

## Article

# The Effect of Food Deprivation on Foraging Behavior and Digestive and Metabolic Capacities of the Chinese Mitten Crab, *Eriocheir sinensis*

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**Abstract:** Food deprivation is a common stress in crustaceans that can affect their behavior and physiology. In this study, a video recording analysis system was used to compare the predation rate and behavior of the Chinese mitten crab, *Eriocheir sinensis*, foraging on the freshwater snail, *Bellamyia quadrata*, under different degrees of food deprivation. The activities of lactate dehydrogenase,  $\alpha$ -amylase, pepsin, and lipase in the hepatopancreas of crabs were determined after food deprivation for 0, 3, 9, and 15 days. The results showed that the predation and encounter rates of *E. sinensis* increased and then decreased with an increase in food deprivation time. The proportion of stationary time of *E. sinensis* initially decreased and then increased, whereas the proportion of searching and handling time increased initially and then decreased. There was a significant difference in the probability of capture upon encountering; however, food deprivation did not significantly affect the probability of consumption upon capture. Under food deprivation, the proportion of *E. sinensis* crushing tactics after nine-day food deprivation was significantly lower than that in the first nine days. Lactate dehydrogenase activity in the *E. sinensis* hepatopancreas after food deprivation was significantly higher than that in those not subjected to food deprivation, and the activities of  $\alpha$ -amylase and pepsin in the hepatopancreas were consistent with the predation rate trend, showing an initial increase followed by a decrease. In conclusion, different degrees of food deprivation significantly affected the predation cycle of *E. sinensis* on *B. quadrata*. These results lay a solid foundation for further studies on the foraging behavioral ecology of *E. sinensis* and provide important information for improving *E. sinensis* production.

**Keywords:** food deprivation; *Eriocheir sinensis*; *Bellamyia quadrata*; foraging behavior; physiology



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## 1. Introduction

The Chinese mitten crab *Eriocheir sinensis* is one of the most economically-important crabs in China. In 2019, the total production of *E. sinensis* was 780,000,000 kg [1]. In the pond culture process, yield can be largely increased when *E. sinensis* reasonably feeds on the freshwater snail *Bellamyia quadrata* [2–4]. Snails are used 3–5 times more annually in organic crab farming. One of the main reasons for this is that when crabs are deprived of food due to insufficient feed supply, seasonal changes, and heterogeneous food distribution, *B. quadrata* can be used as supplementary food [5,6]. In addition, molting, intraspecific competition for food, burrowing to withstand the enemy, and harsh winters can cause food deprivation [7,8].

Foraging of crabs is closely related to food deprivation [9]. For example, starvation causes the risk-averse blue crab *Callinectes sapidus* to forage [10]. Starved Dungeness crabs *Cancer magister* spend more time foraging for food under low-salinity and high-temperature conditions [11]. Starvation can change predation behavior, such as predation rate [12,13]; time distribution including stationary time, moving time, searching time, and handling time [12,14]; the selectivity coefficient [13]; and cannibalism [15]. The predation rate of the swimming crab *Portunus trituberculatus* and the Japanese stone crab *Charybdis japonica* initially increased and then decreased with the rise in food deprivation [12]. The white-leg shrimp *Litopenaeus Vannamei* deprived for 24 h and 48 h was less inactive and showed significantly increased feeding activity [14]. Furthermore, food deprivation causes changes in digestive enzyme activities, including protease, lipase, and amylase activities [16,17]. Li et al. [18] demonstrated that the pepsin, trypsin, and lipase activities of the oriental river prawn *Macrobrachium nipponense* initially decreased and then increased during the recovery growth experiment following different periods. In contrast, the amylase activity initially increased and then decreased significantly. There were similar findings for the red swamp crayfish *Procambarus clarkii* [19]. Predators change their behavior and physiology to adapt to food deprivation. However, the digestive enzyme activity of *E. sinensis* and the predation behavior against *B. quadrata* under different degrees of food deprivation remain unclear. It is arduous to establish a reasonable standard for feeding *E. sinensis* with *B. quadrata*. Therefore, it is essential to examine the predation cycle of *E. sinensis* foraging on *B. quadrata* under different degrees of food deprivation.

In this study, the predation cycle of *E. sinensis* foraging on *B. quadrata* under different degrees of food deprivation was analysed, and the differences in predation rate, encounter rate, probability of capture upon encounter, and probability of consumption upon capture by *E. sinensis* were compared. The digestive and metabolic enzyme activities of *E. sinensis* under different degrees of food deprivation were measured. We hypothesized that food deprivation would change the *E. sinensis* predation cycle and improve the predation rate. We also predicted that digestive and metabolic enzyme activities would decrease with increasing food deprivation.

## 2. Materials and Methods

### 2.1. Resources and Temporary Culture of Experimental Crabs and Snails

*E. sinensis* was obtained from the Chongming Base of Shanghai Ocean University, transported to the laboratory, and maintained in a circulating water system for a 14-day acclimation period. The aerated freshwater temperature was  $25.0 \pm 0.5$  °C, and the photoperiod was maintained at 12 h light/12 h dark.

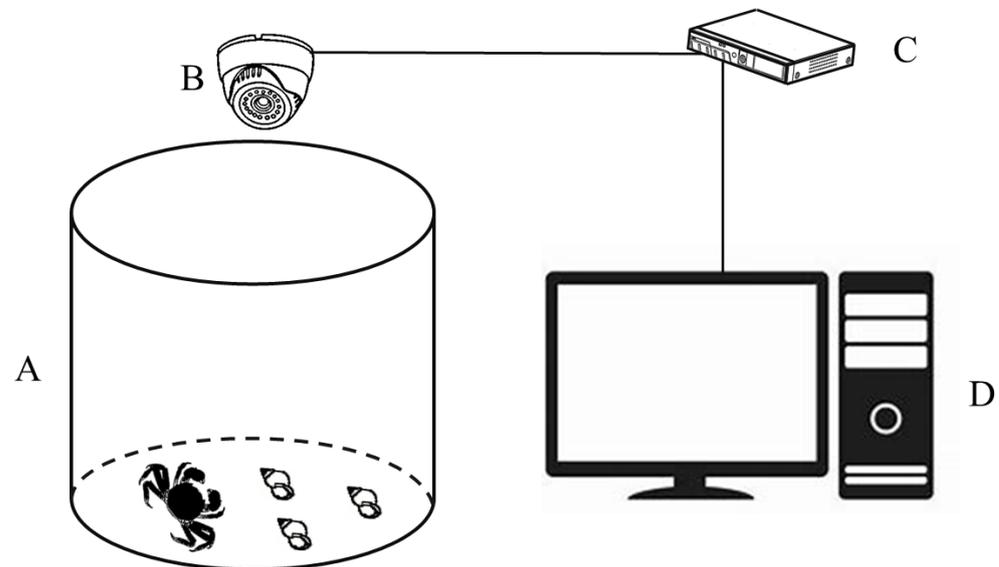
*B. quadrata* (shell width: 7–8 mm) were collected from The Coastal Base of Shanghai Ocean University and held in an aquarium with a water temperature of  $25.0 \pm 1.1$  °C and a photoperiod of 12 h light/12 h dark. The water was changed once per day and continuously aerated. Simultaneously, *B. quadrata* were fed once daily to adapt to the predatory environment.

### 2.2. Experimental Design and Procedures

The experiment was divided into a control group and five hunger treatment groups. In each treatment group, crabs were deprived for 0, 3, 6, 9, 12, and 15 days, represented by  $S_0$ ,  $S_3$ ,  $S_6$ ,  $S_9$ ,  $S_{12}$ , and  $S_{15}$ , respectively, and each treatment was replicated four times. After the acclimation period, digital calipers measured the crab carapace width to the nearest 0.01 mm. Male crabs (carapace width: 35–40 mm) in the intermolt stage with both chela and all walking legs were selected for use in the trials.

The crabs were transferred to a circular aquarium (0.5 m diameter  $\times$  0.7 m depth; Figure 1) using a circulating water system. The video recording analysis system included a camera (DS-2CD3T86FD-L, Hikvision, Shanghai, China), a network video recorder (7932-R4, Hikvision), and a monitor (DS-D5024FQ-NA, Hikvision). The camera was located 1 m above the surface. For each round of the aquarium experiment, one crab was placed

in the aquarium environment 24 h before the experiment, allowing crabs to adapt to the environment. The water temperature was  $25.0 \pm 0.9$  °C, and the photoperiod was 12 h light/12 h dark. No feeding was performed to regulate the crab's hunger levels during this period. Forty snails were placed in the experimental aquarium after starvation for a certain period.



**Figure 1.** Video camera recording analysis system and round experimental aquarium: (A) experimental aquarium, (B) camera, (C) network video recorder, and (D) monitor.

### 2.3. Index and Determination Methods

#### 2.3.1. Foraging Behavior Index

One hour during the day and one hour in the evening were randomly selected to observe and quantify crab foraging behavior [12]. The predation rate was calculated as the number of snails that each crab preyed on every day [20]. The encounter rate was calculated as the number of encounters divided by predator search time [21]. Crabs chose to capture snails or not, or snails would escape capture after encounter; furthermore, crabs chose to reject or consume snails after capture. Therefore, the probability of capture upon encounter ( $\text{Pr}(\text{capture}/\text{encounter})$ ) and the probability of consumption upon capture ( $\text{Pr}(\text{consumption}/\text{capture})$ ) were recorded [21,22]. The measurements were performed as follows:

$$\begin{aligned}\text{Pr}(\text{capture}/\text{encounter}) &= F2/F1, \\ \text{Pr}(\text{consumption}/\text{capture}) &= F3/F2\end{aligned}$$

where  $F1$  is the number of encounters between *E. sinensis* and *B. quadrata* during the observation period,  $F2$  is the number of captures of *B. quadrata* by *E. sinensis* during the observation period, and  $F3$  is the number of *B. quadrata* consumed during the observation period.

An ethogram was developed for *E. sinensis* foraging behavior based on Barbeau and Scheibling [23] (Table 1). The stationary time, moving time, searching time, handling time, and average handling time were quantified according to the statistics of the crab's static state, moving state, searching state, and handling state duration.

**Table 1.** Ethogram of foraging behavior of *Eriocheir sinensis*, adapted from Barbeau and Scheibling [23].

Behavior	Description
Stationary	Crab is in a steady position or grooming.
Moving	Crab movement forward or backward with position change without probing/sweeping.
Searching	Crab's walking leg probes and sweeps over the floor of the aquarium.
Handling	Handling for crabs is the period from capture until the crab moves away after consuming the snails.

### 2.3.2. Shell-Breaking Techniques and the Proportion of Successful Snail Avoidance

This study analysed the methods of shell breaking and the proportion of successful snail avoidance. The handling method was inferred from the shape of the shells of the residual snails after crab predation [12,24,25], which were classified into three categories: probing (i.e., the shell was not damaged), peeling (i.e., some tissues were missing at the edge of the shell), and crushing (i.e., the shell was broken, and only shell fragments were left). The proportion of crushing tactics was the ratio of the number of broken shells to the total number of snail shells. Snails sense chemical cues and climb above the waterline in the presence of active predators. The number of snails that crawled above the waterline was recorded hourly. The proportion of snails that achieved successful avoidance was the ratio between the number of snails above the waterline and the total number of snails [26].

### 2.3.3. Digestive and Metabolic Enzyme Activities

After food deprivation for 0, 3, 9, and 15 days, four crabs were randomly selected and anesthetized by chilling. The hepatopancreas samples were rapidly removed and stored in centrifuge tubes at  $-20\text{ }^{\circ}\text{C}$ . Ice bath homogenization was performed according to the ratio of hepatopancreas tissue mass (g) to extract volume (mL) of 1:5. The homogenates were centrifuged at  $8000\times g$  for 10 min at  $4\text{ }^{\circ}\text{C}$ , and the supernatant was collected and placed on ice for testing. The activities of lactate dehydrogenase (LDH),  $\alpha$ -amylase ( $\alpha$ -AL), pepsin, and lipase (LPS) were determined using commercial lactate dehydrogenase assay kits (A020-1-2),  $\alpha$ -amylase assay kits (C016-1-1), pepsin assay kits (A080-1-1), and lipase assay kits (A054-1-1) (Nanjing Jiancheng Institute of Biotechnology, Nanjing, China), respectively, according to the manufacturer's instructions.

## 2.4. Statistical Analysis

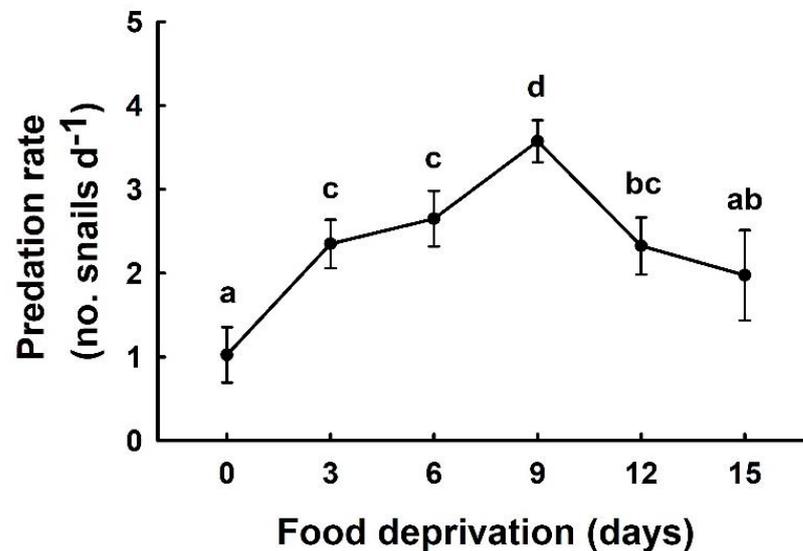
By observing the predation cycle of *E. sinensis* foraging on *B. quadrata*, the obtained indices were expressed as mean  $\pm$  standard deviation (SD). Statistical analysis was performed using IBM SPSS Statistics v.22. The predation rate, behavioral data, and digestive and metabolic enzyme activity data were analysed by analysis of covariance (ANCOVA), using food deprivation as the main factor and experiment date as a covariate. A one-way ANOVA was used to analyse the proportion of state behavior time for *E. sinensis* under different degrees of food deprivation, and Duncan's test was used for multiple comparisons. Differences were considered statistically significant at  $p < 0.05$ .

## 3. Results

### 3.1. Average Predation Rate

As the date of the recording for each treatment was different and may affect the average predation rates and foraging behaviors, it was used as a covariate for the analysis of covariance to exclude confounding interference from the date on subsequent results. The average predation rates of *E. sinensis* under different degrees of food deprivation are shown in Figure 2. The average predation rate of *E. sinensis* after 0, 3, 6, 9, 12, and 15 days of food deprivation ( $S_0$ ,  $S_3$ ,  $S_6$ ,  $S_9$ ,  $S_{12}$ , and  $S_{15}$ , respectively) was  $1.0\text{ snails d}^{-1}$ ,  $2.4\text{ snails d}^{-1}$ ,  $2.6\text{ snails d}^{-1}$ ,  $3.6\text{ snails d}^{-1}$ ,  $2.3\text{ snails d}^{-1}$ ,  $1.9\text{ snails d}^{-1}$ , respectively, showing a trend of increasing at first and then decreasing with an increase in the degree of food deprivation.

The average predation rates of the  $S_0$ ,  $S_3$ ,  $S_6$ ,  $S_9$ , and  $S_{12}$  treatments were significantly higher than that of  $S_0$  ( $p < 0.05$ ), but there was no significant difference between the  $S_{15}$  and  $S_0$  treatments ( $p > 0.05$ ). The average predation rate of the  $S_9$  treatment was significantly higher than that of the other treatments ( $p < 0.05$ ).



**Figure 2.** Predation rate of *Eriocheir sinensis* under different degrees of food deprivation. Different lowercase letters denote significant differences from Duncan's post hoc test.

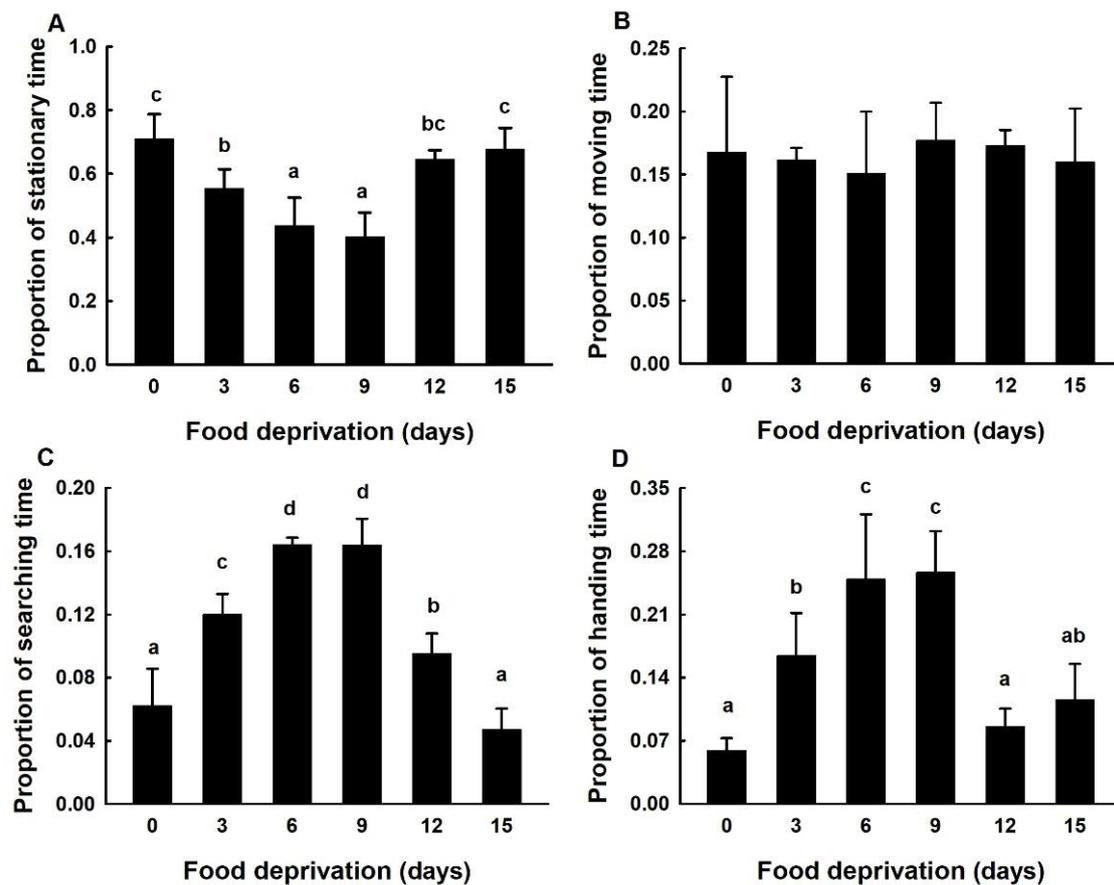
### 3.2. Temporal Distribution of State Behavior

The proportions of stationary time for the  $S_0$ ,  $S_3$ ,  $S_6$ ,  $S_9$ ,  $S_{12}$ , and  $S_{15}$  treatments were 71%, 55%, 44%, 40%, 65%, and 68%, respectively (Figure 3A). With increasing food deprivation time, the stationary time ratio of *E. sinensis* initially decreased and then increased. The highest proportion of stationary time was under the  $S_0$  treatment, and the lowest was under the  $S_9$  treatment.  $S_3$ ,  $S_6$ , and  $S_9$  exhibited a significantly lower proportion of stationary time than  $S_0$  ( $p < 0.05$ ); however, there was no significant difference in the proportion of stationary time among  $S_{12}$ ,  $S_{15}$ , and  $S_0$  ( $p > 0.05$ ). Additionally, the proportion of stationary time was significantly lower under  $S_6$  and  $S_9$  treatments than under  $S_3$ ,  $S_{12}$ , and  $S_{15}$  treatments ( $p < 0.05$ ).

As shown in Figure 3B, the proportion of moving time under the  $S_0$ ,  $S_3$ ,  $S_6$ ,  $S_9$ ,  $S_{12}$ , and  $S_{15}$  treatments was 17%, 16%, 15%, 18%, 17%, and 16%, respectively. The highest proportion of moving time was for  $S_9$ , while the lowest was for  $S_6$ . There were no significant differences among the treatments ( $p > 0.05$ ).

The proportion of search times for the  $S_0$ ,  $S_3$ ,  $S_6$ ,  $S_9$ ,  $S_{12}$  and  $S_{15}$  treatments were 6%, 12%, 16%, 16%, 10%, and 5%, respectively (Figure 3C). The *E. sinensis* searching time proportion increased and decreased with increasing food deprivation time. The highest proportion of search time was for  $S_9$ , and the lowest was for  $S_0$ .

The proportion of handling times for the  $S_0$ ,  $S_3$ ,  $S_6$ ,  $S_9$ ,  $S_{12}$ , and  $S_{15}$  treatments was 6%, 16%, 25%, 26%, 9%, and 11%, respectively (Figure 3D), showing an initial increase followed by a decrease. The highest proportion of handling time was for  $S_9$ , and the lowest was for  $S_0$ . The proportion of handling times for  $S_3$ ,  $S_6$ , and  $S_9$  was significantly higher than that for  $S_0$  ( $p < 0.05$ ), but there was no significant difference among  $S_{12}$ ,  $S_{15}$ , and  $S_0$  ( $p > 0.05$ ). Furthermore, the handling times for  $S_6$  and  $S_9$  were significantly higher than those for  $S_3$ ,  $S_{12}$ , and  $S_{15}$  ( $p < 0.05$ ).



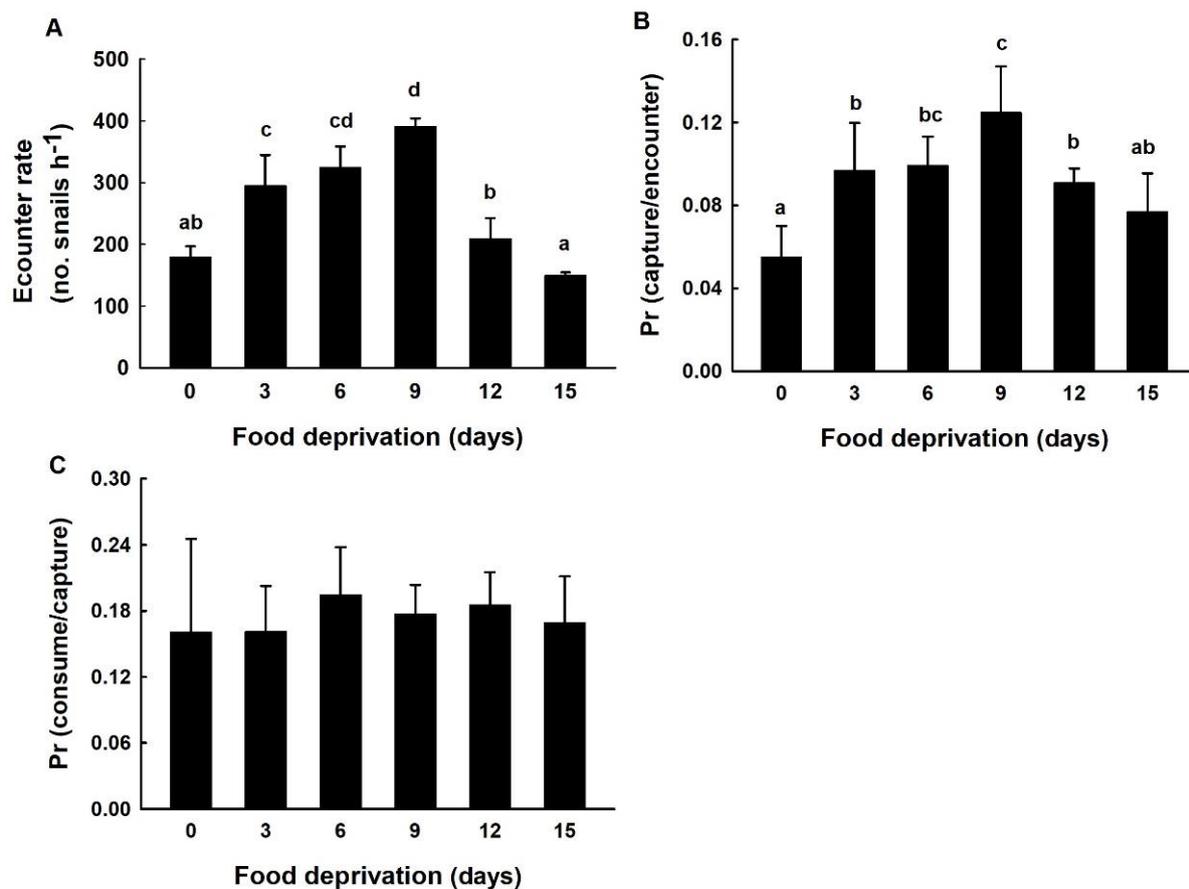
**Figure 3.** Temporal distribution of the behavior state of *E. sinensis* under different degrees of food deprivation: (A) proportion of stationary time, (B) proportion of moving time, (C) proportion of searching time, and (D) proportion of handling time. Different lowercase letters indicate significant differences among treatments ( $p \leq 0.05$ ).

### 3.3. The Encounter Rate, the Probability of Capture upon Encounter, and the Probability of Consumption upon Capture

The encounter rates between *E. sinensis* and *B. quadrata* under different degrees of food deprivation are illustrated in Figure 4A, showing an initial increase and then a decrease with an increasing degree of food deprivation. The encounter rate was significantly higher under the  $S_3$ ,  $S_6$ , and  $S_9$  treatments than under  $S_0$  ( $p < 0.05$ ) but was not significantly different from that under  $S_0$  or between that under the  $S_{12}$  and  $S_{15}$  treatments ( $p > 0.05$ ). In addition, the encounter rate under  $S_9$  was significantly higher than that under  $S_3$ ,  $S_{12}$ , and  $S_{15}$  ( $p < 0.05$ ).

### 3.4. Handling Time per Snail, Proportion of Crushing Tactics, and Successful Avoidance

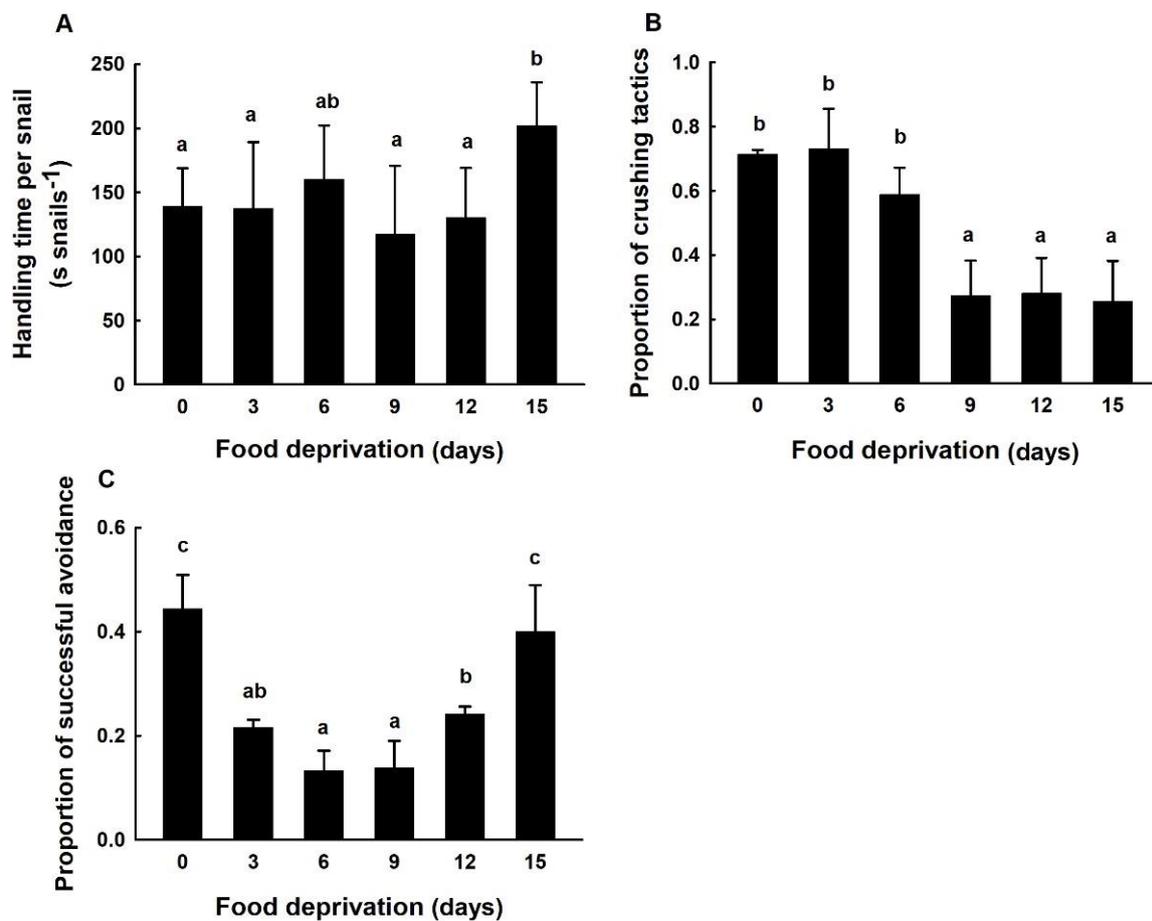
The handling times per snail for  $S_0$ ,  $S_3$ ,  $S_6$ ,  $S_9$ ,  $S_{12}$ , and  $S_{15}$  were 139, 137, 160, 117, 131 and 182 s, respectively, with the highest being for  $S_{15}$  and the lowest being for  $S_9$  (Figure 5A). The handling time per snail under the  $S_{15}$  treatment was significantly higher than that under  $S_0$  ( $p < 0.05$ ), whereas there was no significant difference between  $S_0$  and the other treatments ( $p > 0.05$ ). In addition, the handling time per snail under  $S_{15}$  was significantly higher than that under the other treatments ( $p < 0.05$ ).



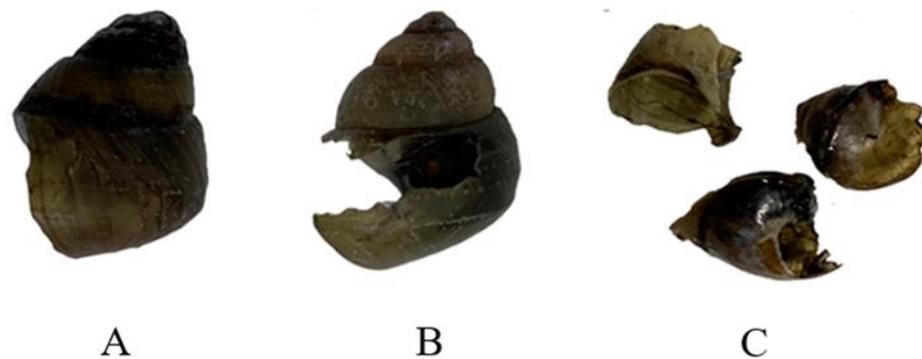
**Figure 4.** Changes of (A) the encounter rate, (B) the probability of capture upon encounter, and (C) the probability of consumption upon capture under different degrees of food deprivation in *E. sinensis*. Lowercase letters indicate significant differences among treatments ( $p \leq 0.05$ ).

*E. sinensis* preyed on *B. quadrata* using the three shell-breaking techniques (Figure 6). After the experiment, we found three snail shell stations: probing, in which the shell was not damaged and the flesh was consumed (Figure 6A); peeling, in which some tissues were missing at the edge of the shell (Figure 6B); and crushing, in which the shell was broken, and only shell fragments were left (Figure 6C). The proportion of crushing tactics used by *E. sinensis* foraging on *B. quadrata* under different degrees of food deprivation is shown in Figure 5B. The proportion of crushing tactics under  $S_0$ ,  $S_3$ ,  $S_6$ ,  $S_9$ ,  $S_{12}$ , and  $S_{15}$  were 71%, 73%, 59%, 27%, 28%, and 26%, respectively. The highest proportion of crushing tactics was under  $S_3$ , whereas the lowest was under  $S_{15}$ . The proportion of crushing tactics under the  $S_9$ ,  $S_{12}$ , and  $S_{15}$  treatments was significantly lower than that under  $S_0$  ( $p < 0.05$ ), but there were no significant differences among the  $S_3$ ,  $S_6$ , and  $S_0$  treatments ( $p > 0.05$ ). Additionally, the proportion of crushing tactics under the  $S_3$  and  $S_6$  treatments was significantly higher than that under the  $S_9$ ,  $S_{12}$ , and  $S_{15}$  treatments ( $p < 0.05$ ).

The proportion of successful avoidance of *B. quadrata* under different degrees of food deprivation is shown in Figure 5C. The proportion of successful avoidance under  $S_0$ ,  $S_3$ ,  $S_6$ ,  $S_9$ ,  $S_{12}$ , and  $S_{15}$  were 44%, 22%, 13%, 14%, 24%, and 40%, respectively. The highest proportion of successful avoidance was under  $S_0$ , whereas the lowest was under  $S_6$ . A significant reduction in the proportion of successful avoidance was observed under the  $S_3$ ,  $S_6$ ,  $S_9$ , and  $S_{12}$  treatments compared to that under  $S_0$  ( $p < 0.05$ ), but there was no significant difference between the  $S_{15}$  and  $S_0$  treatments ( $p > 0.05$ ). In addition,  $S_6$  and  $S_9$  treatments resulted in a significantly lower proportion of successful avoidance than  $S_{12}$  and  $S_{15}$  ( $p < 0.05$ ).



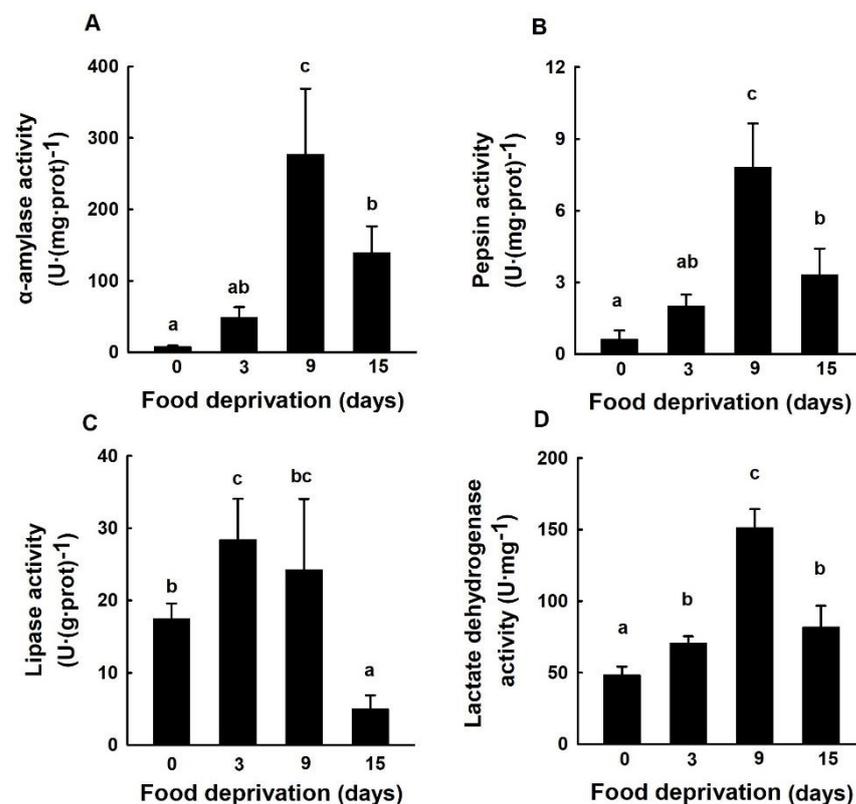
**Figure 5.** (A) The handling time per snail, (B) the proportion of crushing tactics, and (C) the proportion of successful avoidance under different degrees of food deprivation in *E. sinensis*. Different lowercase letters indicate significant differences among treatments ( $p \leq 0.05$ ).



**Figure 6.** Illustration of shell conditions used to infer the handling method of *E. sinensis*: (A) probing, (B) peeling, and (C) crushing.

### 3.5. The Digestive and Metabolic Enzyme Activities

The  $\alpha$ -amylase activity in the hepatopancreas of *E. sinensis* under different degrees of food deprivation is shown in Figure 7A. The activity of  $\alpha$ -amylase was affected by the food deprivation time, with a tendency to increase first and then decrease. The  $\alpha$ -amylase activity under S<sub>9</sub> was significantly higher than under other treatments ( $p < 0.05$ ).



**Figure 7.** The activities of the digestive enzymes (A)  $\alpha$ -amylase, (B) pepsin, and (C) lipase and the activity of the metabolic enzyme (D) lactate dehydrogenase of *E. sinensis* under different degrees of food deprivation. Different lowercase letters indicate significant differences among treatments ( $p \leq 0.05$ ).

As shown in Figure 7B, pepsin activity tended to increase initially and decrease with increasing food deprivation time.  $S_0$ ,  $S_3$ , and  $S_{15}$  pepsin activities were significantly lower than that of  $S_9$  ( $p < 0.05$ ), with no significant difference among them ( $p > 0.05$ ).

Lipase activity in the hepatopancreas of *E. sinensis* under different degrees of food deprivation is shown in Figure 7C. The lipase activity under  $S_3$  was significantly higher than that under  $S_0$  ( $p < 0.05$ ), and the lipase activity under  $S_{15}$  was significantly lower than that under  $S_0$  ( $p < 0.05$ ), whereas there were no significant differences between the  $S_9$  and  $S_0$  treatments ( $p > 0.05$ ). The lipase activity under  $S_3$  and  $S_9$  was significantly higher than that under  $S_{15}$  ( $p < 0.05$ ). The pepsin activity in the hepatopancreas of *E. sinensis* was lower than that of  $\alpha$ -amylase and lipase.

The lactate dehydrogenase activity in the hepatopancreas of *E. sinensis* under  $S_3$ ,  $S_9$ , and  $S_{15}$  treatments was significantly higher than that under  $S_0$  ( $p < 0.05$ ). In addition, the lactate dehydrogenase activity under  $S_9$  was significantly higher than that under  $S_3$  and  $S_{15}$  ( $p < 0.05$ ; Figure 7D).

#### 4. Discussion

##### 4.1. Effects of Different Degrees of Food Deprivation on the Foraging Behavior of *E. sinensis*

There is a growing recognition of the importance of animal behavior in aquaculture, but this has not been explored concerning *E. sinensis* farming. This study demonstrates that different degrees of food deprivation can affect the *B. quadrata* predation cycle of *E. sinensis*. Previous studies have suggested that the crab predation rate is closely related to hunger levels [12]. Under food deprivation stress, the predation rates of *P. trituberculatus* and *C. japonica* initially increased and then decreased with an increase in the degree of food deprivation, which is similar to our findings [12]. This result may be because, in the early stage of food deprivation, *E. sinensis* exhibits increased searching time and handling time to obtain food to meet its energy demand, resulting in an increased predation rate.

However, after nine-day food deprivation, *E. sinensis* may actively regulate their energy budgets, utilize the limited reserve energy for primary physiological metabolism [27,28], and reduce the searching time and handling time, which leads to a decline in predation rate. The predation rate was the highest when deprived of food for nine days in our study, while that of *P. trituberculatus* and *C. japonica* was six days [12]. This indicates that different crustaceans have different starvation tolerances, and the same species also has different starvation tolerances in different growth stages [29], which may be due to different body fat content [30] and physiological functions [31]. The study on predation cycles can facilitate improvements in feeding techniques and management levels.

Studies have suggested that stationary behavior indicates low feeding motivation [14]. Lee and Meyers [32] found that the levels of stationary behavior decreased after food deprivation by stimulating foraging for *L. vannamei* through the feed. *E. sinensis*, under short-term food deprivation, has a stricter selection criterion for prey [33]; accordingly, it tends to spend more time and energy on feed acquisition. The decrease in searching behavior levels after nine days may be because starvation regulates energy allocation by reducing search and handling time to reduce foraging investment in snails. Searching behavior during long-term food deprivation may cause excessive energy consumption, and such excessive searching should be reduced in production by increasing prey density to increase the encounter rate. Handling time includes shell breaking time and feeding time, and shell breaking time is related to the presence of an operculum and shell thickness [24]. In future studies, the handling time can be combined with the energy content of the prey to evaluate the net energy intake per unit of handling time to achieve the optimal foraging theory.

Predation rates can be analysed using three components: encounter rate, the probability of capture upon encounter, and the probability of consumption upon capture [23]. Food deprivation causes the frequency of crustacean activity to increase and then decrease [34], resulting in the same trend in the encounter rate with snails. The probability of capture upon encounter was determined by prey size and/or predator autonomy. Prey size was almost identical in our study; consequently, food deprivation influenced whether the predator preyed on, and thus, the probability of capture upon encounter. These results indicate that food deprivation affected *E. sinensis* predation rates mainly by affecting the encounter rate and whether it could capture snails after the encounter. The avoidance behavior of snails resulted in a lower encounter rate between crabs and snails. In addition, the odorant of hungry crabs possibly drives away snails and thus the patterns of avoidance behavior. The warning substance in the urine of blue crabs could scare off their prey, which would respond more to the scent of a predator consuming the same species [35]. We propose that in *E. sinensis*, food deprivation drives an increase in the release of a substance that causes avoidance behavior in the snails, but this is only conjecture and requires further experimental verification.

#### 4.2. Effects of Food Deprivation on the Shell-Breaking Techniques of *E. sinensis*

Crabs mainly prey on snails by using three shell-breaking techniques [24,25,36]. In the probing technique, the crab removes the flesh through the aperture of the shell using a chela. In the shell-peeling technique, the crab breaks the shell of the snail along the lip of the aperture using chelae to remove flesh from the shell. In the shell-crushing technique, the crab squeezes the posterior or middle portion of the shell. A similar phenomenon was observed in the present study: *E. sinensis* preyed on *B. quadrata* using these three shell-breaking techniques. This suggests that shell-breaking techniques of *E. sinensis* are influenced by food deprivation and that they have flexible handling techniques for prey, which may be linked to their ability to acquire food successfully and invade new habitats [37]. The crushing shell-breaking technique seems to be more efficient, since this technique can crush prey to obtain tissues and obtain more energy per unit time [38]. However, handling techniques changed after food deprivation for a certain period. This

preference may be because *E. sinensis* uses the probing shell-breaking technique to save energy with increasing food deprivation.

The avoidance behavior of snails is influenced by predator activity, which in turn influences crab predation [26]. In this study, the avoidance behavior of snails was conspicuously affected by the different food deprivation states of *E. sinensis*, with the avoidance behavior of snails showing opposite trends to the predation rate and activity frequency of the crab, owing to chemical cues from active predators and dead conspecifics [26]. However, this study did not find that the avoidance behavior of *B. quadrata* had a significant impact on the *E. sinensis* foraging behavior, which may be because there were 40 snails in this experiment. Thus the crabs had plenty of prey to choose from, and few crabs actively chose to prey on the snails that had successfully evaded. In nature, the predator–prey relationship is complex and is worth further study.

#### 4.3. Effects of Food Deprivation on Crab Digestive and Metabolic Enzyme Activities

In addition to behavioral responses to food deprivation, crustaceans have physiological regulatory mechanisms, including changes in the activities of digestive and metabolic enzymes [39,40]. The metabolic and digestive enzymes of the hepatopancreas in *E. sinensis* respond significantly to food deprivation. Evidence that food deprivation affects the predation behavior of *E. sinensis* is shown by different lactate dehydrogenase activities, which have been proven to be one of the key enzymes for measuring the metabolic status of crustaceans [41]. Lactate dehydrogenase activity in the hepatopancreas of food-deprived *E. sinensis* was significantly higher than that in crabs not subjected to food deprivation, which indicated that the metabolic rate of *E. sinensis* drastically decreased under food deprivation. In addition, food deprivation conspicuously affects digestive enzyme activities [42,43]. Complying with food deprivation is one strategy crustaceans use to reduce digestive enzyme activity [44]. The digestive enzyme activities measured in this study showed an upward and downward trend, slightly different from the expected downward trend. It is reasonable to assume that digestive enzyme activity may decrease during food deprivation when there is no substrate load in the intestine [17]. This may be due to the lack of food stimulation and induction, which reduces the secretion of digestive enzymes [45]. Alternatively, the tissue structure may be atrophied and stunted owing to food deprivation, resulting in a decline in function and even a cessation of digestive enzyme production [46]. However, digestive enzyme activities increased in the early stages of food deprivation in this experiment, probably because of the use of residual food in the *E. sinensis* intestine or the mobilization of nutritional reserves to sustain life when food is scarce [47]; however, digestive enzyme activity tended to decrease under long-term starvation [44]. Nonetheless, starvation had no significant effect on the activities of protease and amylase in *Cherax quadricarinatus* [48]; therefore, comparisons between seawater and freshwater species should be performed with caution. Moreover, the peak lipase activity in the crab hepatopancreas appeared under the S<sub>3</sub> treatment, possibly because lipids are utilized to provide energy first and are the main energy source for *E. sinensis* during starvation, followed by carbohydrates and proteins [49]. Increased enzyme activity is generally associated with decreased reserves, and decreased fat storage is considered a typical feature of short-term starvation [48]. However, this study failed to detect changes in the content of energy substances under food deprivation, which could be further explored in juvenile *E. sinensis*. Furthermore, the diversity of responses to starvation in different crustaceans shows no preference for resource utilization [50]. Some species, such as the Japanese tiger prawn *Penaeus japonicus*, utilized glycogen first as a resource [51]. Subsequently, lipase activity decreased with an increase in food deprivation, which is consistent with previous work [52]. Calvo et al. found that lipase activity decreases during starvation, suggesting that lipase may not be synthesized when food is unavailable [48]. This concurs with the hypothesis of digestive lipase activity regulation proposed by Sacristan et al. [52], which states that when food is absent for a long time, the de novo synthesis of intracellular lipase is stimulated; therefore, lipids stored as energy reserves are mobilized.

## 5. Conclusions

These results suggest that food deprivation significantly affects the foraging behavior and metabolic and digestive enzyme activities of *E. sinensis*. These properties initially increased and then decreased with an increase in food deprivation time. Additionally, food deprivation affects the handling of *E. sinensis*. After food deprivation for nine days, the proportion of crushing tactics decreased significantly, and more attention was paid to the probing shell-breaking technique. These results will help to determine the feeding strategy of crabs to improve production and provide a reference for future research on the foraging behavior of *E. sinensis*.

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