sale of these snails may adversely affect ecological resources. Therefore, this study aims to develop an artificial propagation and breeding method for *C. diadema*.

2. Materials and methods

2.1. Experimental animals

C. diadema were purchased from an aquatic wholesaler/distributor in Taiwan. Each tank (45 × 45 × 45 cm) had 50 adult snails (shell length, 9.09 ± 0.87 mm), which were picked up using a sieve (mesh size: 8 and 8 mm) and acclimatized at 25℃ ± 1℃. Specific lipids such as long-chain polyunsaturated fatty acids can promote gonadal development of *Haliotis fulgens* (Nelson et al., 2002, Bilbao, 2012), therefore to prevent reproduction, the snails were starved for 2 weeks before the experiment (Bilbao et al., 2012). *C. diadema* deposits its capsule onto the shell of another *C. diadema* and then uses tweezers to peel off a portion of the shell with the capsule from the snail. Snail capsules (length: 815.61 ± 20.87 μm) were cultured in 9-cm plates at 25℃ ± 1℃. Larvae (shell length: 154.85 ± 6.03 μm) hatched from these capsules were cultured in 12-well plates.

2.2. Identification of the optimal feed for broodstock

2.2.1. Experimental feeds

Diatoms were cultured in a fiber reinforced plastics (FRP) tank (2000 L), containing 48 clean plastic plates (60 × 30 cm). During the cultivation period, seawater was continuously filtered and supplied through a 20-μm filter bag and seaweed powder (A51A16; Chuan Kuan Enterprise, Taiwan) was added to promote diatoms' growth and their attachment to plastic plates. Using a microscope (Olympus BX41), we conducted diatom identification, revealing a predominant presence of *Nitzschia sp.*, *Navicula sp.*, and *Cylindrotheca sp*. These diatoms were found in a ratio of 2:2:1, respectively. Diatoms were removed from the plastic plate and centrifuged at 800 ×*g* for 20 min at 25℃, measured the weight to make sure the wet weight of diatoms at least 6 g. The daily feeding amount of the diatom group was a plastic plate (60 × 30 cm) covered with diatoms. The *Penaeus monodon* commercial feed obtained from Tairoun Products Co., Ltd., Taiwan. The daily feeding amount of the *P. monodon* commercial feed group was 5% of the total body weight. *Ulva lactuca* and *Cladophora fascicularis* were obtained from the Aquatic Animal Center of National Taiwan Ocean University, Taiwan. The daily feeding amount of the *U. lactuca* group and *C. fascicularis* group were 15% of the total body weight (Prihadi *et al.*, 2018). Furthermore, the snails may be eating throughout the day, adequate food were placed in the tank. The tank will be checked the following day to confirm whether there is any remaining diet, to determine if the previous day's feeding quantity was sufficient.

2.2.2. Proximate composition analysis

Proximate composition analysis was carried out as follows: Untreated experimental diets were used to determine the moisture content, and the moisture content was determined by oven drying the samples at 110 °C for 4 h to a constant weight. The experimental diets were treated at 40°C for 48 h and then converted to dry matter for analysis of crude protein, crude lipid and ash content. Ash content was determined by burning samples at 600 °C for 24 h. Crude protein was analyzed by the Kjeldahl procedure using the Kjeltec System from Tecator (Höganäs, Sweden). Crude lipids were determined using the soxhlet method (Señoráns and Luna, 2012).

2.2.3. Experimental procedure

Each tank (45 × 45 × 45 cm) had 50 adult snails (shell length, 9.09 ± 0.87 mm), which were picked up using a sieve (mesh size: 8 and 8 mm) and acclimatized at 25℃ ± 1℃. Adult snails were fed with different feeds for 4 weeks: diatoms, *P. monodon* commercial feed, *U. lactuca*, or *C. fascicularis*. The broodstock was divided into two groups: one group for dissection and the other group for assessing capsule productivity. Each group was divided into four subgroups on the basis of treatment; each treatment was performed using a tank (45 × 45 × 45 cm) containing 50 adult snails. These experiments were performed in triplicate. In the dissection group, 10 snails were sampled (in triplicate) from each treatment subgroup on day 0, 7, 14, 21, and 28 of the experiment. In the assessing group, the capsules of 10 snails were observaed from each treatment subgroup were observed (in triplicate) on days 0, 7, 14, 21 and 28 of the experiment. Animals maintained in closed containers, with each tank is aerated and having a daily water exchange percentage of 50% to maintain good water quality. The water source for the exchange was stored and aerated for 1 day to ensure that the water quality (e.g. temperature and dissolved oxygen) didn't differ much before and after the water exchange. *C. diadema* are fed once a day and record the number of capsules laid on the snail shell. The experimental period was four weeks.

2.2.4. Feed intake

For diatom treatment, the remaining diatoms were removed from the plastic plate, centrifuged at 800 ×g for 20 minutes at 25℃, and their weight was measured. For commercial feed treatment, the remaining feeds were collected by siphoning them at the end of feeding with 300 mesh net; then, these were dried at 40℃ for 1 day. Then, their weight was measured. For *U. lactuca* and *C. fascicularis* treatments, the remaining seaweeds were collected and moisture was removed from them with the help of a hand towel. To minimize weight loss due to osmotic pressure, a control treatment was included; this treatment involved the use of different feeds in freshwater without broodstock. Additionally, 50% of the water will be changed, and residue will be removed to calculate feed intake every other day. The weight of the feeds before and after treatment was recorded. Feed intake was calculated everyday using the following formula:

Daily feed intake = (initial daily feed weight in the experimental tank – final daily feed weight in the experimental tank) – (initial daily feed weight in the control tank – final daily feed weight in the control tank)

2.2.5. Gonad–hepatopancreas somatic index

The gonad somatic index (GSI) is often used to describe the maturation of the gonads. However, because *C. diadema* is very small, it is extremely difficult to separate the gonads and hepatopancreas. Therefore, this study uses the ratio of the combined weight of the gland and hepatopancreas to the overall soft tissue weight as an indicator of gland

maturity. Specimens of *C. diadema* were immersed in 10% formalin for 1 week. The relative reproductive effort of the experimental *C. diadema* was determined by the Gonad–Hepatopancreas Somatic Index (GHSI) method (Griffen and Norelli, 2015). *C. diadema* were dissected under a stereomicroscope and the gonad–hepatopancreas and the remaining soft tissue were separated and weighed to calculate the index using the following formula:

GHSI = (weight of gonad and hepatopancreas / weight of the remaining soft tissue) × 100

2.3. Determination of optimal salinity changes for the embryonic development and larval survival of C. diadema

2.3.1. Experimental feeds

The daily feeding amount of every group was a plastic plate (60 × 30 cm) covered with diatoms. The preparation of diatoms is as described in Section 2.2.1.

2.3.2. Experimental procedure

The broodstock was cultured in freshwater. After the broodstock laid capsules on the shells of other snails, the capsules were collected from shells by using tweezers. Then, the capsules were transferred from a 0‰ salinity condition to 0‰, 5‰, 10‰, 15‰, 20‰, 25‰, 30‰ and 35‰ salinity conditions. The duration of embryonic development was recorded. The hatched larvae were subsequently not provided with food, and daily larval mortality at each salinity level was recorded. Each treatment involved the use three of a 9-cm plate each containing one capsule and was performed in triplicate. The experimental period of embryonic development was four weeks, and period of larval survival was four weeks.

2.3.3. Embryonic and larval development

Embryonic development was divided into seven stages: blastula, rapid division, gastrula, pre- veliger, veliger I, veliger II, and hatching (Barroso and Matthews-Cascon, 2009).

Embryonic and larval development were monitored using a microscope (Olympus BX41) and digital camera (WXCAM-4KMPA).

2.4. Determination of the optimal salinity for broodstock, capsule hatching, and larval survival

2.4.1. Experimental feeds

The daily feeding amount of every group was a plastic plate (60 × 30 cm) covered with diatoms. The preparation of diatoms is as described in Section 2.2.1.

2.4.2. Experimental procedure

In this broodstock experiment, we tested different reared salinity levels (0‰, 5‰, 10‰, 15‰, 20‰, 25‰, 30‰, and 35‰) on reproduction and capsule hatching. The broodstock was fed with diatoms once a day and take 50% water exchange every other day. Before feeding would take one plastic plate, remove all diatoms and were centrifuged at 800 ×g for 20 min at 25℃ to make sure the wet weight of diatoms at least 6 g. Each treatment involved the use of a tank (45 × 45 × 45 cm) containing 50 adult snails and was performed in triplicate. After feeding 2 weeks, the broodstock started to laid capsule on the shells of other snails. Recorded the number of capsules and collected from shells by using tweezers. Capsule hatching was examined at 0‰, 5‰, 10‰, 15‰, 20‰, 25‰, 30‰, and 35‰ salinity. The duration of embryonic development was recorded. Hatched larvae were then unfed, and daily larval mortality at salinity level was recorded. Each experiment involved the use of a 9-cm plate containing one capsule and was conducted in triplicate. Each tank operates within a closed system, with a daily water exchange rate of 50%. The water in the tanks is aerated, and *C. diadema* are fed once a day. The experimental period of broodstock reproduction was four weeks, period of *embryonic development* was four weeks, and period of larval survival was four weeks.

2.5. Identification of the optimal microalgae and salinity level for larval culture

2.5.1. Experimental feeds

Isochrysis galbana or *Chaetoceros muelleri* were cultured with F/2 medium (Guillard and Ryther, 1962) in plant incubator (SS-980, Tominaga) under 12-h light/dark cycle at 25℃ ± 1℃. Hatched larvae from capsules were fed with different microalgae, *I. galbana* or *C. muelleri* (concentration: 5×10^4 cell/mL).

2.5.2. Experimental procedure

Based on best result of previously experiment, broodstock reared at 20‰ salinity and fed with diatoms. After broodstock laid capsules which were collected and hatched at 20‰ salinity. When larvae hatched were transfer to different salinity levels: 20‰, 25‰, 30‰, and 35‰. The larvae were cultured at different salinity levels: 20‰, 25‰, 30‰, and 35‰. The larvae were fed daily with 100% water change and added algae (*I. galbana* or *C. muelleri*) every other day. The survival rate and shell length of the larvae were recorded every 4 days. Each treatment involved the use of a 12-well plate, and each well contained one larvae (1 larvae/3 mL). Each experiment was performed in triplicate. The experimental period was ten weeks. During the experimental period, 100% water was changed and algae were added into each well daily.

2.6. Identification of the optimal inducer for larval metamorphosis

2.6.1. Experimental feeds

The preparation of *I. galbana* is as described in Section 2.5.1. Hatched larvae from capsules were cultured at 25‰ salinity and fed with *I. galbana* (concentration: 5×10^4 cell/ mL).

2.6.2. Experimental inducers

To prepare various inducers, such as diatoms (Salas-Garza et al., 2009), mucus (Salas-Garza *et al.*, 2009), sediment (Cob et al., 2010), and KCl (5 mM) (Sigma) (Cob et al., 2010), the following procedures were followed. Diatoms were removed from the plastic plate to a 9-cm plate and cultured for 1 week to facilitate their attachment to the 9-cm plate. Mucus from adult snails was obtained by maintaining one snail in a 9-cm plate overnight, allowing its mucus to adhere to the plate. Sediment which including snail's feces, diatoms and detritus was collected by siphoning it out from adult broodstock tanks with 300 mesh net, and weighted 1 g added into 9-cm plate. The stocking KCl solution (180 mM) was prepared by dissolving 6.70 g of KCl in 0.5 L of distilled water, followed by autoclaving.

2.6.3. Experimental procedure

Based on best result of previously experiment, broodstock reared at 20‰ salinity and fed with diatoms. After broodstock laid capsules which were collected and hatched at 20‰ salinity. Hatched larvae from capsules were cultured at 25‰ salinity and fed with *I. galbana* (concentration: 5×10^4 cell/ mL). When the larvae (shell length: 440.76 ± 43.81 μm) developed propodium, different inducers were added—control, diatoms, mucus, sediment, and KCl (5 mM)—to induce metamorphosis. In the control treatment, no inducer was added. The rate of larval metamorphosis was recoded for 7 days after the addition of inducers. Each treatment involved the use of a 9-cm plate containing 6 larvae (1 larvae/3 mL) and was performed in triplicate. During the experimental period, a complete water change of 100% of the water volume was performed daily. In addition, fresh inducers were added after each water change. The larvae were fed daily with *I. galbana* at a concentration 5×10^4 cell/mL. The experimental period was seven days, the rate of larval

Table 1
Proximate composition of the experimental diets for *Clithon diadema* broodstock.

Proximate composition (%)	Diet			
	D	F	U	C
Moisture	80.47 ± 0.42	16.00 ± 0.00	88.38 ± 2.23	83.31 ± 1.34
Ash*	62.10 ± 1.93	10.00 ± 0.00	14.53 ± 2.87	29.01 ± 0.35
Crude Protein*	13.07 ± 2.59	42.00 ± 0.00	16.41 ± 1.69	22.94 ± 0.14
Crude Lipid*	11.03 ± 1.50	6.00 ± 0.00	1.28 ± 0.49	6.55 ± 0.36

D: diatom; F: Penaeus monodon commercial feed; U: Ulva lactuca; C: Cladophora fascicularis.
Data are presented in terms of the mean ± standard deviation values (n = 3).
* indicates dry matter form.

Table 2
Daily feed intake and estimated protein and lipid intake of *Clithon diadema* broodstock fed with an experiment diet.

		Diet			
		D	F	U	C
Feed intake (g/ day)	Weight	3.830 ± 0.288^{a}	0.082 ± 0.016^{c}	0.902 ± 0.077^{b}	0.058 ± 0.015^{c}
Estimated amount (g/ day)	Protein	0.501 ± 0.038	0.035 ± 0.007	0.148 ± 0.013	0.013 ± 0.003
	Lipid	0.422 ± 0.032	0.005 ± 0.001	0.012 ± 0.001	0.004 ± 0.001

D: Diatom; F: Penaeus monodon commercial feed; U: Ulva lactuca; C: Cladophora fascicularis.
Data are presented in terms of the mean ± standard deviation values (n = 3).
Mean values with different letters (a, b, and c) indicate significant difference ($p < 0.05$).

Table 3
Effects of different diets on the gonad hepatopancreas somatic index (GHSI) and capsule number of *Clithon diadema* broodstock in freshwater during a 4-week period.

Treatment		Weeks				
		0	1	2	3	4
GHSI	D	13.50 ± 4.84$^{D}_{a}$	25.68 ± 8.48$^{C}_{a}$	29.90 ± 7.89$^{C}_{a}$	38.69 ± 5.43$^{B}_{a}$	48.02 ± 5.91$^{A}_{a}$
	F	12.60 ± 5.12$^{C}_{a}$	21.42 ± 6.29$^{B}_{a}$	25.57 ± 7.45$^{B}_{ab}$	31.49 ± 7.49$^{A}_{b}$	32.00 ± 7.41$^{A}_{b}$
	U	14.93 ± 5.51$^{C}_{a}$	22.28 ± 6.94$^{BC}_{a}$	22.77 ± 8.38$^{B}_{bc}$	25.89 ± 7.39$^{AB}_{c}$	31.65 ± 10.22$^{A}_{b}$
	C	12.78 ± 4.81$^{B}_{a}$	22.21 ± 5.93$^{A}_{a}$	20.30 ± 7.51$^{A}_{c}$	20.63 ± 7.44$^{A}_{d}$	19.52 ± 6.93$^{A}_{c}$
Capsule number	D	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	1.67 ± 0.55^{a}	33.00 ± 22.72^{a}	71.33 ± 42.55^{a}
	F	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.33 ± 0.58^{b}	5.33 ± 4.04^{b}
	U	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	1.33 ± 2.31^{b}
	C	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{b}	0.00 ± 0.00^{b}

D: Diatom; F: Penaeus monodon commercial feed; U: Ulva lactuca; C: Cladophora fascicularis.
Data are presented in terms of the mean ± standard deviation values (n = 30).
Mean values with different uppercase letters (A, B, and C) in the same row indicate significant difference ($p < 0.05$)
Mean values with different lowercase letters (a, b, c, and d) in the same column indicate significant difference ($p < 0.05$).

Table 4
Effects of different diets on the total capsule number, capsule parameters, and egg parameters of *Clithon diadema* broodstock.

Treatment	Total capsule number	Capsule length (μm)	Capsule width (μm)	Egg number per capsule	Egg diameter (μm)	Sex ratio
D	105.00 ± 63.51^{a}	829.31 ± 65.31^{a}	401.44 ± 64.40^{a}	26.40 ± 2.23^{a}	99.44 ± 12.10^{a}	1: 2.74
F	5.67 ± 3.51^{b}	803.36 ± 18.11^{a}	370.62 ± 57.04^{a}	28.61 ± 1.00^{a}	105.76 ± 4.58^{a}	1: 2.24
U	1.33 ± 2.31^{b}	805.02 ± 11.13^{a}	364.95 ± 39.45^{a}	26.72 ± 1.53^{a}	94.35 ± 3.80^{a}	1: 2.17
C	0.00 ± 0.00^{b}	-	-	-	-	1: 2.28

D: diatom; F: Penaeus monodon commercial feed; U: Ulva lactuca; C: Cladophora fascicularis.
Sex ratio: male/ female number (n=150).
Data are presented in terms of the mean ± standard deviation values (N=3, n = 3).
Mean values with different letters (a and b) indicate significant difference ($p < 0.05$).
- indicates no capsule deposition.

metamorphosis was recoded.

2.7. Data analysis

Data were analyzed using the one-way analysis of variance test. The survival rate and shell length of the larvae were calculated using the two-way analysis of variance test. Tukey's honestly significant difference test was used for between-treatment comparisons. All statistical analyses were performed using SPSS (version 26.0; IBM, Armonk, NY, USA). A *P* value of <0.05 indicated statistical significance.

3. Results

3.1. Effects of different diets on gonadal development and capsule productivity of adult C. diadema

Table 1 presents the daily diatom, *Penaeus monodon* commercial feed, *U. lactuca*, and *C. fascicularis* intake of *C. diadema*. Among these diets, the diatom-fed group exhibited the highest daily intake. Further insights into the composition and daily intake of these diets reveal that the diatom-fed group also displayed the highest daily intake of crude protein and crude lipid (Table 2). Table 3 presents the gonad hepatopancreas somatic index (GHSI) of *C. diadema* feeding on different baits. The GHSI of groups fed on diatoms, commercial feed, and *U. lactuca* increased with feeding time. In week 2, the GHSI of the diatom-fed group was 29.90; the snails in this group produced 1.67 ± 0.55 capsules. In the weeks 3 and 4, the diatom-fed group continued to produce capsules and its GHSI was significantly higher than those of the other groups ($p < 0.05$). The GHSI of the *Penaeus monodon* commercial feed-fed group reached 31.49 in the third week, and it produced 0.33 ± 0.58 capsules. Similarly, in the fourth week, the GHSI of the *U. lactuca*-fed group was 31.65, and it produced 1.33 ± 2.31 capsules. The GHSI of the *C. fascicularis*-fed group initially increased to 22.21 in week 1 but subsequently stagnated and even decreased to 19.52 in week 4. Table 4 presents the results regarding the total capsule number, capsule parameters, and egg parameters of *C. diadema* after the 4-week feeding period. The total number of capsules was significantly higher in the diatom-fed group than in the other groups ($p < 0.05$). However, no significant differences in capsule length or width or egg quantity or diameter were noted among the groups ($p > 0.05$).

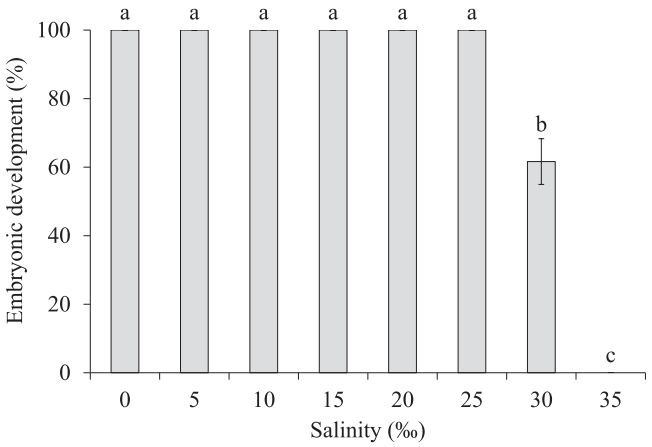

Fig. 1. Effects of acute salinity changes on *Clithon diadema* embryonic development The letters above the bars indicate significant differences in means (N=3, n = 9) between salinity levels.

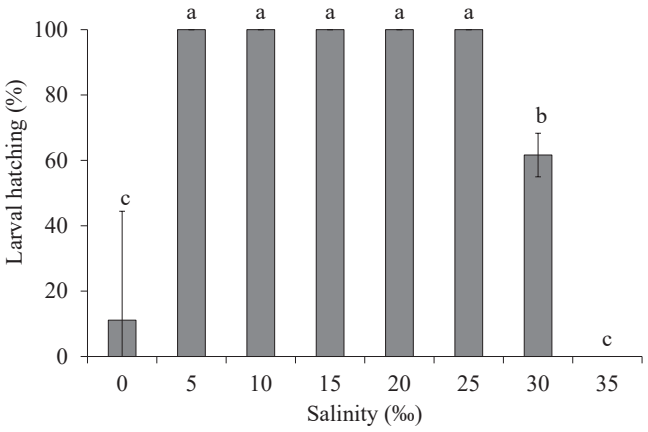

Fig. 2. Effects of acute salinity on *Clithon diadema* larval hatching. The letters above the bars indicate significant differences in means (N=3, n=3) between salinity levels.

Table 5
Effect of acute salinity change on embryonic development times of *Clithon diadema*.

Salinity (‰)	Embryonic development time (days)						
	S1	S2	S3	S4	S5	S6	S7
0	1.00 ± 0.00[a]	2.44 ± 0.53[b]	4.44 ± 0.53[b]	6.67 ± 0.50[b]	8.89 ± 0.78[a]	13.22 ± 1.20[ab]	15.00★
5	1.00 ± 0.00[a]	2.33 ± 0.50[b]	4.44 ± 0.53[b]	6.44 ± 0.53[ab]	8.33 ± 0.50[a]	13.67 ± 0.71[ab]	15.33 ± 0.50[a]
10	1.00 ± 0.00[a]	2.00 ± 0.00[a]	3.00 ± 0.00[a]	5.11 ± 0.78[a]	7.33 ± 0.50[a]	13.78 ± 0.83[ab]	15.33 ± 1.12[a]
15	1.00 ± 0.00[a]	2.00 ± 0.00[a]	3.00 ± 0.00[a]	5.33 ± 0.50[ab]	8.00 ± 0.71[a]	12.56 ± 1.13[a]	14.89 ± 0.33[a]
20	1.00 ± 0.00[a]	2.00 ± 0.00[a]	3.00 ± 0.00[a]	5.00 ± 0.00[a]	7.89 ± 0.33[a]	11.11 ± 0.33[a]	14.00 ± 0.71[a]
25	1.33 ± 0.58[a]	3.00 ± 0.00[b]	4.00 ± 0.00[b]	9.00 ± 0.00[c]	12.11 ± 0.78[b]	15.11 ± 0.78[b]	19.67 ± 0.87[b]
30	2.00 ± 0.00[b]	3.43 ± 0.53[b]	4.43 ± 0.53[b]	10.00 ± 0.58[c]	12.57 ± 0.79[b]	15.57 ± 0.53[b]	20.50 ± 0.71[b]
35	-	-	-	-	-	-	-

S1: Blastula stage; S2: Rapid division stage; S3: Gastrula stage; S4: Pre- veliger stage; S5: Veliger I stage; S6: Veliger II stage;
S7: Hatch stage/ hatching time.
Data are presented in terms of the mean ± standard deviation values (N=3, n = 9), except for ★, which represents the only sample that hatched at S7.
Mean values with different letters (a, b, and c) indicate significant difference ($p < 0.05$).
- indicates undeveloped.

3.2. Effects of changes in acute salinity on embryonic development and larval survival of C. diadema

C. diadema was cultured in freshwater and fed diatoms. After the formation of *C. diadema* capsules, they were subsequently hatched various salinity levels, ranging from 0‰ to 35‰. Fig. 1 depicts the effects of salinity changes on the embryonic development of *C. diadema*. The embryonic development ratio were consistently 100.00% ± 0.00% for snails cultured at 0‰ to 25‰ salinity. However, the embryonic development decreased to 61.64% ± 0.00% and 0.00% ± 0.00%, at 30‰ and 35‰ salinity respectively. Table 5 presents the effects of salinity changes on the time required for each embryonic development stage. Embryo development to the blastula stage (S1) took less time in the 0‰ to 25‰ groups compared to the 30‰ group ($p < 0.05$). However, embryos in the 35‰ group did not develop due to excessive variations

Table 6
Effects of acute salinity changes on the shell length and height of *Clithon diadema* larvae on posthatching day 1.

Salinity (‰)	Larval shell length (μm)	Larval shell height (μm)
0	140.00★	121.98★
5	142.88 ± 4.23[a]	118.59 ± 2.70[a]
10	142.47 ± 7.43[a]	124.53 ± 5.29[a]
15	142.06 ± 2.03[a]	122.23 ± 0.39[a]
20	144.19 ± 4.40[a]	121.06 ± 1.30[a]
25	142.28 ± 2.81[a]	118.51 ± 5.86[a]
30	145.62 ± 5.93[a]	121.84 ± 6.10[a]
35	-	-

Data are presented in terms of the mean ± standard deviation values (N=3, n = 9).
Mean values with the same letter (a) indicate nonsignificant difference ($p > 0.05$).
★indicates only sample that hatched at 0‰ salinity.
- indicates unhatched sample.

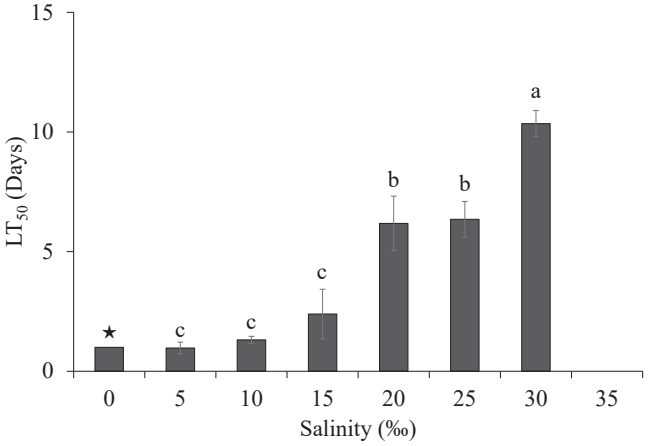

Fig. 3. Effects of acute salinity changes on the median lethal time (LT50) of *Clithon diadema larvae.* The letters above the bars indicate significant differences in means (N=3, n=9) between salinity levels. ($p < 0.05$) between salinity levels. ★ represents the only sample that hatched at 0‰ salinity.

Table 7
Effects of different salinity levels on the survival and total capsule number of Clithon diadema broodstock in week 4.

Salinity (‰)	Broodstock survival rate (%)	Total capsule number
0	96.67 ± 1.15^{a}	28.33 ± 6.03^{c}
5	97.33 ± 1.15^{a}	36.67 ± 4.93^{bc}
10	98.00 ± 0.00^{a}	53.33 ± 10.41^{b}
15	98.00 ± 2.00^{a}	82.00 ± 14.80^{a}
20	97.33 ± 1.15^{a}	79.00 ± 9.85^{a}
25	96.67 ± 2.31^{a}	54.00 ± 6.56^{b}
30	90.67 ± 4.16^{b}	19.33 ± 2.08^{c}
35	86.00 ± 2.00^{b}	14.33 ± 5.13^{c}

Data are presented in terms of the mean ± standard deviation values (N=3, n = 3).
Mean values with different letters (a, b, and c) indicate significant difference ($p < 0.05$).

Table 8
Effects of different salinity levels on the embryonic development time of *Clithon diadema*.

Salinity (‰)	Embryonic development time (days)						
	S1	S2	S3	S4	S5	S6	S7
0	1.00 ± 0.00^{a}	2.33 ± 0.50^{ab}	4.33 ± 0.50^{bc}	7.44 ± 0.53^{b}	9.44 ± 0.53^{c}	13.56 ± 0.53^{b}	-
5	1.00 ± 0.00^{a}	2.22 ± 0.44^{ab}	4.33 ± 0.50^{bc}	7.44 ± 0.53^{b}	8.89 ± 0.33^{bc}	14.44 ± 0.53^{bc}	15.89 ± 0.78^{bc}
10	1.00 ± 0.00^{a}	2.00 ± 0.00^{a}	3.00 ± 0.00^{a}	4.89 ± 0.93^{a}	7.67 ± 0.50^{ab}	14.56 ± 0.53^{bc}	15.89 ± 0.33^{bc}
15	1.00 ± 0.00^{a}	2.00 ± 0.00^{a}	3.00 ± 0.00^{a}	4.67 ± 0.50^{a}	7.44 ± 0.53^{a}	13.33 ± 0.50^{b}	14.33 ± 0.50^{ab}
20	1.00 ± 0.00^{a}	2.00 ± 0.00^{a}	3.22 ± 0.44^{ab}	5.33 ± 0.50^{a}	8.44 ± 0.53^{abc}	11.22 ± 0.44^{a}	13.56 ± 0.53^{a}
25	1.00 ± 0.00^{a}	2.00 ± 0.00^{a}	3.11 ± 0.33^{a}	5.11 ± 0.33^{a}	8.22 ± 0.44^{abc}	15.33 ± 0.50 ^{cd}	17.22 ± 0.44^{c}
30	2.00 ± 0.00^{b}	2.56 ± 0.53^{ab}	4.67 ± 0.50^{c}	8.44 ± 0.53^{bc}	11.78 ± 0.67^{d}	16.78 ± 0.67^{d}	20.00 ± 0.71^{d}
35	2.00 ± 0.00^{b}	3.44 ± 0.53^{b}	5.44 ± 0.53^{c}	9.56 ± 0.73^{c}	12.22 ± 0.67^{d}	18.11 ± 0.60^{e}	21.00 ± 0.87^{d}

S1: blastula stage; S2: rapid division stage; S3: gastrula stage; S4: pre- veliger stage; S5: veliger I stage;
S6: veliger II stage; S7: hatch stage/ hatching time.
Data are presented in terms of the mean ± standard deviation values (N=3, n = 9).
Mean values with different letters (a, b, c, d, and e) indicate significant difference ($p < 0.05$).
- indicates undeveloped.

(0‰ to 35‰) in salinity. *C. diadema* cultured at 10‰, 15‰, and 20‰ salinity exhibited significantly shorter development times for the rapid division stage (S2) and gastrula stage (S3) compared to the 0‰, 5‰, 25‰, and 30‰ groups ($p < 0.05$). Moreover, the 20‰ group showed the fastest progression to veliger II stage (S6) and hatch stage (S7); however, no significant difference was observed between the 5‰ and 20‰ groups. Statistical analysis could not be performed in the 0‰ group due to only one capsule hatching. The effect of acute changes in salinity on the larval hatching rate of *C. diadema* is presented in Fig. 2. All larvae hatched successfully in the 5‰ to 25‰ groups. In the 0‰, 30‰, and 35‰ groups, the hatching percentages were 11.11% ± 33.33%, 61.64% ± 6.67%, and 0.00% ± 0.00%, respectively, which were significantly lower than those in the 5‰ to 25‰ groups. The effects of acute changes in salinity

Table 9
Effects of different salinity levels on the shell length and height of *Clithon diadema* larvae on posthatching day 1.

Salinity (‰)	Larval shell length (μm)	Larval shell height (μm)
0	-	-
5	140.45 ± 4.34^{a}	122.35 ± 2.60^{a}
10	141.82 ± 1.88^{a}	118.59 ± 2.81^{a}
15	141.58 ± 1.89^{a}	119.40 ± 2.60^{a}
20	140.57 ± 4.13^{a}	120.45 ± 2.63^{a}
25	143.96 ± 4.09^{a}	119.33 ± 1.95^{a}
30	140.81 ± 4.08^{a}	120.74 ± 3.17^{a}
35	142.39 ± 2.49^{a}	121.69 ± 3.57^{a}

Data are presented in terms of the mean ± standard deviation values (N=3, n = 9).
Mean values with the same letter (a) indicate nonsignificant difference ($p > 0.05$).
- indicates no larva hatched after treatment.

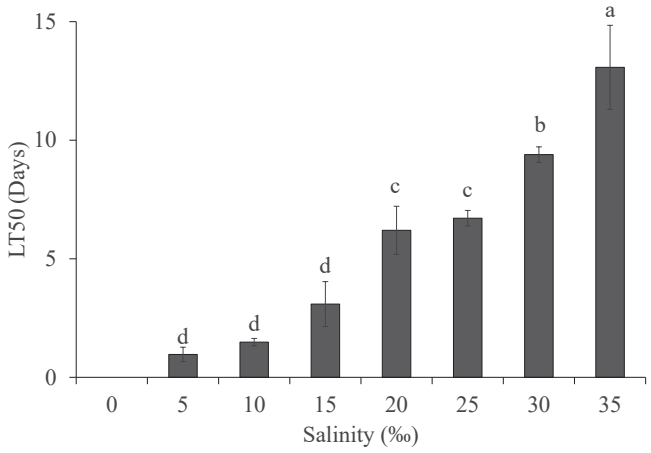

Fig. 4. Effects of different salinity levels on the median lethal time (LT50) of *Clithon diadema* larvae. The letters above the bars indicate significant differences in means (N=3, n=9) between salinity levels ($p < 0.05$) between salinity levels.

on the larval shell length and height of *C. diadema* at post- hatching day 1 were illustrated in Table 6. No significant differences were noted among the groups ($p > 0.05$). Fig. 3 presented the effect of acute changes in salinity on the median lethal time (LT50) of *C. diadema* larvae. No larvae hatching was noted in the 35‰ group. The 30‰ group exhibited the highest LT50 value, but no significant difference was noted between the 30‰ and 25‰ groups.

3.3. Effects of different salinity on the adult survival, capsule productivity, embryo development, and larval survival of C. diadema

Table 7 presented the adult survival and total capsule production of *C. diadema* cultured for 4 weeks at salinity levels of 0‰ to 35‰, with diatoms provided as their food source. The 10‰ and 15‰ groups had better survival rates, but they did not significantly differ from the 0‰, 5‰, 20‰, and 25‰ groups ($p < 0.05$). The total capsule production increased with increasing salinity, reaching its peak in the 15‰ group. However, total capsule production began to decrease at salinity levels of >20‰. The embryonic development ratio in each group consistently reached 100.00% ± 0.00% (data no show). Table 8 presented the effects of salinity on the time required for each stage of embryonic development. The 20‰ group exhibited the most fast development, although no significant difference was observed compared to the 15‰ group. None of the larvae in the 0‰ group hatched, whereas all the larvae in the other groups successfully hatched. The shell length and height of the

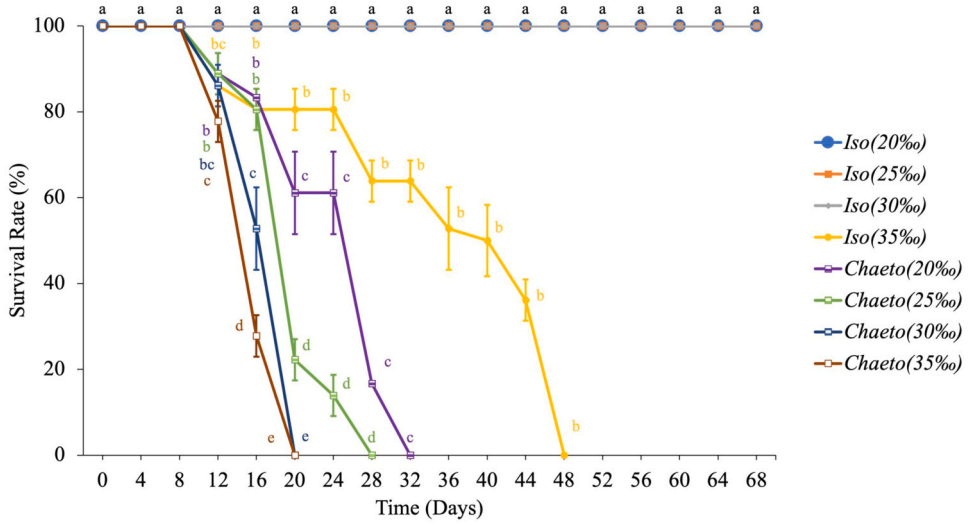

Fig. 5. Effects of two microalgae and different salinity levels on the survival of *Clithon diadema* larvae during a 68-day period. (N=3, n=3).

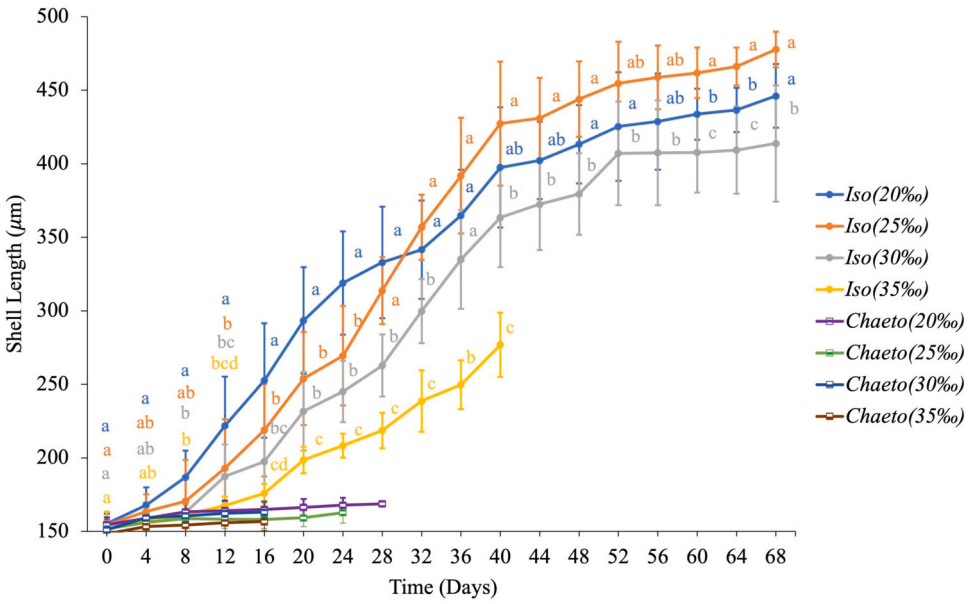

Fig. 6. Effects of two microalgae and different salinity levels on growth performance of *Clithon diadema* larvae during a 68-day period. (N=3, n=12).

hatched larvae did not no vary significantly among the salinity groups ($p > 0.05$; Table 9). Fig. 4 presents the effects of different salinity levels on the LT50 of *C. diadema* larvae. No larvae hatched in the 0‰ group. The 35‰ group had the highest LT50 value, but no significant difference was observed between the 35‰ and 30‰ groups.

3.4. Effects of different microalgae and salinity on the growth of larval C. diadema

The larvae were cultured at 25‰ or 30‰ salinity and fed with either *I. galbana* or *C. muelleri*. They were referred to as *Iso*(20‰), *Iso*(25‰), *Iso*(30‰), *Iso*(35‰), *Chaeto*(20‰), *Chaeto*(25‰), *Chaeto*(30‰), and *Chaeto*(35‰). The survival rates of these larvae are shown in Fig. 5. From day 0–8, the survival rate of each group was 100%. However, after day 8, the survival rate of the *Iso*(35‰), *Chaeto*(20‰), *Chaeto*(25‰), *Chaeto*(30‰), and *Chaeto*(35‰) groups gradually declined. The *Iso*(20‰), *Iso*(25‰), and *Iso*(30‰) groups sustained a 100% survival rate on day 68, whereas all other groups have already perished. Fig. 6 shows the effects of different microalgae and salinity on the growth of the larvae. The larval growth performance of the *Iso*(20‰), *Iso*(25‰), and *Iso*(30‰) groups exhibited a gradual increase. On day 68, the larval growth performance of the *Iso*(25‰) group was the highest, although no significant difference was observed between this group and the *Iso*(20‰) group ($p > 0.05$). Fig. 7 presents the morphology of larval *C. diadema* from hatching to metamorphosis. Table 10 presents the time required and proportion for the larvae to develop propodium and undergo metamorphosis. All larvae in the *Iso*(35‰), *Chaeto*(20‰), *Chaeto*(25‰), *Chaeto*(30‰), and *Chaeto*(35‰) groups died; thus, they were excluded from the analysis. No propodium development or metamorphosis was observed in the *Iso*(30‰) group during the experimental period; thus, this group could not be analyzed. No significant difference was noted between the *Iso*(20‰) and *Iso*(25‰) groups in the time required for propodium development and metamorphosis ($p > 0.05$). However, the ratio of propodium development and metamorphosis in the *Iso*(25‰) group was significantly higher than that in the *Iso*(20‰) group ($p < 0.05$).

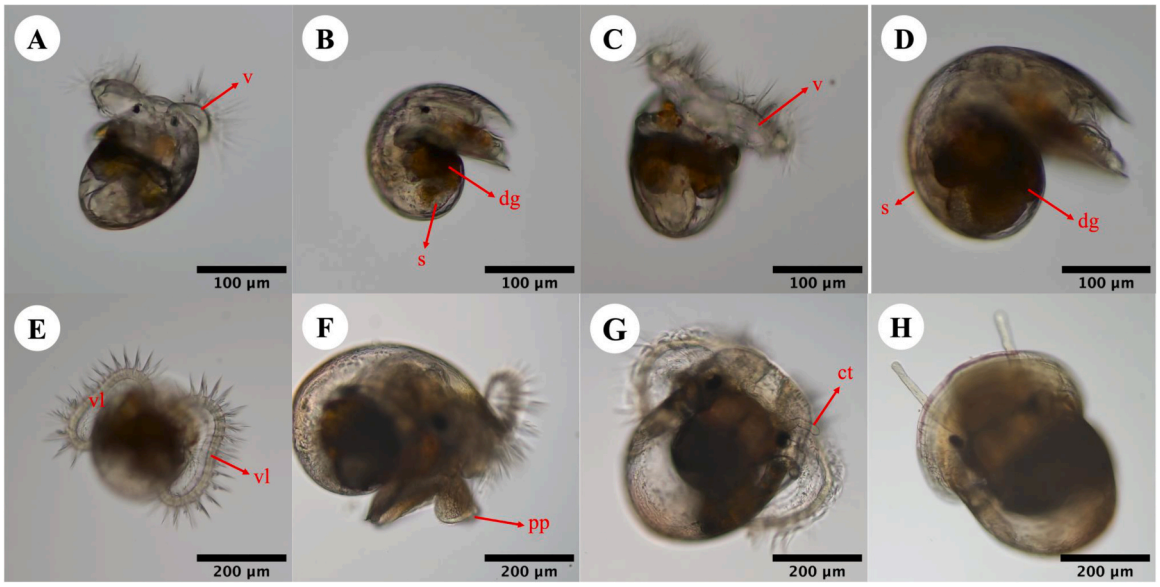

Fig. 7. Development of *Clithon diadema* larvae fed with *Isochrysis galbana* at 25℃ and 25‰ salinity during a 68-day period. (A) After 0 day posthatching (dph), semicircular velum appeared in a veliger larva. (B) After 2 dph, the digestion gland and stomach were filled with microalgae. (C) After 10 dph, the semicircular velum enlarged. (D) After 12 dph, the shell and digestion gland enlarged. (E) After 14 dph, the velum comprised two velar lobes. (F) After 49 dph, the propodium appeared (lateral view). (G) After 49 dph, the cephalic tentacle appeared and crawling behavior was observed. (H) After 67 dph, the larva metamorphosed into a juvenile snail (dorsal view). v, velum; dg, digestive gland; s, stomach; vl, velar lobes; pp, propodium; ct, cephalic tentacle.

Table 10
Effects of *Isochrysis galbana* and different salinity levels on the propodium development and metamorphosis of *Clithon diadema* larvae.

Variable	Days		Percentage (%)	
	Propodium	Metamorphosis	Propodium	Metamorphosis
Iso (20‰)	50.40 ± 2.88^{a}	72.00 ± 4.83^{a}	30.56 ± 4.81^{b}	13.89 ± 4.81^{b}
Iso (25‰)	56.43 ± 5.77^{a}	71.00 ± 3.79^{a}	69.44 ± 4.81^{a}	44.44 ± 12.73^{a}
Iso (30‰)	-	-	-	-
Iso (35‰)	-	-	-	-

Iso: Isochrysis galbana.
Data are presented in terms of the mean ± standard deviation values (N = 3; n = 12).
Mean values with different letters (a and b) indicate significant difference ($p < 0.05$).
- indicates undeveloped.

3.5. Effects of different inducers on the metamorphosis of larval C. diadema

Fig. 8 presents the effects of diatom, mucus, sediment, and KCl on the metamorphosis rate of *C. diadema* larvae. The larvae in the mucus and KCl groups initiated metamorphosis on day 1, whereas the sediment group exhibited larval metamorphosis on day 2. In the control group, larvae metamorphosis occurred on day 7. However, no larval metamorphosis was observed in the diatom group on day 7. On day 7, the mucus group exhibited the highest rate of larval metamorphosis. The sediment group had the second highest rate of larval metamorphosis. However, no significant differences were observed among the other groups.

4. Discussion

Aquatic organisms have specific temperature, salinity, and nutritional requirements that are crucial for their cultivation. Therefore, establishing appropriate water quality parameters and diets is essential for the cultivation of aquatic organisms. The feeding habit varies across snails depending on their species and life cycle stages. For instance, abalone *Haliotis discus hannai* undergoes a transition from microbial diets to macroalgal diets as it matures (Kawamura et al., 2001). Thus, it is imperative to identify the preferred diet of snail broodstock, which may include diatoms, *Penaeus monodon* commercial feed, *U. lactuca*, and *C. fascicularis*. Diatoms are microalgae, and most diatoms tend to attach to surfaces and form a thin film (Armstrong et al., 2000). In this study, we observed that diatoms were attached to the surface of a plastic plate. *C. diadema* uses its radula to scrape off the attached diatoms when it crawled on the surface of the plastic plate. Diatoms are unicellular algae and thus can be easily digested and absorbed by *C. diadema*. Commercial *P. monodon* feed contains cholesterol, which can stimulate gonad development in organisms. However, it is speculated tha *P. monodon* commercial feed is available as cylindrical pellets; grasping these pellets is difficult for *C. diadema*, which interferes with feed consumption (Qi et al., 2010; Lebata-Ramos et al., 2023). Furthermore, the feed disintegrates in water within 4 hours, reducing *C. diadema* intake. While feces and disintegrated feed are indeed accumulated together by the net, it is noteworthy that the ingestion of feed by the snails in the experimental group was notably inadequate. This diminished consumption directly translates to a reduced production of feces. As a result, the quantity of fecal matter excreted has a negligible effect on the data collection pertaining to disintegrated feed. *U. lactuca* is composed of bilayered cells and appears flat and blade-like (Postma et al., 2018). Despite its hard and rough texture, *C. diadema* consumes *U. lactuca* by using the radula. *C. fascicularis*, a filamentous macroalgae, is unsuitable for *C. diadem*'s crawling and feeding. Our results suggest that a planar bait structure benefits for *C. diadema* crawling and feeding, while a columnar and filamentary structure hinders snail crawling, leading to poor palatability. Polyunsaturated fatty acids act as precursors for the biosynthesis of sterols and hormones. Thus, these fatty acids are crucial for the development of the reproductive system and the formation of yolk (Meunpol et al., 2005). Mollusks, with limited ability to synthesize polyunsaturated fatty acids (Monroig and Kabeya, 2018), depend on essential fatty acids from their food (Laing et al., 1990). Our results indicated the diatom-feeding *C. diadema* group exhibited the fastest capsule production and the highest total count, reaching 105 capsules at the second week. The rich lipid and polyunsaturated fatty acids of diatoms can promote the gonad development and capsule production of

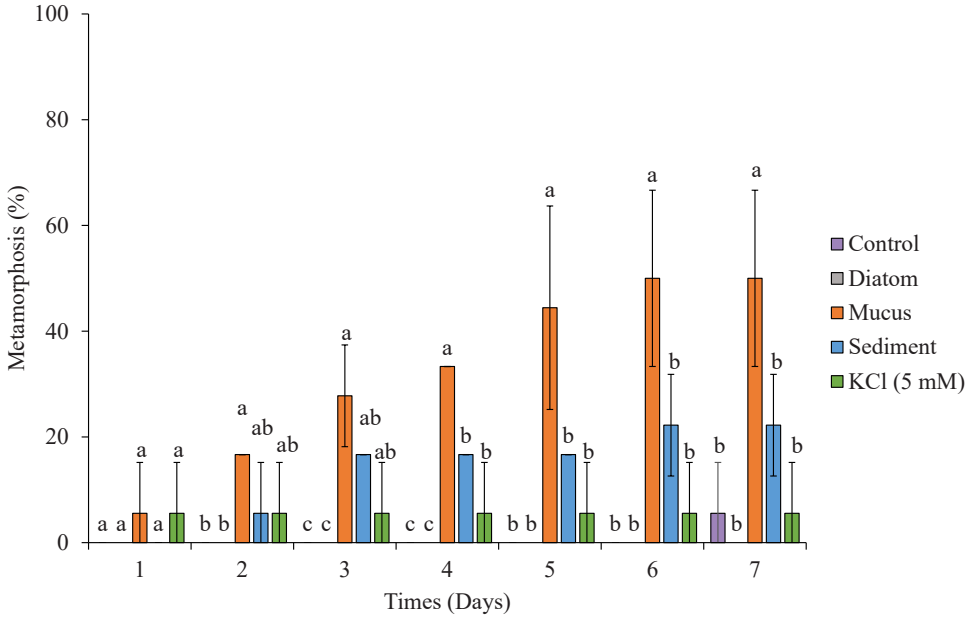

Fig. 8. Effects of different inducers on the metamorphosis rate of *Clithon diadema* larvae during a 7-day period. The letters above the bars indicate significant differences in means (N=3, n=3) between between inducers ($p < 0.05$).

C. diadema (Hendriks et al., 2003; Suhnel et al., 2012). Therefore, diatoms are the most suitable bait for promoting the reproduction and capsule production of *C. diadema*. Although *P. monodon* commercial feed can easily disintegrate in water and the food intake of *C. diadema* is low, a feed rich in eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) can promote the gonadal development and capsule production of *C. diadema*. The contents of EPA and DHA in *U. lactuca* are low (Tabarsa et al., 2012) (Supplementary Table 1). However, the lamellar structure of *U. lactuca* is favorable for *C. diadema* consumption. Thus, the *C. diadema* gonads of *U. lactuca* can mature and produce capsules. The 1 mm diameter filamentous structure of *C. fasciculari*s is insufficient for *C. diadema*, whose foot is approximately 15 mm in diameter. This size difference makes it difficult for *C. diadema* to navigate over such fine filaments for both crawling and feeding (Qi et al., 2010). Thus, the poor palatability of *C. fasciculari*s leaded to immature gonads and capsules in *C. diadema*. Comparing the diatom group, there was no significant difference in the appearance of capsules and eggs between the *P. monodon* commercial group and *U. lactuca* group. This finding indicates that the nutrient composition of these diets only affects the production of capsules but not the sizes of capsules and the number and diameter of eggs. The results of this study also found that egg capsules can be produced when the GHSI of *C. diadema* is above 29.90. Therefore, the value can be used as the basis for the maturation of *C. diadema* gonads.

Environmental salinity affects the physiology, growth, survival, and larval development of aquatic organisms (Boeuf and Payan, 2001; Pourmozaffar et al., 2020). When the environment changes substantially, the embryos of aquatic organisms fail to develop or even die. Gastropods produce egg masses, like gelatinous eggs or capsules, that control water and salt flow during reproduction (Kangas and Skoog, 1978). However, excessively high and low salinity will still cause the retardation of embryonic development (Pechenik et al., 2003; Przeslawski, 2004). Freshwater snails, acute bladder snail (*Physa acuta*) and pouched snail (*Glyptophysa gibbosa*), exhibit longer hatching times and lower egg survival with increasing salinity (0‰ to 5‰) after laying their egg masses in freshwater (Zukowski and Walker, 2009). Areola babylon (*Babylonia areolata*), a marine snail, lays capsules at 32‰ salinity, then moves to 20% and 25%, all embryos fail to hatch (Sreejaya, 2008). This study examined *C. diadema* capsules produced in freshwater, then incubated and hatched at salinities ranging from 0‰ to 35‰. If the embryo is kept in a 0‰ environment, it will not be able to hatch. This finding indicates that because *C. diadema* is a migratory organism, its capsules exhibit superior adaptability to fluctuations in environmental salinity. Howerev, when actue salinity changes by >30‰ (0‰ to 30‰), several adverse effects can occur, including slow embryonic development, failed embryonic development, prolonged hatching time for larvae, and decreased hatching rate.

Aquatic organisms adjust osmotic pressure through inorganic ions (e.g., Na^+, K^+, and Cl^-) and organic substances (urea and free amino acids). In invertebrates, free amino acids maintain isotonic pressure with the surrounding environment (Larsen et al., 2011). When exposed to extreme salinity, bivalves and gastropods close their shells, reducing metabolic function and conserving energy for osmotic pressure adjustment. (Veiga et al., 2016; Rola et al., 2017; Qin et al., 2020). Estuarine snails, like *Stramonita brasiliensis* and asian hornsnail (*Batillaria attramentaria*), survive in a broad salinity range (20‰–55‰ and 13‰–33‰) and exhibit normal activity without shell closure. (Veiga et al., 2016; Ho et al., 2019). *C. diadema* survived at 0‰–35‰ salinity; over a 4-week period, the survival rate was 86% at 35‰ salinity. This result indicates that *C. diadema* is a euryhalophilic snail and has a high tolerance to salinity. However, when salinity exceeds 25‰, capsule production of *C. diadema* begins to decline as the increased salinity forces *C. diadema* to use energy to adjust osmotic pressure. This diversion of energy away from essential processes such as gonad maturation ultimately leads to population decline (Wiesenthal et al., 2019). Neritidae species vary in salinity requirements for hatching. *Neritina zebra*, an estuary snail, thrives in 5‰–34‰ salinity. However, exposing their capsules to 15‰ salinity increases hatching time by 7 days compared to 5‰ salinity. (Barroso and Matthews-Cascon, 2009). Transferring *C. diadema* capsules from 0‰ salinity to 15‰–20‰ resulted in the fastest embryo development and shortest hatching time, but no development occurred at 30‰ salinity. This finding indicates that the embryo had high tolerance to salinity. Nevertheless, capsules cannot adjust in the presence of excessive changes in salinity, resulting in embryo development failure (Table 3). *C. diadema* embryos can develop at 0‰ salinity, but they cannot hatch successfully because the capsules require stimulation in a saline environment to trigger their opening and release the larvae. The embryos exhibited the fastest development and hatching rates at 15‰–20‰ salinity. Embryos could develop and hatch at 35‰ salinity, but the process required more time. The half-lethal time of larva without feeding after hatching was 1–3 days at 5‰–15‰ salinity and >6 days at

20‰–35‰ salinity. This finding is consistent with the characteristics needed by two-sided migratory larvae to survive in a salinity-rich environment (Kano, 2006). These results indicate that 15‰–20‰ salinity is the most suitable for *C. diadema* breeding and larva hatching.

The form, size, and nutritional content of bait affect the palatability and growth of larva. *Isochrysis* and *Pavlova* species without cell walls are easily digested by larvae (Martinez-Fernandeza et al., 2004; Tibbetts and Patelakis, 2022). Diatoms with large spines may hinder the intake of larvae (Lora-Vilchis and Maeda-Martinez, 1997). The larva of each species have their own nutritional requirements. Seawater larva cannot synthesize polyunsaturated fatty acids by themselves; thus, they need to eat the bait with high contents of EPA and DHA (Richmond, 2004). Freshwater larva have higher requirements for linoleic acid and α-linolenic acid (Richmond, 2004). *I. galbana* is rich in DHA, and *C. muelleri* is rich in EPA. Therefore, *I. galbana* and *C. muelleri* are commonly used as bait for raising the larva of snail and shellfish to improve their growth, survival, and metamorphosis (Hikihara et al., 2020; Yang et al., 2020). In this study, the *C. diadema* larva was found to utilize the velar cilia to capture *I. galbana* and *C. muelleri*, resulting in their stomachs and digestive glands being filled with these two microalgae (data no show). The survival rate and shell length of the *I. galbana* group were better than those of the *C. muelleri* group, indicating that high DHA levels have a positive effect on the survival and shell length of *C. diadema* larva. Salinity can affect the survival and growth performance of larva (Montory et al., 2014). When salinity exceeds the optimum range for larva, the larvae grow slowly and their survival rate decreases. For example, *Crassostrea nippona* larva had the highest survival rate and the best growth at the salinity of 26‰. At the salinity of 14‰ and 34‰, the larva close the shells and isolate from the external environment to reduce the energy required to adjust osmotic pressure. However, due to long-term nonfeeding and anaerobic metabolism, the growth of seedlings is slow and the survival rate is reduced (Wang et al., 2018).

C. diadema larvae had the highest survival rate and growth rate at 20‰–25‰ salinity. However, the survival rate of larvae exposed 35‰ salinity for 48 days was 0‰. This may be related to the regulation of larva osmotic pressure. The prolonged consumption of energy required to maintain osmotic pressure causes a decline in the survival rate and growth even when sufficient *I. galbana* is consumed. This result is the same as the optimum salinity range for lined nerite (*Nerita balteata*) larva, which belongs to the same family Neritidae (Dai Vu et al., 2021). Based on these findings, the optimal rearing conditions for *C. diadema* larvae involve culturing them at 25‰ salinity and providing a diet of *I. galbana* at a concentration of 5×10^4 cells/ mL.

Mollusk larva require a suitable diet or environment for metamorphosis (Gosling, 2008). Metamorphosis of snail larvae is primarily triggered by natural stimulants present in their living environment, such as their food, mucus, excrement, and debris residues of other snails (Cob et al., 2010; Hadfield et al., 2000; He et al., 2017; Yang et al., 2015). The presence of these stimulants in the environment indicates that the area is conducive to the survival of snails, providing an abundance of food and making it a suitable habitat for juvenile snails after metamorphosis (Cob et al., 2010). *N. atramentosa* larva can use pebbles to induce metamorphosis (Lesoway and Page, 2008). KCl can induce metamorphosis in most snail larva. When potassium ions are introduced into the sensory organ through the sodium–potassium pump, the intracellular membrane potential rises, causing depolarization and inducing larval metamorphosis (Hadfield et al., 2000; Yang et al., 2015; He et al., 2017). Sediments, macroalgal extracts, and KCl induced *S. canarium* larva to achieve 100% metamorphosis within 48 h. In addition, gamma-aminobutyric acid (GABA) secreted by diatoms can induce metamorphosis in small abalone (*Hediste diversicolor*) larva (Bryan and Qian, 1998). The mucus of wavy turban snail (*Lithopoma undosa*) induced a 78% increase in the metamorphosis rate of its larvae. *C. diadema* larvae developed forefeet and tentacles and began crawling at 48 days after hatching. The metamorphosis rate of larvae ranged from

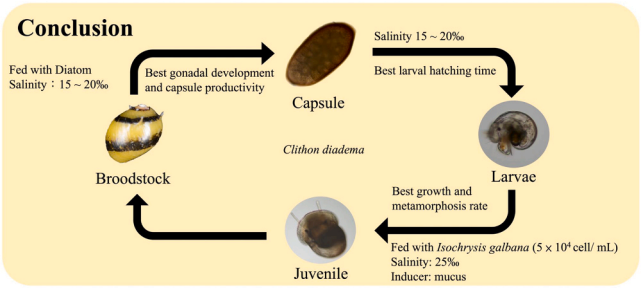

Fig. 9. Artificial reproduction and breeding method for *Clithon diadema*.

13.89% to 44.44% after providing them with a plastic plate covered with diatoms for 20–27 days. This study identified stimulants that can accelerate larval metamorphosis. The results indicated that mucus and sediments could promote the metamorphosis of larva and shorten larva development time, with mucus having the greatest effect. The sediment composition is complex, comprising food, mucus, excrement, and debris residues of other snails. However, the effect of mucus alone is superior. This finding suggests that mucus is the primary factor in inducing metamorphosis. KCl did not effectively induce the metamorphosis of larvae, and its higher concentration may be required. Diatoms can induce metamorphosis in *H. diversicolor* larvae ((Bryan and Qian, 1998), albeit with a lower capacity observed in *C. diadema* larvae, possibly due to variations in diatom species or species type.

In conclusion, this study successfully established an artificial propagation method for *C. diadema* (Fig. 9). Feeding diatoms to *C. diadema* broodstock at 15‰–20‰ salinity was observed to enhance gonad maturation and increase capsule production. The most rapid embryonic development and larvae hatching were observed at 15‰–20‰ salinity. The larvae were cultured at 25‰ salinity and fed with *I. algae* as their primary food source. Once the larvae developed propodium, the mucus from *C. diadema* broodstock was employed to induce larval metamorphosis.

CRediT authorship contribution statement

Yeh-Fang Hu: Methodology. **Fan-Hua Nan:** Supervision. **Hsieh-Yen Lee:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Yu-Ru Lin:** Formal analysis. **Huai-Ting Huang:** Writing – review & editing, Writing – original draft, Methodology.

Declaration of Competing 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.

Data availability

Data will be made available on request.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.aqrep.2024.102145.

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