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RESEARCH ARTICLE

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ADAPTIVE SELF-DEFENSE: THE STRATEGIC SIGNIFICANCE OF LIMB AUTOTOMY IN FRESHWATER CRABS *OZIOTELPHUSA BILOBA*

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ABSTRACT

Background: Freshwater crabs *Oziotelphusa biloba* in paddy fields of Kerala exhibit extensive defensive self-amputation of limbs and understanding these autotomy patterns can provide insights into ecological and evolutionary significance of higher rate of autotomy response ($41.4 \pm 0.86\%$) than the marine crabs in nearby sea shore. **Results:** The morphometric characteristics in relation to the self-amputation process analyzed with observing 956 crabs during June to October 2022. The variability in rates of amputation among crabs with males ($37.54 \pm 1.14\%$) exhibiting slightly higher rates than females ($35.96 \pm 0.89\%$). The seasonal data in level of autotomy implies a higher autotomy rates in September and October, Right-sided limbs were more prone to injury (71.5% in males, 65.3% in females), Carapace width (30-35mm) and weight (15-16g) correlated with higher autotomy rates and Chelate legs were most susceptible to autotomy, particularly the right chelate leg in males ($21.82 \pm 1.6\%$) and both legs in females ($25.87 \pm 1.57\%$ and $22.22 \pm 1.91\%$). **Conclusion:** This study provides valuable insights into autotomy patterns and correlates in *O. biloba*, shedding light on the ecological and evolutionary significance of this defense strategy. The findings not only enlighten the predatory behavior in the field but also the energy conservation strategy during the injuries in limbs due to intraspecific interactions and cave building.

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INTRODUCTION

Animals living in a natural environment will certainly experience a wide range of attacks from other species or even attacks from inside their species. However, most animals typically take measures to defend themselves in response to prey or cannibalism (Li and Sun, 2013). Among these defence mechanisms, various species frequently use limb autotomy. Autotomy is an effective and quick reflex action when encountering predatory attack or intraspecific aggression that involves self-amputation of limb along a predetermined breakage plane (Knoppe and Larson, 2014), in which the functional departments within the organism are coordinated within a short time period. Additionally, autotomy is thought to be a reliable and universal escape mechanism that helps to somewhat reduce the severity of the injury (Yang et al., 2018). Many aquatic invertebrate animals capable of a wide range of autotomy forms include rodents, arachnids, crustaceans, and echinoderms (He et al., 2016). Multiple studies show that most crustaceans, including crabs and shrimp, can avoid danger by autotomizing their chelipeds, walking limbs, and swimming legs. Despite the fact that some of their appendages have been completely or partially damaged by their predators and competitors, shellfish are willing to cut off the injured part of their appendages to avoid further infections (McVean and Findlay, 1979). And the broken parts of the injured limbs can be quickly regenerate 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). Regeneration is a natural ability of living organisms to replace injured or worn-out parts, repair or renew damage or lost body parts, or to reconstitute the whole body from a small fragment during the post embryonic life of an organism. It involves differentiation, morphogenesis and regrowth for the complete functional recovery. The ability to regenerate lost parts have observed throughout the animal kingdom but to various extent in different phyla. In the case of invertebrates, sponges, coelenterates, flatworms exhibit more power of regeneration. Within vertebrates it is limited to regeneration of some body parts such as limbs, gills and tail. There are five different types of regeneration mechanisms have been observed in different organisms such as Morphallactic (Hydra), Epimorphic (Amphibians), Heteromorphic (Palinurus), Super (Planaria) and Compensatory regeneration (mammalian liver). Crustacean limb regeneration involves Autotomy in a predetermined region later dedifferentiation of adult structures in order to form an undifferentiated mass of cells. The highly proliferating cells become accumulated under the wound epidermis and within two days, bulge transforms into a conical lump. This lump of dedifferentiated cells along with the epidermal covering is called regeneration bud or regeneration blastema. The dedifferentiated cells continue to proliferate and finally re-differentiated to form a rudiment of the limb. The rudiment eventually transforms in to a complete limb as in epimorphic regeneration.

Many crustaceans are well known for their ability to regenerate the same organ after autotomy. Crustaceans can also reproduce limbs in accordance with the molting cycle. The speed of regeneration is primarily determined by gender, age, molting period, and the type or number of limbs lost. Although limb autotomy occurs throughout the shellfish lifecycle, regeneration occurs only during the molting phase of larvae or juveniles (Stueckle *et al.*, 2008). In *Uca*, autotomy-induced resetting allows for the emergence and early growth of autotomized limb buds. Limb and emergence and basal growth are independent of pro-ecdysis and are inhibited if autotomy occurs during later stages (Penny M. Hopkins, 1982).

In most cases, this antipredator defense would cause many negative effects. The effects of limb autotomy and regeneration on many crustaceans include feeding rate, survival rate, immune function, growth rate, reproduction, conspecific competition, and the ability to resist predators (He *et al.*, 2016). While there are few objective studies comparing the effects of limb autotomy and ablation (imitating the limb-impaired crabs under natural conditions) on the cellular and biochemical functional parameters in crustaceans, previous researchers frequently believed that the innate nature of autotomy is similar to limb ablation and acquired injury (Sloset *et al.*, 2009). Autotomy response is a uni-segmental reflex. The damaged or constrained limb is generally the one that gets autotomized (Easton, 1972). Limb loss may negatively affect feeding and mating (Fujaya *et al.*, 2020). Autotomy is the amputation of an appendage at a predetermined breakage plane via a reflex that is usually uni-segmental. Its main characteristic is that the limb is always amputated at a preformed breakage plane, also known as breakage-joint, or autotomy plane (Adiyodi, 1972). Break age points are either permanent sites of weakness that are damaged by external pressures or possible sites of weakness that only lose tensile strength during autotomy. An autotomy plane comprises a number of separate breakage zones. (Wilkie, 2001). It allows for a clean break during autotomy, while structural characteristics reduce stress and encourage rapid fluid compartment sealing, wound healing, and wound closure (Lawrence *et al.*, 1986 & Wilkie, 2001). As a result, a small amount of hemolymph was lost from the basiischium region or the removed leg (Fleming *et al.*, 2007).

The autotomy response is high in crustaceans, where a special type of autotomy muscle is responsible for autotomy (Hopkins, 1982). Specialized autotomy muscles that are inserted on the proximal edge of the basi-ischiopodite (BI) segment of the walking leg are responsible for the autotomy reflex (Mac Vean, 1984). A crucial part of autotomy also involves the rotator levator muscle. The quickest and most efficient regeneration is ensured by limb loss at the predetermined fracture plane. Many species that lose limbs, on the other hand, have the ability to regenerate them (McVean, 1976). The trend of an animal to autotomize a limb depends on the functions and central control of autotomy decisions by nervous centers.

The freshwater species *Ozotetelphusa biloba* lives in a habitat with gravel and stones in waterways, commonly found in rice fields at relatively low elevations throughout Kerala. The species is widely distributed in different parts of South India and grows up to a size of 4.5 to 4.8 cm carapace width (Pushpalatha *et al.*, 2014). In terms of feeding, this species is classified as an opportunistic omnivore. Because of its abundance and multiple roles as a scavenger, predator, and prey to higher vertebrates, this is an important crustacean in the freshwater food chain. This is an ecologically and economically significant tropical species with exceptional potential as a sentinel organism (Padmanaban & Mohan, 2013). The crab exhibits remarkable regeneration capacity (Bliss, 1950) but very few works have been conducted in the mechanism of autotomy and regeneration. Crustaceans can amputate their appendages neatly at a preformed autotomy plane on a nervous reflex resulting from injury or discomfort to their distal segments. An inflexible exoskeleton covered with the body of crustaceans; In *Ozotetelphusa biloba* regeneration of lost appendages is intimately linked with molt. Molting affects regeneration and regeneration affects molting. Amputation behavior of *Ozotetelphusa biloba* done in two conditions, normal and anesthetized

crab. Anesthetized crab showed delayed autotomy. Amputated leg under normal condition shows autotomy within seconds. (Pushpalatha, 2014). The crabs are in intimate contact with aquatic environment rich in pathogenic microbes and are prone to infection by those microbes at various stages of growth, an effective system developed in *Ozotetelphusa biloba* for eliminating and detecting harmful microorganisms. In crustaceans the endocrine control of somatic and retinal pigmentations, molting, metabolism, reproduction, heart rate and activity rhythms recognized. In *Ozotetelphusa biloba* endocrine mechanisms involved in the larval life of crustaceans opposed to the wealth of information available on insect molt control. This species is of interest to researchers due to its unique characteristics, such as its ability to regenerate lost limbs. Studies on *Ozotetelphusa biloba* have provided valuable insights into the molecular mechanisms underlying limb regeneration and tissue repair. The autotomy frequency and pattern in the freshwater crab *Ozotetelphusa biloba* can give away the basic modulation of the autotomy and recovery rate happening in the field and that can be correlated with the experimental autotomy patterns. The study focuses on the variability in autotomy rates, ultrastructure of the autotomy plane and variations with respect to the field and experimental autotomy rates.

RESULTS

Regeneration of walking legs in the *Ozotetelphusa biloba* is most efficient when it follows autotomy (The reflexive loss of a limb). Closure of the wound and wound healing occur immediately following autotomy and visible regeneration begins within a few days. Regeneration of the walking leg occurs in two distinct stages: The first stage, called basal growth, involves mitosis and differentiation. The second stage involves primarily protein synthesis and water uptake and is called Pro-ecdysial Growth. Pro-ecdysial Growth involves direct hormonal stimulation.

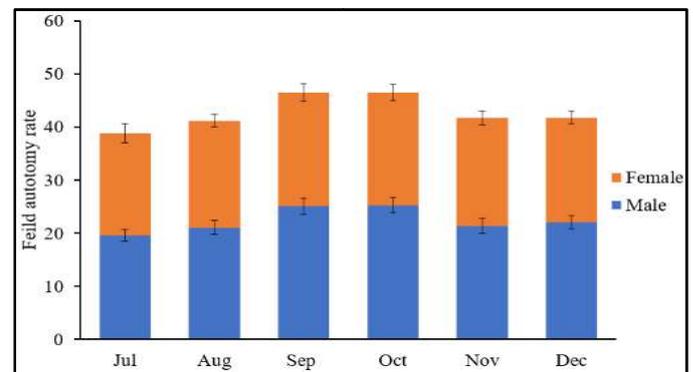


Figure 1. The autotomy rates of freshwater crab *Ozotetelphusa biloba* in the year 2022 at the paddy fields of Kerala, India

Incidental limb autotomy rate of juvenile freshwater crab *Ozotetelphusa biloba*: The incidental autotomy response of freshwater crab *Ozotetelphusa biloba* was studied in the freshwater fields of Chettiyarmad. A total of 956 crabs were observed in which 448 male and 508 female crabs during the study period June to December 2022. The crabs observed in the field have more injuries (582 injured crabs) during the period after monsoon compared to other months. The autotomy in *Ozotetelphusa biloba* observed from July to December was recorded in the study. The limb autotomy happens in the autotomy plane located between the basis and the ischium region. Incidence autotomy rates of *O. biloba* in the paddy fields show 41.4 ± 0.86%. The male crabs (37.54 ± 1.14%) (Figure 3) showed a higher autotomy rate than females (35.96 ± 0.89%). No significant difference was observed between male and female crabs in autotomy rates in the Paddy fields ($P > 0.05$). The autotomy response rate shows a steady rate of autotomy response along the year compared with male freshwater crab. But there has been an increase in autotomy rate to female crabs after the monsoon and a highest in the months of September (21.4 ± 1.7) and October 2022 (25.3 ± 1.48%).

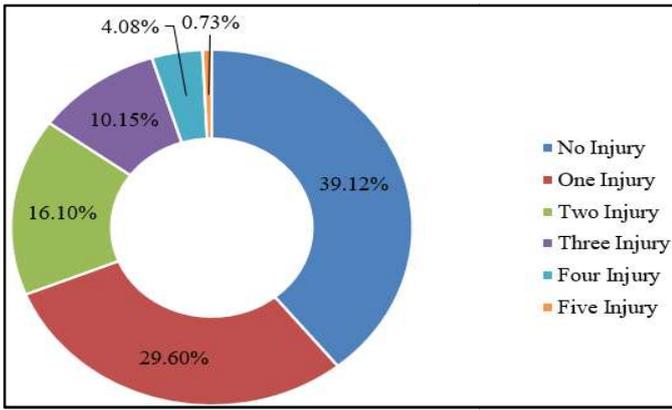


Figure 2. Showing the autotomy response frequency with respect to the number of injured appendages in freshwater crabs *Oziotelphusa biloba* from the paddy fields

The rate of autotomy observed varied correspondingly to the crab batches collected in the corresponding months during the experiment period. In September and October, the autotomy injury was higher (Figure 1). There have observed 39.12% of uninjured crabs from the field the remaining injured crabs have mostly with single appendage injuries (29.60%). We observed a maximum of five injuries per crab in the field but fewer (0.73%) were observed. But the rest of the crabs with multiple injuries were two injuries (16.10%), three injuries (10.15%), four injuries (4.08%) respectively in the freshwater fields of Chettiyarmad (Figure 2). More than five injuries per individual crabs were negligible in the paddy fields of the study area.

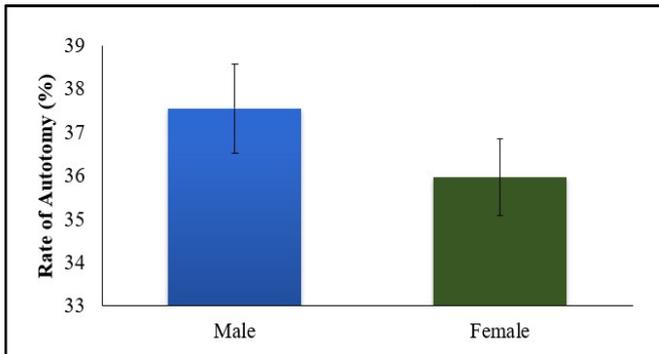


Figure 3. Showing the autotomy response rate with respect to sex of the crab in *Oziotelphusa biloba* in the paddy fields

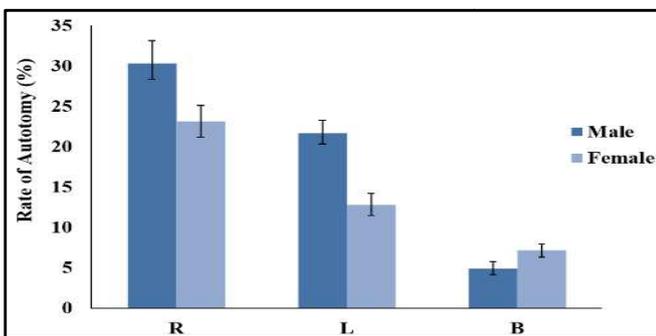


Figure 4. Showing the male and female rates are given.5) The autotomy injury with respect to the Right (R) and Left (L) side of the *Oziotelphusa biloba* in the paddy fields

The autotomy rates of *Oziotelphusa biloba* were significantly differ in their injury happened to the right and let side of the limb to get injured. The male *O. biloba* have higher rates of autotomy on both the right (30.29± 2.84) and left (21.67± 1.96) sides compared to females (23.15±1.96 and 12.81, respectively). In individual crabs with both sided injuries, the right side is more frequently affected than the left side. Females, however, have a slightly higher incidence of autotomy on both sides (7.14± 0.79) compared to males (4.93± 0.84) (Figure 4).

These findings indicate a possible lateral preference or vulnerability in the crabs, with the right side being more prone to autotomy.

Effect of Carapace width (CW) and Weight of the crab (W) in autotomy patterns offreshwater crab *Oziotelphusa biloba*: The size of the crab and autotomy rate in *Oziotelphusa biloba* crabs were related to carapace width (CW) along with weight (W) of the crab. Both the male and female freshwater crabs with carapace width 30-35mm shows a high autotomy rate compared with other juvenile crabs. The autotomy response rate observed to be males 19 ± 0.07% and female crabs 13.64 ± 0.9% respectively in the freshwater paddy fields. In male crabs a gradual increase in autotomy rates as the carapace width advances up to a peak width of 30-35cm (Figure 5). The autotomy rate also decreases in males with an increase in carapace width more than 35cm. In females the autotomy rates almost similar initially and a high altitude attains at the crabs with carapace width of 30-35mm and decreases steadily over 35cm sized crabs in the paddy fields. The autotomy response appears to be variable with respect to the different walking legs as well as different weight of each crab. The higher autotomy frequency observed in medium sized crabs of weight range from 8g to 11g and 11 g to 14g. The female crabs of medium weight showing some higher autotomy rate than the male crabs in the field. The female crabs observed 21.75 ± 1.69% of autotomy response rate and male crabs with 15.79 ± 1.39% within crabs of weight range of 8g to 11g (Figure 6). The autotomy rates show a dome shaped graphical patterns in relation to the weight of the crab.

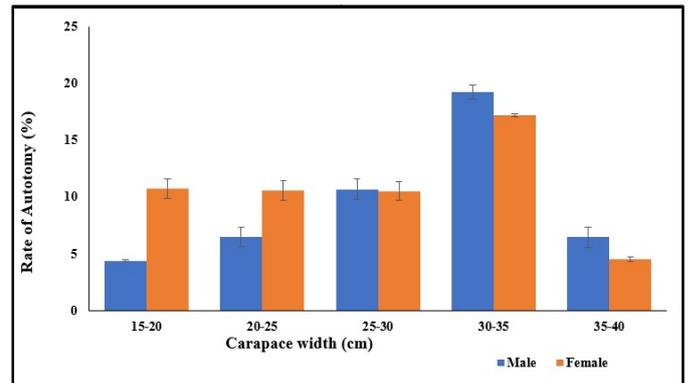


Figure 5. The autotomy injury in relation to the Carapace width of *O. biloba*.

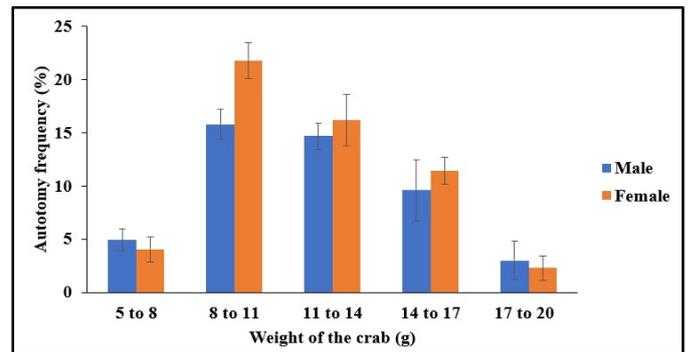


Figure 6. The autotomy injury and weight of *O. biloba*

The chelate legs getting injured in most of the crabs along with other swimming legs for the freshwater crabs *Oziotelphusa biloba* in the paddy fields of Chettiyarmad. The rate of autotomy observed higher in left chelate leg (LCH) of male crab 21.82 ± 1.6%. The rate of autotomy observed lower in the left and right swimmerets 6.34 ± 0.84% for right third swimmeret (R3) and left first swimmeret (L1) 7.5 ± 0.62% respectively (Figure 7). The female right and left chelate legs getting injured frequently 25.87 ± 1.57% (RCH) and 22.22 ± 1.91% (LCH), while the right sided third swimmeret (R3) getting minimal injury 1.98 ± 0.7% and left sided fourth swimmeret (L4) 4.98 ± 0.8% respectively.

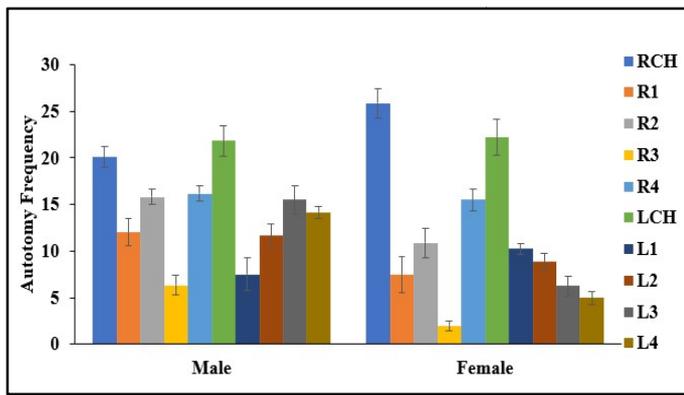
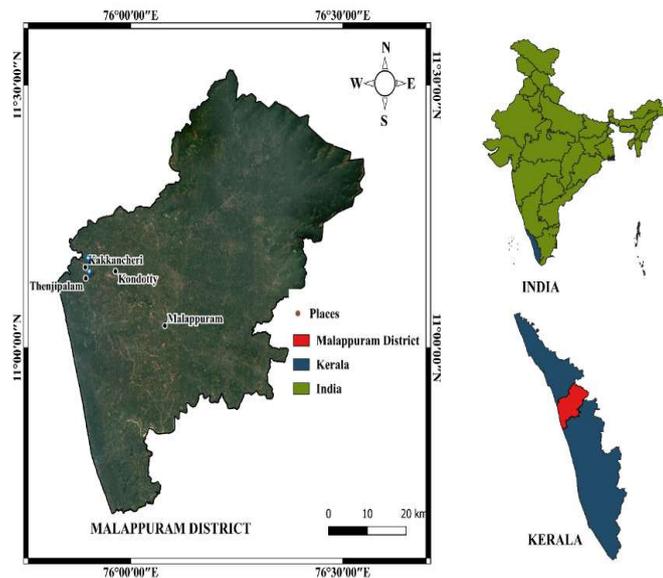


Figure 7. Autotomy frequency of individual Appendages in *O. biloba*. (Where, chelate leg (RCH), walking legs R1, R2, R3 and R4. Similarly on the left-side with Left chelate (LCH) leg, walking legs L1, L2, L3 and L4)

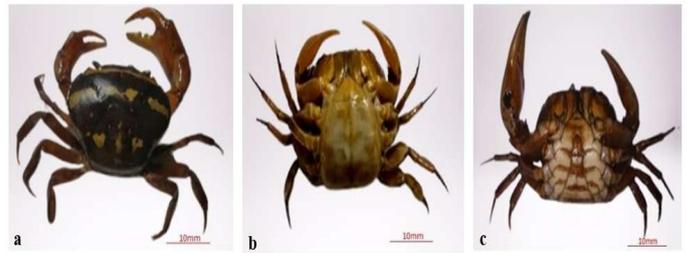
METHODS

Study area: The experimental animal fresh water crab *O. biloba* was collected from paddyfields of Idimuzhikkal (11.170⁰ N; 75.882⁰ E) and Chettiyarmad (11.1408; 75.8947 E) near the University of Calicut, Malappuram district, Kerala, from July to December 2021. The fields were regularly used for the cultivation of Paddy, Banana, Cassava, and some tubers (Figure 8). The water-shedding on the banks of the fields was well occupied with four different species of Fresh water crabs.



Data collection: The level of autotomy depends on various factors in crustaceans. It varies with respect to the crustaceanspecies, natural condition, feeding as well as the age of the crabs. The autotomy rate (or level) in this study was analyzed according to Dvoretzky and Dvoretzky (2009), and He *et al.* (2016).

Morphoparametrics correlation to the autotomy frequency: The autotomy rate with respect to the sex of the freshwater crab in the field was observed along with the relative rate of autotomy to the size of the crab *O. biloba* (Figure 9). The Carapace Width (CW) and Weight of the crab were used for the analyses. The individual limb autotomy rates were analyzed in both sexes, as right-side limbs, such as chelate leg (RCH), and walking legs R1, R2, R3, and R4. Similarly, on the left side with the Left chelate (LCH) leg, walking legs L1, L2, L3, and L4. The weight of the crabs with corresponding Carapace width ranged from 5g to 20g in each group.



Data were presented as mean \pm standard deviation (SD). One-way analysis of variance (ANOVA) and t-test were used to determine the differences between different treatment groups. All statistical analyses were performed using SPSS computer software (SPSS, version 26.0), and a significant difference was regarded as existing if $P \leq 0.05$.

DISCUSSION

The freshwater crab *Oziotelphusa biloba* inhabited in shallow to deep burrows along banks of rice field, embankment and underneath the fences bordering paddy field. Autotomy appears to be a means of adaptation to escape from predators and also from skipping loss of hemolymph and energy and ultimately from death Raja *et al.*, (1976). Various physicochemical and enzymatic studies related to autotomy has been extensively done in crustaceans in recent years (Juanes and Smith 1995; Fleming *et al.*, 2007; Lindsay, 2010). However, the natural autotomy patterns and the defense mechanisms were comparatively lesser studied in freshwater crabs. Juvenile freshwater crabs were the mostly studied experimental animal in laboratory for the early autotomy responses. The incidental autotomy rates of the *Oziotelphusa biloba* crabs in the freshwater fields of Chettiyarmad was $63.77 \pm 1.46\%$ from January to December 2022, a higher rate, when compared to the marine crabs *Paralithodes camtschaticus* (Dvoretzky and Dvoretzky, 2009; Zhao *et al.*, 2015). The male crabs ($37.54 \pm 1.14\%$) (Figure 4). have more autotomy rate than females ($35.96 \pm 0.89\%$). No significant difference observed in male and female *Oziotelphusa biloba* crabs in autotomy rates in the Paddy fields ($P > 0.05$).

The significant proportion of the crabs collected from the freshwater paddy fields, (39.12%) were uninjured, while the remaining 60.88% had sustained injuries. This finding suggests that injuries are a common occurrence in this species, and that autotomy may be a frequent defense mechanism employed by these crabs. Among the injured crabs, the majority had single appendage injuries (29.60%). This is consistent with previous studies that have shown that autotomy is a common defense mechanism in crustaceans, including crabs (McLaughlin, 1983; Faulkner, 1995). The fact that single appendage injuries were the most common type of injury observed suggests that these crabs are able to effectively autotomize their appendages to escape from predators. The freshwater crab *Oziotelphusa biloba* with multiple injuries were not uncommon. Specifically, 16.10% of crabs had two injuries, 10.155% had three injuries, and 4.08% had four injuries. This finding suggests that these crabs are able to survive and even thrive despite sustaining multiple injuries. However, injuries exceeding five per individual crab were negligible. This suggests that there may be a limit to the number of times a crab can autotomize its appendages before it becomes vulnerable to predators or other environmental stressors (Govind, 1995). This is consistent with previous studies that have shown that repeated autotomy can have negative consequences for crustaceans, including reduced growth rates and increased mortality (Faulkner, 1995). Overall, the findings of this study provide insight into the frequency and patterns of injury in *Oziotelphusa biloba* crabs, and highlight the importance of autotomy as a defense mechanism in this species. According to Zhang *et al.* 2016 the incidence of autotomy in crustaceans generally varies among species to species. Similarly, autotomy levels have no significant differences between females and males of swimming crab juveniles (Zhao *et al.*, 2015).

The male crabs shown 71.5% increase in injury of right-side limbs in compared with left-side limbs meanwhile in females have 55.3% increase. But in both sexes right sided limbs are often get injured in crabs of freshwater paddy field in Chettiyarmad. Both sexes showed similar trends, that is, body weight increment was the lowest in the full limb autotomy groups, it was significantly higher in the partial limb autotomy groups, and the highest in unmutated crabs (Yushinta fujaya, 2020). In small *Carcinusmaenas*, only 1.7% of individuals lost their appendages (Sekkelsten, 1988). While in female *C. angulatus*, the injury rate was about 80% (Spivak & Politis, 2011). The possibility for the variation in autotomy frequency between the sexes of crabs in the field cannot be neglected to an extent. The defensive mechanism against predators primarily involves chelate legs and this creates injuries higher in the chelate legs compared to the other walking legs (Robinson et al., 1970). The size and age have some role in frequency of limb autotomy along with the injury happens to the legs. Among the juvenile crabs, a higher autotomy frequency observed in male and female crabs with weight of 8g to 11g and having a carapace width of 30mm to 35mm. Crustacean autotomy patterns were generally found to be independent of sex, which might imply causes of autotomy, such as predation, are sex independent. Nevertheless, exceptions were found in some species, such as *C. angulatus* (Spivak & Politis, 1989), and *C. maenas* (McVean, 1976). The size of right chelate leg is comparatively larger than the left one, also right chelate leg is primarily used for attachment and catching the prey. The rate of autotomy observed higher in left chelate leg (L_{CH}) of male crab $21.82 \pm 1.6\%$. The rate of autotomy observed lower in the left and right swimmerets $6.34 \pm 0.84\%$ for right third swimmeret (R_3) and left first swimmeret (L_1) $7.5 \pm 0.62\%$ respectively (Figure 8). The female right and left chelate legs getting injured frequently $25.87 \pm 1.57\%$ (R_{CH}) and $22.22 \pm 1.91\%$ (L_{CH}), while the right sided third swimmeret (R_3) getting minimal injury $1.98 \pm 0.7\%$ and left sided fourth swimmeret (L_4) $4.98 \pm 0.8\%$ respectively. Due to the delayed feeding, molting initiation and growth arrests after the loss of large limb may be the reason for 10-15% higher autotomy rates for right chelate leg than the left in *Oziotelphusa biloba*. Skinner and Graham (1972) indicated that removal of two chelipeds and four pereopods was sufficient to stimulate a precocious molt in *C. sapidus*. Since times to ecdysis for their control animals were not reported, the degree to which limb removal shortened the molt cycles in their treatment group is unclear. Minimal injury occurs in autotomy as the autotomy membrane, which internal partition within the basi-ischial segment separating the basis and ischium, restrict the loss of body fluid.

CONCLUSION

The study reveals that *Oziotelphusa biloba* in paddy fields exhibits a significant autotomy rate of $41.4 \pm 0.86\%$, with no notable difference between males and females. The autotomy rate varies across different months, with higher rates observed in September and October. The right-sided limbs are more frequently injured in both males and females, and crabs with a carapace width of 30-35mm and specific weight ranges show higher autotomy rates. The study also highlights leg-specific autotomy patterns, with chelate legs being more prone to autotomy. These findings contribute to our understanding of the ecological and behavioral significance of autotomy in *O. biloba* and have implications for the management and conservation of this species in paddy fields.

List of Abbreviations: Not applicable.

Declarations

Declaration of generative AI in scientific writing: The authors haven't used any AI tool for the writing, Literature review, data analysis or content preparation throughout the journal preparation and presentation of items in this article.

Ethics approval and consent to participate

- The authors declare that this study complies with all relevant ethical guidelines and regulations. No ethical issues or violations were identified during the conduct of this research.
- The authors declare that all applicable institutional and/or national guidelines for the care and use of animals were followed during this study. This article does not contain any studies with human participants performed by any of the authors.

Animal Ethics declaration

Animal Ethics declaration: Not applicable.

Consent for publication: The authors declare that the manuscript has been read and approved by all authors, and that the submission has not been previously published or is not under consideration for publication elsewhere. The authors transfer copyright of the manuscript to "International Journal of Development Research" upon acceptance for publication.

Availability of data and material: Data have been represented as graphical as well tables along with the results. No additional data to be presented in any manner regarded to the study

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Authors' contributions: Author 1 conceptualized the study, collection and data analysis from the field, Author 2 assisted in sample collection and field data generation and Author 3 Supervised the study.

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