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Effects of limb autotomy on growth, feeding and regeneration in the juvenile *Eriocheir sinensis*

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ABSTRACT

Eriocheir sinensis, as an emerging aquaculture species, has become more popular in Chinese pond culture. However, high density stocking has usually caused high limb autotomy rate among juvenile crabs, and there are still very few reports about the effects of autotomy on both survival and growth rates in the juvenile *E. sinensis*. Therefore, we aimed at the effects of limb autotomy on the growth of *E. sinensis* through the individual cultivation method. The results showed that almost all injured juvenile crabs could feed and molt as normal crabs, and could manage to regenerate new limbs. The duration of the 1st molt had a negative correlation with the number of autotomized limbs. After the 2nd molt, the size of the new limb was comparable to that of the normal limb and the weight growth rate decreased with the increasing number of autotomized limbs. However, a positive correlation was found between the specific growth rate and the number of autotomized limbs during the 1st molting period. In this period, the feeding rate decreased slightly, and the crabs with more limb loss tended to have the lower feeding rates, but this had no significant difference ($P > 0.05$). Besides, feed efficiencies in experimental sets were slightly higher than the control, and those crabs that autotomized more limbs correspondingly had the higher feed efficiencies ($P < 0.05$). Meanwhile, the results also revealed that limb autotomy had no significant effects on the duration of the 2nd molting, the weight growth rate, the specific growth rate as well as the feeding rate in *E. sinensis* juveniles.

Statement of relevance: Eriocheir sinensis, as one emerging aquaculture species, has become more popular in Chinese pond culture. However, the high culture density has usually caused a high rate of autotomy among the juveniles and there are still few reports on the effects of autotomy on the survival and growth rate in *E. sinensis*. The present study was to assess the effect of autotomy on the growth of *E. sinensis* such as the molting period, weight growth rate, specific growth rate, feeding rate as well as feed efficiency in *E. sinensis* juveniles, through the individual cultivation method.

1. Introduction

Animals living in natural environment will inevitably encounter a variety of attacks from other species or even their own species. However, in response to the prey or cannibalism, most animals usually take some countermeasures to ensure their safety (Li and Sun, 2013). Among these countermeasures, limb autotomy is commonly adopted by many animals to confuse their opponents and then escape from the dangers, such as rodents (Maginnis, 2006; Fleming et al., 2007), Arachnida (Arnold, 1988), crustaceans (Smith and Hines, 1991), and echinoderms (Mauzey et al., 1968; Bingham et al., 2000). For example, lizards would autotomize their tails (Lawrence, 1992), spiders would autotomize their legs (Maginnis, 2006), and sea stars would autotomize their arms (Ramsay et al., 2001) while being in danger. Moreover, many shellfish, such as crabs and shrimps, can also escape from the risk by autotomizing their chelipeds, walking limbs and swimming legs. Although some of their appendages are damaged totally or partially by their predators and competitors, shellfish are willing to cut off the injured part of appendages so as to avoid further infections (McVean and Findlay, 1979).

It is well known that many animals are able to spontaneously regenerate the same organ after the autotomy. Shellfish can also reproduce limbs via the action of molting. The speed of regeneration depends mainly on the gender (Mohrherr, 1987), the age (Mariappan et al., 2000), the molting period (Hopkins, 1982; Quinitio and Estepa, 2011), and the type or number of limbs lost (Bennett, 1973). Although limb autotomy is throughout the shellfish lifecycle, their regeneration exists in the molting phase of larva or juveniles only (Stueckle et al., 2008). The effects of limb autotomy and regeneration on shellfish physiology or ecology have been concerned and investigated extensively, and these

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include feeding rate (Edgar, 1990; Juanes, 1992), survival rate (Parsons and Eggleston, 2005; Frisch and Hobbs, 2011), immune function (Plaistow et al., 2003; Slos et al., 2008), growth rate (Frisch and Hobbs, 2011; Quinitio and Estepa, 2011), reproduction (Smith, 1992), conspecies competition (Daleo et al., 2009) and the ability to resist predators (Davenport et al., 1992; Smith, 1995). All these researches have laid a good theoretical foundation for the individual biology and population ecology.

Due to its high nutritional value and market demand, Chinese mitten crab (*Eriocheir sinensis*) has increasingly become a promising commercial aquaculture species in China (Sui et al., 2011). The aquaculture cycle of *E. sinensis* normally takes two years: in the first year growing from the megalopae to the "coin-sized" crab (3–10 g/ind.) and in the second year growing from the "coin-sized" crab to the market-sized crab (80–200 g/ind.) (He et al., 2014). The stocking density during the first year is very high (35–50 ind./m^2), high density crabs are very vulnerable to the predator invasion. Additionally, the aggressive instinct of *E. sinensis* results in a frequent autotomy among juvenile crabs (Sui et al., 2011). Zhao et al. (2015) found that limb autotomy rate of coin-sized crabs reached up to 30%, but by now effects of autotomy on juvenile survival and growth rates have not been well studied.

In this study, our objective is to determine the influences of limb autotomy and regeneration on the molting period, growth rate, feeding rate, and feed efficiency in the juvenile *E. sinensis*. To achieve this, juvenile crabs were selected from the earth pond and then they were randomly divided into six groups. Crabs in each group were forced to autotomize the given limbs. Thereafter, each crab was separately stocked under the same condition to investigate the above parameters within two molting periods using the individual cultivation method. This study would well expand the knowledge of individual biology and breeding ecology of *E. sinensis*, and would also further uncover the effects of autotomy on the physiology and ecology of *E. sinensis*.

2. Materials and methods

2.1. Source of juvenile crabs

In early July 2015, approximately 400 *E. sinensis* juvenile crabs had been selected from the earth pond at the Chongming research base of Shanghai Ocean University. They had the same morphological characteristics (e.g. crack in trailing edge and yellow shell), which indicated these crabs would molt shortly (Fig. 1). All crabs were temporarily stocked in a tank(length × width × depth = 2 m × 1 m × 0.5 m) with a recirculating water supply and some *Elodea nuttallii* for juveniles' concealment while molting. After 6 h, 128 soft-shelled crabs in the post-molting period were collected and transferred into another tank under the same condition. After 12 h, all crab shells became hard.

Fig. 1. The soft-shelled and the normal *E. sinensis* juvenile crabs under the same condition. Soft-shelled juvenile crabs usually have a black shell while the crabs usually have a yellow shell would molt shortly. The belly of soft-shelled crabs is whiter than that of the normal crabs.

2.2. Experimental design

Ninety-six hard-shelled crabs were selected (48 individuals for each gender) from the above 128 soft-shelled crabs and then randomly divided into six groups (A0, A1, A2, A3, A4, and A5; males:females = 1:1). Crab surface was gently dried with a towel and then weighed. Afterwards, they were forced to autotomize the given limbs as below: A0 did not autotomize any limb (the control), A1 randomly autotomized 1 limb, A2 randomly autotomized 2 limbs, A3 randomly autotomized 3 limbs, A4 randomly autotomized 4 limbs, and A5 randomly autotomized 5 limbs. This work could be done by gently grasping limbs with fingers and then the crab would autotomize the corresponding limbs spontaneously. Finally, they were raised separately in 96 tanks (length × width × depth = 30 cm × 20 cm × 18 cm, 7 L water). During this experiment, water depth was maintained at 12 cm and the light was provided overhead by a fluorescent under a 12 h:12 h (light:dark) cycle. All tanks were aerated gently and 8 cm PVC tubes (a diameter of 5 cm) were provided as shelters. Water conditions were maintained at 26 ± 2 °C, pH 7.8 ± 0.2 and DO less than 5 mg/L. These water parameters were the optimum levels for the juvenile *E. sinensis* (Kang et al., 2012). The experimental crabs were excessively fed with commercial formulated diets (Xinxin Diet 1# and 2# in year 2) once per day at 17:00. The uneaten feed was completely removed after 2 h and then 1/3 volume of water was exchanged, and the weight of eaten feed was calculated as exactly as 0.001.

2.3. Data collection and calculation

Over the experiment, the number of death and the molting time were recorded every day and crabs were weighted 24 h after molting. This experiment would not be terminated until all crabs had completed the 2nd molt. The weight growth rate (WGR), specific growth rate (SGR), feeding rate (FR), and feed efficiency (FE) in each molting period were separately calculated on the basis of the following formulas:

$$\mathrm{WGR}(\%) = 100 \times (W_n - W_{n-1})/W_{n-1};$$
$$\mathrm{SGR}\left(\%\mathrm{day}^{-1}\right) = 100 \times (\ln Wn - \ln Wn - 1)/t;$$
$$\mathrm{FR}(\%) = 100 \times C/[t \times (Wn + Wn - 1)/2];$$
$$\mathrm{FE}(\%) = 100 \times (Wn - Wn - 1)/C.$$

Where W_n is the mean body weight after molting, W_{n-1} is the mean body weight before molting, C is the amount of feed intake in the whole molting period, and t is the length of molting period.

2.4. Statistical analysis

Data were presented as mean ± standard deviation (SD). Homogeneity of variance of data was evaluated using the Levene's test. When required, an arcsine-square root or logarithmic transformation would be performed prior to the analysis. One-way ANOVA was used to determine the difference between different treatments. If any significant difference was detected, Duncan's multiple range test was employed. When a normal distribution or homogeneity of the variances was not achieved, data were subjected to the Kruskal–Wallis H nonparametric test, followed by the Games–Howell nonparametric multiple comparison test. $P < 0.05$ was regarded as the statistically significant level. All statistics were performed using the SPSS package (version 12.0).