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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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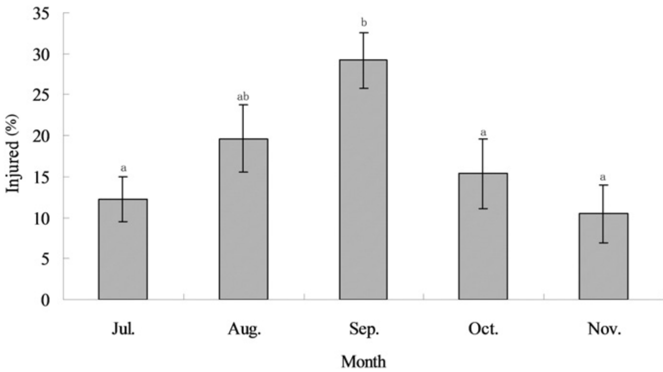

Fig. 1. Inter-monthly variability in autotomy levels of swimming crab juveniles in earth ponds. Note: columns without the same letter on the top indicate there are significant differences among them ($P < 0.05$).

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

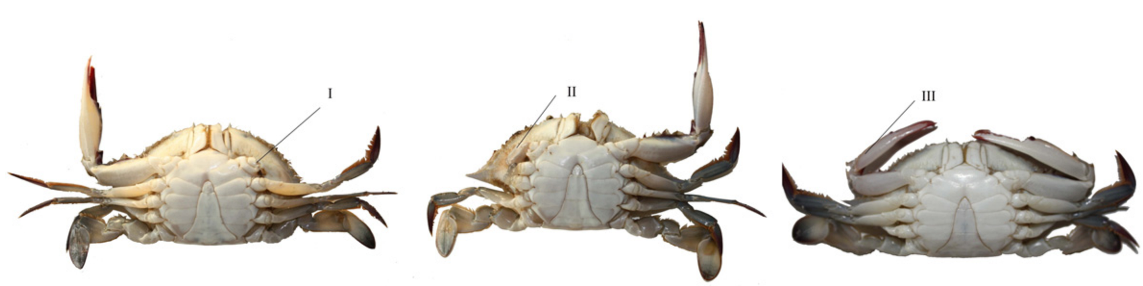

Fig. 2. Three stages of limb regeneration after autotomy in swimming crab juveniles.