# Limb autotomy patterns in the juvenile swimming crab (Portunus trituberculatus) in earth ponds

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# Limb autotomy patterns in the juvenile swimming crab (*Portunus trituberculatus*) in earth ponds

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ABSTRACT

The patterns of limb autotomy in the juvenile swimming crab (*Portunus trituberculatus*) were investigated in earth ponds. High incidence of limb autotomy was found in these juvenile crabs accounting for about 28%, and no significant differences were observed between the males and females ($P > 0.05$). Both the males and females with different body sizes performed the autotomy behavior, and the frequency of autotomy remained almost at the same level. Forelimbs (especially, chelipeds) were lost more often than posterior limbs ($P < 0.05$), and the rate of cheliped loss was the highest. The majority of crabs (about 87%) were more prone to autotomizing the single-side limbs than the other crabs (about 13%) that lost both-side limbs equivalently and averagely ($P < 0.05$). Most juvenile individuals usually lost one to two chelipeds or walking legs or swimming legs, while fewer lost three or more than three limbs, and the fewest lost five limbs.

*Statement of relevance:* Swimming crab aquaculture is one of the most significant aquacultural industries in China. Although there are many reports on limb autotomy patterns of crabs, little is known about the autotomy levels in swimming crab when reared in earth ponds. To determine factors affecting injury levels in swimming crab, we address this issue for the first time in earth ponds by quantifying patterns of limb loss, such as frequency of injured individuals and frequency of injured limbs, and by evaluating sex, size and monthly differences in autotomy levels. Based on this study, we expect to provide some references about limb autotomy patterns in swimming crab, which will pave the way to further studies on the biology and group ecology of swimming crab. Next, we also expect to give some guidance about how to lower the incidence of autotomy and even how to optimize the aquaculture process in earth ponds.



## 1. Introduction

Autotomy, the reflexive amputation of a body part of an animal, is a common escape mechanism when encountering predatory attack or intraspecific aggression. Aquatic invertebrate animals capable of a wide range of autotomy forms include some echinoderms (Bingham et al., 2000; Ramsay et al., 2001), molluscs (Marzo et al., 1993), and crustaceans (He et al., 2016). Commonly, these animals in the natural environment can apply autotomy to protect them from predatory attack or intraspecific aggression (Juanes and Smith, 1995). For example, to escape from the predators, many decapods would autotomize any of their limbs including chelipeds, walking limbs, and swimming legs. And the injured limbs can be soon regenerated at the previous breakage point. Although limb autotomy is a mechanism that allows animals to have a clear immediate survival advantage during predatory attacks (Smith, 1990) or that protects from contracting an infection in the case of a damaged limb (McVean and Findlay, 1979; Slos et al., 2009), in most cases, this antipredator defense would cause many negative effects. In many crustaceans, the consequences of limb loss involve effects on feeding, growth, regeneration, reproduction, competitive ability, predator avoidance, and survival ability (Juanes and Smith, 1995). Moreover, limb autotomy would also pose a threat to the stabilization and development of a group especially when there is a low rate of reproduction and survival.

Swimming crab (*Portunus trituberculatus*), a species of decapods, is one of the most important marine economic crabs in China. As a result of the increase of market demand and the decrease of wild swimming crab, the aquaculture of this crab is developing rapidly. However, the low productivity of swimming crab in earth ponds still limits the development of this industry. The low productivity can be mainly attributed to the aggressive instincts of swimming crab. These crabs are characteristic of bullying and intraspecific aggression, and they therefore have a high incidence of autotomy and a low rate of survival when reared in earth ponds. By now, however, there are fewer reports relating to the patterns of limb autotomy and the effects of autotomy on the growth in swimming crab.

The objective of this study was to determine autotomy patterns in the juvenile swimming crab in earth ponds. We aimed to quantify

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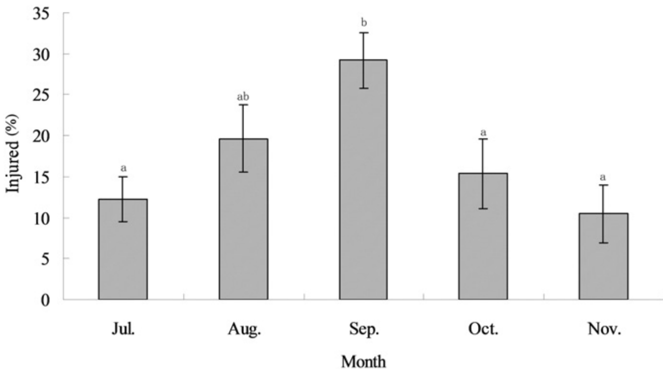


**Fig. 1.** Inter-monthly variability in autotomy levels of swimming crab juveniles in earth ponds. Note: columns without the same letter on the top indicate there are significant differences among them ($P < 0.05$).

patterns of limb loss, such as frequency of injured individuals and frequency of injured limbs, evaluating sex, size and monthly differences in autotomy levels, and to determine factors affecting injury levels in swimming crab. Based on this study, we expect to provide some references about limb autotomy patterns in swimming crab, which will pave the way to further studies on the biology and group ecology of swimming crab. Next, we also expect to give some guidance about how to lower the incidence of autotomy and even how to optimize the aquaculture process in earth ponds.

## 2. Materials and methods

### 2.1. Site and time of study

This study was conducted in Dengbu Island, one test base of Marine Fisheries Research Institute of Zhejiang province (MFRIZ), Zhoushan. Megalopa larva (Stage II) of swimming crab was prepared at the breeding site in Xixuan Island, the other test base of MFRIZ, Zhoushan. The larvae were stocked averagely (~1.5 kg each) in four equivalent-size earth ponds. The square of each pond was about 6000 $m^2$ with a water depth of 0.8 m. And we examined the autotomy patterns during warm months (July–November).

### 2.2. Sampling and experimental design

We regarded each of the four ponds as one experimental group. During the stocking period, larvae were fed with fresh fish and 30% of fresh water was exchanged weekly to guarantee a good quality of water in each pond. About two hundred juvenile crabs were caught from each pond on the 15th of each month. And the rates of autotomy were analyzed according to Dvoretsky and Dvoretsky (2009). Meanwhile, three parameters (i.e. body weight, sex, limb injury) were measured and recorded for each juvenile when sampled. If limb injury happened to any crab, the details (e.g., the breakage point, left or right side) would be recorded carefully. The five left-side limbs from cheliped were defined as 1L, 2L, 3L, 4L, and 5L. And it was similar to the right-side limbs (1R, 2R, 3R, 4R, and 5R). According to body weight, the samples were divided into five sizes: 30–30.9 g, 40–49.9 g, 50–59.9 g, 60–69.9 g, and 70–79.9 g. We finally quantified the rates of autotomy of females and males, the proportions of limb loss categories, and the autotomy levels of females and males with different body sizes. The autotomy rate (or level) in this study was calculated on the basis of the following formula:

$$R(\%) = n/N \times 100$$

where R = the autotomy rate, n = the number of injured individuals, and N = the total number of samples. All limbs were assumed for this comparison to have equal loss probabilities.

### 2.3. Statistical analyses

Data were presented as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was used to determine the differences between different treatment groups. Homoscedasticity of groups was checked using Levene's test. When required, an arcsine-square root or logarithmic transformation would be performed prior to the analysis. If any significant difference was detected, Duncan's multiple range test was employed. When a normal distribution and/or homoscedasticity of the variances were not achieved, data were subjected to the Kruskal-Wallis H nonparametric test followed by the Games-Howell nonparametric multiple comparison test. All statistical analyses were performed using SPSS computer software (SPSS, version 13.0, USA), and a significant difference was regarded to exist if $P < 0.05$.

## 3. Results

The autotomy occurred in earth ponds each month over the whole experiment (Fig. 1). From July to November, the percentage of injured crabs increased rapidly and then decreased dramatically. The percentage in July was 12% and then increased gradually to 20% in August, peaking at ~30% in September. Thereafter, this percentage halved to 15% in October and finally dropped to 10% in November.

There were three stages for swimming crab juveniles to regenerate injured limbs after autotomy: I) the stage that crabs just completed autotomy with no new limb buds observed; II) the stage that crabs were at the beginning of the first molt with new limb buds observed; III) the stage that crabs completed the first molt with new slightly smaller limbs observed (Fig. 2). Overall, although the autotomy level reached the highest in September, there were no significant differences between females and males ($P > 0.05$, Fig. 3). In addition, all different sized crabs in this study had autotomy behavior, and their autotomy levels had no significant differences ($P > 0.05$, Fig. 4).

Interestingly, the majority of swimming crab juveniles (about 87%, left plus right) just injured one-side limbs while only 13% of juveniles injured two-side limbs ($P < 0.05$, Fig. 5). Among them, both left-side and right-side limbs had almost equal loss probabilities ($P > 0.05$). Most juvenile individuals usually lost one to two chelipeds or walking

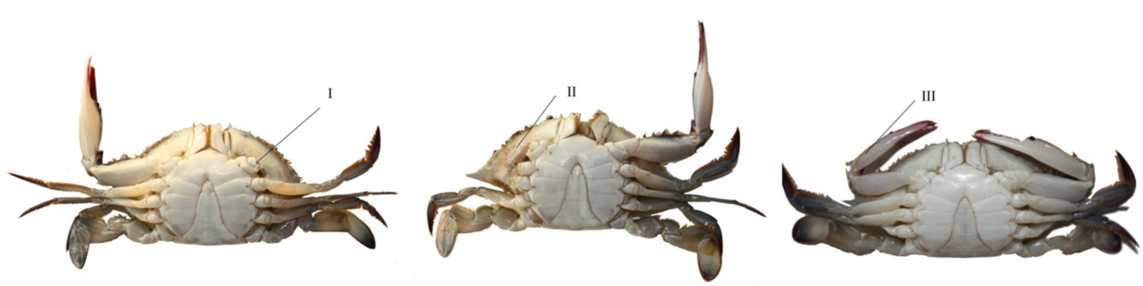


**Fig. 2.** Three stages of limb regeneration after autotomy in swimming crab juveniles.

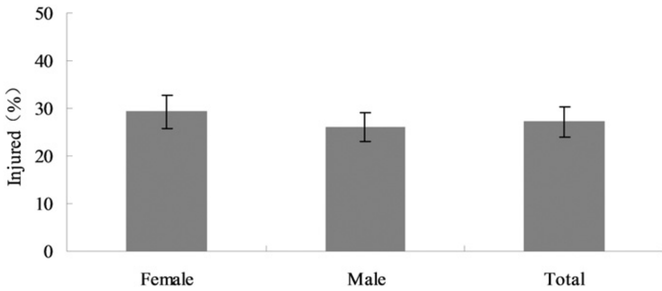


**Fig. 3.** Autotomy rates of female and male crabs.

legs or swimming legs, while fewer lost three or more than three limbs, and the fewest lost five limbs (Fig. 6). The juveniles that autotomized more than five limbs were not observed. Although any limb of swimming crab could be autotomized, the loss probability of each limb was different (Fig. 7). Generally, limb loss probabilities decreased gradually from claws to swimming legs. The loss probability of claw was the highest, which was significantly higher than those of the 3rd, 4th and swimming legs ($P < 0.05$). However, the loss probability of swimming legs was significantly lower than those of all other limbs ($P < 0.05$). The autotomy levels in both females and males had no significant differences ($P > 0.05$).

## 4. Discussion

Autotomy in crustaceans like shrimp and crab is a reflexive loss of a limb at a predetermined breakage point following by a high-speed scarring process, as a response to injury or their threat (Dvoretsky and Dvoretsky, 2009). As a physiological mechanism or escape policy, crustaceans can use it to survive from predatory attack or intraspecific aggression. Zhao et al. (2015) reported high autotomy levels in *Eriocheir sinensis* could be attributed to the predators such as mice, frogs or birds in earth ponds. However, these predators rarely existed in our ponds while the autotomy level in swimming crab juveniles was still higher in this study. Therefore, we speculated the main reason for this may be intraspecific aggression. Many researches showed the factors of intraspecific aggression of crustaceans mainly focus on aggressive instincts (He et al., 2016), lack of shelters (Quinitio and Estepa, 2011), overstocking (Rodriguez et al., 2007), insufficient feed (Thomas et al., 2003) as well as out-of-step molts (Lipcius and Herrnkind, 1982).

Our estimate of injury incidence (20–30%) was similar to the ones reported for the other populations (Dvoretsky and Dvoretsky, 2009; Zhao et al., 2015). With the stocking time prolonging, the injury incidence in swimming crab juveniles increased, and then peaked in September, declined finally. We suspected one possible reason might be that the diversity of body size enlarged in September and then caused out-of-step molts, plus the increasing aggression among individuals. Moreover, the high frequency of copulation usually occurred in September, which led to increasing intraspecific aggression and further to high autotomy levels. After September, water temperature lowered

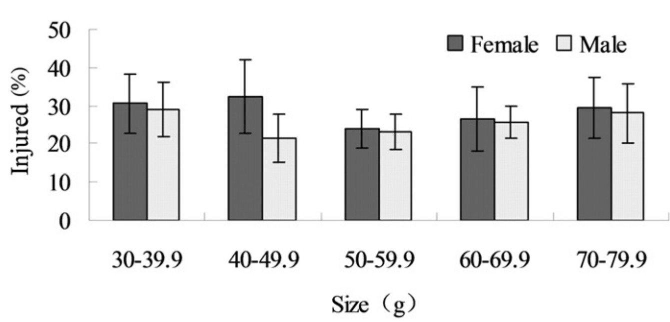


**Fig. 4.** Comparison of autotomy levels in different sized swimming crab juveniles.

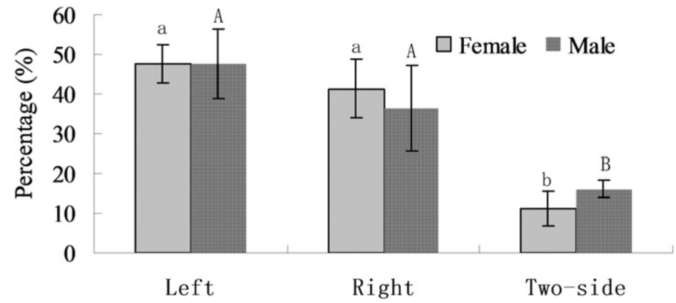


**Fig. 5.** Autotomy frequency of limbs on two sides of juvenile swimming crab. Note: columns without the same letter on the top indicate there are significant differences among them ($P < 0.05$).

gradually, and then the activity and feeding of crabs decreased slowly. In addition, most individuals had completed the last molt after September, and there were fewer soft-shell individuals vulnerable to predators. Therefore, the incidence of autotomy decreased dramatically after September.

Kestemont et al. (2003) deemed that intraspecific aggression includes tail-first type and head-first type. Most crabs would fight using their right claws when threatened (Robinson et al., 1970). Although this behavior would easily damage their forelimbs, the survival rate can significantly increase in this situation. In this study, the loss probabilities of forelimbs (especially, claws) were significantly higher than those of posterior limbs, and limb loss levels on two sides were nearly equal. It is therefore observed that the attack of swimming crab belongs to the head-first type. However, it is claw that crabs need to prey, defend or mate, without which their survival and reproduction may lower. Some reports showed that the feeding rate of swimming crab decreases after it lost claws, and that the crushing stress of claws is significantly smaller than that of the normal claw (Smith and Hines, 1991). Moreover, the development of ovary of swimming crab can not start until copulation completed, it has been proved that crabs without claws attract the opposite sex less easily than the normal crabs (Claxton et al., 1994). Therefore, the wounding of claws may be one of the main reasons for low survival rate and gonadal dysgenesis. Some studies found that the right claw of *Paralithodes camtschaticus* is better developed than the left one, which is usually used as a crusher and to defend. And therefore its right claw is vulnerable to wounding (Dvoretsky and Dvoretsky, 2009). However, in this study autotomy probabilities of left and right claws (1L and 1R) were equal, which further proved that swimming crab uses both claws simultaneously for prey or defense. Like *Paralithodes camtschaticus* (Dvoretsky and Dvoretsky, 2009) and *E. sinensis* (Zhao et al., 2015), most swimming crab juveniles autotomize only 1–2 limbs, and some juveniles also autotomize more limbs

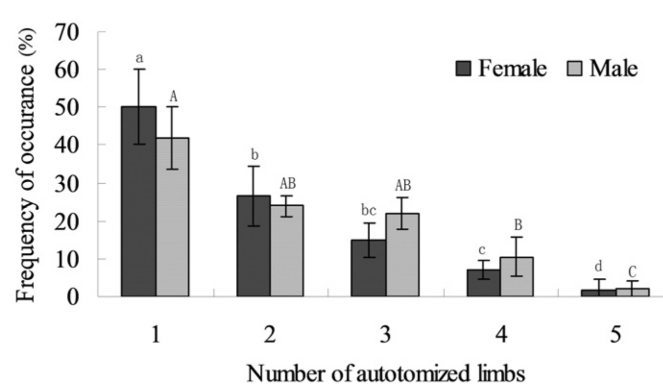


**Fig. 6.** Comparison of observed frequencies of the crabs that were missing 1, 2, …, 5 limbs (of 10 possible missing limbs including chelipeds, walking legs and swimming legs). Note: columns without the same letter on the top indicate there are significant differences among them ($P < 0.05$).

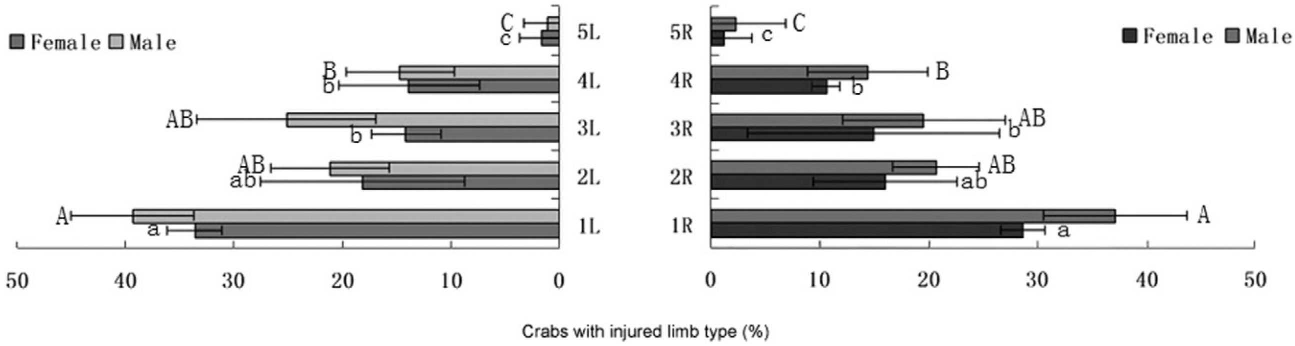


**Fig. 7.** Histogram of the percentage of juvenile crabs missing different types of limbs. 1 — Cheliped, 2–4 — Walking legs, and 5— Swimming legs. R, L — Right and left sides, respectively.

probably because they are vulnerable to injury with a lower their defense after autotomy. Although these juveniles may injure more than two limbs, there are few juveniles that lost more than five limbs. The main reason for this may be that these individuals cannot survive or grow if they lose more than five limbs.

The autotomy levels in different crabs have different correlations with body sizes. For example, the autotomy rates of *Callinectes sapidw* and *Carcinus maenas* increase with rising body sizes while that of *Paralithodes camtschatica* decrease (Smith and Hines, 1991). And the autotomy rate of *E. sinensis* decreases and then increases over the growth (Zhao et al., 2015). Like *Cancer pagurus* and *Portunuspelagicus* (Juanes and Smith, 1995), swimming crab in our study performs the same pattern that the autotomy rate is independent of body size. In other words, individuals with different sizes have equal probabilities to be attacked. Therefore, it is further confirmed that there is no selectivity for size and sex when swimming crab encounters intraspecific aggression, and that the attacked subjects are mainly soft-shell crabs. In addition, like *E. sinensis* (Zhao et al., 2015), autotomy levels have no significant differences between females and males of swimming crab juveniles. There may be two factors: 1) claws and defense of juveniles are comparative since the diversity of size is not obvious at this stage; 2) juveniles cannot fight for mating because their development has not yet entered the mating period. However, it still needs further studies in terms of the diversity of autotomy levels between females and males after late September.

Limb loss in crustaceans has usually affected their feeding, growth and reproduction. Thus, how to reduce the incidence of limb autotomy is the key to the aquaculture of swimming crab. Here, we would like to give some suggestions: 1) ensure a mean size of larvae to reduce the incidence of bullying; 2) provide crabs with some shelters for inhabiting and hiding; 3) guarantee a sufficient and average feeding and an appropriate stocking density to avoid intraspecific aggression.

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