# **Original Article**

# Nauplius

The Journal of The Brazilian Crustacean Society

> e-ISSN 2358-2936 www.scielo.br/nau www.crustacea.org.br

Cheliped loss and abnormalities of the narrow-clawed crayfish, *Pontastacus leptodactylus* (Eschscholtz, 1823) (Crustacea: Decapoda: Astacidae)

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# ABSTRACT

Heterochely is an important phenomenon in decapod crustaceans. Nevertheless, it was rarely examined in freshwater crayfish. Therefore, the aim of this study was to investigate cheliped loss and abnormalities of the narrow-clawed crayfish, Pontastacus leptodactylus. The crayfish samples were captured using 17 mm mesh-sized fyke-nets from Atikhisar Reservoir in Çanakkale, Turkey between July 2020 and June 2021. The cheliped loss was classified and compared between sexes and length groups. The cheliped surface was calculated for each specimen for both the right and left cheliped. Results of the study indicate that the percentages of the sampled individuals were 5.46% for the right cheliped missing group, 5.23% for the left cheliped missing group, 10.37% for both chelipeds missing group, and 78.94% for both chelipeds present group. There was a statistical difference between cheliped loss and size groups (p < 0.05). Although cheliped loss is almost non-existent in low-size groups (10.0–29.9 mm), it reaches high values in the 40.0–69.9 mm size groups. The most intense loss occurs in the 40.0–49.9 and 50.0-59.9 mm size groups. It was determined that 10.69% of the sampled individuals examined had a single cheliped (10.03% female, 11.14% male). A statistically significant difference was found between cheliped loss and sex (p < 0.05). The percentage of female and male individuals with no chelipeds is 9.83% for females and 10.73% for males, and with both chelipeds it is

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Submitted 14 February 2023 Accepted 24 August 2023 Published 26 February 2024

DOI 10.1590/2358-2936e20240502

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Nauplius, 32: e20240502

80.14% for females and 78.14% for males. While the number of abnormalities observed in chelipeds was higher in males, abnormalities in both chelipeds were higher in females. Moreover, it was found that cheliped loss significantly differed according to the months of collection (p < 0.05). Cheliped losses increased in July, August, and September when feeding was comparatively intense. In conclusion, the fact that the individuals were obtained alive, was an indication that the abnormalities detected did not significantly affect their vital activities directly. However, morphological abnormalities in the appendages, especially in the chelipeds, may impair their functional use compared to a healthy cheliped. This abnormal condition is assumed to share the disadvantageous limitations experienced by the absence of a cheliped.

#### **KEYWORDS**

Abnormalities, asymmetry, claw, heterochely, limb loss

# INTRODUCTION

The narrow-clawed crayfish, *Pontastacus leptodactylus* (Eschscholtz, 1823), is considered an economically important fishery in Europe, and has found an important place in the center of biology as a model organism in physiology and ecology (Furshpan and Potter, 1959; Wald, 1967; Stein, 1977; Douglass et al., 1993; McMahon, 2001).

Defined as one of the largest types of decapod crustaceans inhabiting freshwater ecosystems, crayfish are easily distinguished from the others by large chelipeds as the first of five pairs of pereopods. This versatile appendage is mainly used in feeding (Stein, 1976), mating and shelter acquisition (Guan, 2010), defense against predators (Wilson et al., 2007), and agonistic behaviors (Brown et al., 1979; Lee, 1995). Meral spreading, cheliped extension, grasping, lifting, scissoring, striking, pushing, nipping, fending, and thrusting are some of the known cheliped mediated displays (Mariappan et al., 2000). The size of the cheliped is one of the important factors in determining the dominance-subordinate hierarchical order in the population, especially in male individuals (Garvey and Stein, 1993; Rutherfold et al., 1995; Gabbanini et al., 1995; Barki et al., 1997; Bywater et al., 2008). In addition to these important functions in the life history of crayfish, the chelipeds are also used in taxonomic nomenclature. Narrow-clawed crayfish P. leptodactylus, white-clawed crayfish (Austropotamobius pallipes (Lereboullet, 1858)), thickclawed crayfish (*Astacus pachypus*, Rathke, 1837), and red claw crayfish (*Cherax quadricarinatus* (Von Martens, 1868)) are some common names of species.

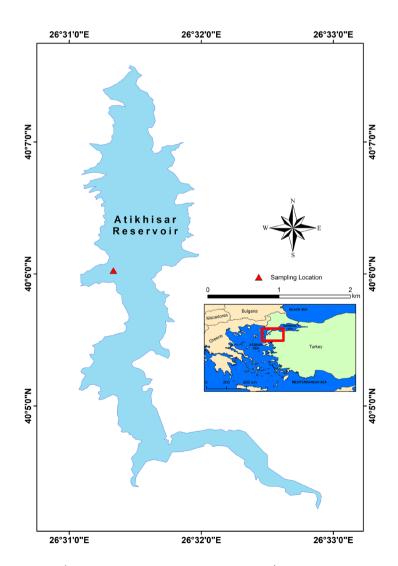
Chelipeds perform many important functions in the lifespan of crayfish, but are the most vulnerable appendages to aggression and mutation. Their absence, has important effects on the life of crayfish (Coughran, 2008). Events, such as reflex severance (autotomy), replacement (regeneration), or malformation (abnormality) of one or more of the limbs (Okada et al., 1997), may result from different factors. Crayfish, which have lost their chelipeds, experience disadvantages in self-defense, mating, feeding, and sheltering (Mariappan et al., 2000; Seebacher and Wilson, 2007). The ability to regenerate lost body parts is one of the unique features that decapods possess. However, the energy they use for the regeneration of the body part will come at a balanced cost of the energy that they normally would have used for growth and reproduction (Vogt, 2012). This energetic cost of limb replacement results in a smaller growth rate compared to individuals without any limb loss.

Cheliped dimorphism is widespread in decapod crustaceans, and many decapods have a pair of different-sized chelipeds, a phenomenon known as heterochely (Hamasaki and Dan, 2022). Although significant in some marine decapod crustaceans, this phenomenon has rarely been examined in freshwater crayfish (Lele and Pârvulescu, 2019). Therefore, the aim of this study was to investigate the loss of chelipeds between sexes and among size groups and to reveal possible effects on the life history in freshwater crayfish. In addition, the abnormalities were examined by image analysis.

# **MATERIAL AND METHODS**

#### Sampling

The crayfish, *P. leptodactylus* were captured using 17 mm mesh-sized fyke-nets from Atikhisar Reservoir in Çanakkale, Turkey (Fig. 1) between July 2020 and June 2021. The reservoir was constructed to supply water for drinking, agricultural and domestic use (Kale and Acarlı, 2019a; 2019b). The total surface area of the reservoir changes between 1.72 km<sup>2</sup> and 3.84 km<sup>2</sup> (Kale and Acarlı, 2019a). The normal water level of the reservoir is 61 m and its volume is 40 hm<sup>3</sup> (Kale, 2019). The occurrence of several species of fish such as European chub Squalius cii (see Akbulut et al., 2008; Koca, 2011; Selvi et al., 2015), common carp Cyprinus carpio (see Akbulut et al., 2008), European eel Anguilla anguilla (see Koca, 2011), northern pike *Esox lucius* (see Selvi and Kaya, 2013), spined loach Cobitis taenia (see Akbulut et al., 2008), black goby Gobius niger (see Akbulut et al., 2008), and big-scale sand smelt Atherina boyeri (see Kale et al., 2022; 2023), the narrow-clawed crayfish P. leptodactylus (see Kale et al., 2020; 2021a), and the western Caspian turtle Mauremys rivulata (see Kale et al., 2021b), have been reported.



**Figure 1.** Map of the sampling area (Atikhisar Reservoir in Çanakkale, Turkey) where *Pontastacus leptodactylus* individuals were collected. The red triangle indicates the sampling location within the lake.

## Data analysis

Carapace Length ( $L_c$ ), Carapace Width ( $W_c$ ), Abdomen Length ( $L_A$ ), Abdomen Width ( $W_A$ ), Right Cheliped Propodal Length ( $L_{RCh}$ ), Left Cheliped Propodal Length ( $L_{LCh}$ ), Right Cheliped Propodal Width ( $W_{RCh}$ ), and Left Cheliped Propodal Width ( $W_{LCh}$ ) were measured using a vernier caliper to the nearest 0.1 mm (Rhodes and Holdich, 1984). Total Wet Weight ( $W_{WT}$ ), Carapace Weight ( $W_C$ ), Abdomen Weight ( $W_A$ ), Right Cheliped Weight ( $W_{RCh}$ ), Left Cheliped Weight ( $W_{RCh}$ ) were weighed using a digital scale to the nearest 0.01 g accuracy.

The cheliped loss in the examined narrow-clawed crayfish samples were classified as right missing, left missing, both missing, and both present. The cheliped losses were compared between sexes and length groups by performing Pearson chi-square tests.

The cheliped surface area was calculated for each specimen using Equation 1 for both the right and left cheliped (Plato et al., 1980; Anagnostou and Schubart, 2014). The difference (DIF) values between the right and left cheliped surface area were also calculated. The chelipeds were considered homochelous if the DIF varied  $\pm 2.5\%$  from the sum of the left and right CS, the left cheliped was considered larger if the DIF was > 2.5% from the sum of left and right CS, and the right cheliped was considered larger in the remaining individuals; the threshold of 5% between the CS of both chelipeds was applied according to Masunari et al. (2015) and Lele and Pârvulescu (2019).

$$S_c = L_{Ch} \times W_{Ch} \tag{1}$$

In this formula,  $S_{\rm C}$  is the cheliped surface,  $L_{\rm Ch}$  is the cheliped length and  $W_{\rm Ch}$  is the cheliped width for both left and right chelipeds.

The proportions of crayfish were compared according to their cheliped size, represented by DIF classes, and between males and females using Fisher's exact tests (Lele and Pârvulescu, 2019) using R (R Core Team, 2022) and SPSS statistical software. Then, to investigate whether heterochely differs with size class of crayfish, we categorized the measured specimens according to their carapace length (CL), into three classes of five (Holdich, 2002; Maguire et al., 2004; Maguire and Klobučar, 2011). Classes 1 and 2 were omitted because they included juvenile crayfish. Class 3 consisted of crayfish with a CL between 50 and 75 mm, class 4 with a CL between 75.1 and 100 mm, and class 5 with a CL larger than 100 mm.

## RESULTS

In this study, chelipeds of 6,444 crayfish, 2,593 females, and 3,851 males, were examined. Depending on the cheliped loss number and side, the examined narrow-clawed crayfish samples were classified into four groups. The percentages of the observed individuals were 5.46% for the right cheliped missing group, 5.23% for the left cheliped missing group, 10.37% for the both chelipeds missing group, and 78.94% for the both chelipeds present group. In addition, the groupings were further classified into sexes and the basic statistics are provided in Tab. 1.

The cheliped presence or absence of the sampled freshwater crayfish were analyzed by classifying them into 10 mm size groups according to their carapace lengths and the details are displayed in Tab. 2. Although cheliped losses are almost non-existent in low size groups (10.0–29.9 mm), they reach high values in the 40.0–69.9 mm size groups. Fisher's exact test showed that 52.79% of the specimens had left and right chelipeds that were equal in size within 5,087 (2,078 F; 3,009 M) crayfish with both chelipeds present. This rate was estimated as 55.0% in males while was 49.5% in female individuals. The proportion of individuals with larger left (24.65%) or right (22.59%) chelipeds was found to be relatively similar.

**Table 1.** Summary of the number and percentage of cheliped types as a function of size for male and female crayfish from the *Pontastacus leptodactylus* population sampled in Atikhisar Reservoir, Çanakkale, Turkey (Pearson chi-square test value,  $\chi^2 = 102.5$ , p = 0.00) (N, number of individuals; EC, equal cheliped; LL, larger left; LR, larger right; MR, missing right; ML, missing left; MB, missing both; and NM, none missing).

| 0   | , ,  | 0,    |       |       |      |      |      |             |       |
|-----|------|-------|-------|-------|------|------|------|-------------|-------|
| Sex | Ν    | % EC  | % LL  | % LR  | Ν    | %MR  | %ML  | % <b>MB</b> | %NM   |
| F   | 2078 | 49.52 | 27.72 | 22.76 | 2593 | 5.55 | 4.47 | 9.83        | 80.14 |
| М   | 3009 | 55    | 22.53 | 22.47 | 3851 | 5.01 | 6.13 | 10.73       | 78.14 |
| F+M | 5087 | 52.76 | 24.65 | 22.59 | 6444 | 5.23 | 5.46 | 10.37       | 78.94 |

It was observed that the highest missing values for right, left, and both chelipeds were observed in freshwater crayfish sampled from the field in September 2020. The highest number of crayfish with both chelipeds present was also determined to be in these samples. The presence or absence of cheliped data by months is given in Tab. 3. The numbers and percentage of freshwater crayfish with right, left, and both chelipeds with abnormalities for 6,444 individuals who were visually examined are displayed in Tab. 4. While the number of abnormalities observed in chelipeds was higher in males, abnormalities in both chelipeds were higher in females.

**Table 2.** The presence or absence of chelipeds according to size classes from the *Pontastacus leptodactylus* population sampled in Atikhisar Reservoir, Çanakkale, Turkey (Pearson chi-square test value,  $\chi^2 = 36.31$ , p = 0.02) (CL, carapace length, MR, missing right; ML, missing left; MB, missing both; NM, none missing; LL, larger left; LR, larger right; EC, equal cheliped; F, female; and M, male).

| CL (mm) | MR |    | ML |     | MB  |     | NM  |     |     |     |     |     |
|---------|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|         |    |    |    |     |     |     | LL  |     | LR  |     | EC  |     |
|         | F  | М  | F  | М   | F   | М   | F   | М   | F   | М   | F   | М   |
| 10–19.9 |    |    |    | 1   |     | 1   | 1   |     |     | 1   | 1   |     |
| 20-29.9 | 2  | 3  | 1  | 2   | 12  | 7   | 6   | 3   | 3   | 5   | 2   | 1   |
| 30-39.9 | 9  | 10 | 7  | 12  | 22  | 31  | 23  | 22  | 15  | 30  | 18  | 31  |
| 40-49.9 | 49 | 33 | 42 | 53  | 130 | 113 | 218 | 181 | 195 | 170 | 385 | 320 |
| 50-59.9 | 68 | 84 | 46 | 105 | 64  | 186 | 244 | 332 | 175 | 321 | 467 | 938 |
| 60–69.9 | 13 | 57 | 15 | 58  | 22  | 69  | 74  | 128 | 76  | 132 | 145 | 353 |
| 70–79.9 | 3  | 6  | 5  | 4   | 5   | 6   | 9   | 11  | 8   | 15  | 11  | 11  |

**Table 3.** Monthly variation in the individual numbers of missing chelipeds from the *Pontastacus leptodactylus* population sampled in Atikhisar Reservoir, Çanakkale, Turkey (Pearson chi-square test value,  $\chi^2 = 880$ , p = 0.00) (F, female; M, male; MR, missing right; ML, missing left; MB, missing both; and NM, none missing).

| Month     | Sex | MR | ML | MB  | NM  |
|-----------|-----|----|----|-----|-----|
| T 1       | F   | 6  | 5  | 83  | 246 |
| July      | Μ   | 4  | 7  | 139 | 375 |
| A         | F   |    |    | 10  | 231 |
| August    | М   |    |    | 21  | 337 |
| Santambar | F   | 40 | 37 | 91  | 316 |
| September | М   | 45 | 49 | 163 | 480 |
| Ostalian  | F   | 10 | 8  | 3   | 217 |
| October   | М   | 21 | 29 | 14  | 238 |
| November  | F   | 9  | 9  | 6   | 284 |
| November  | М   | 16 | 18 | 5   | 377 |
| December  | F   | 3  | 3  | 2   | 20  |
| December  | М   | 26 | 29 | 6   | 267 |
| [         | F   | 24 | 13 | 8   | 155 |
| lanuary   | М   | 22 | 37 | 15  | 242 |
| February  | F   | 16 | 14 | 6   | 192 |
| redruary  | М   | 6  | 13 | 4   | 123 |
| March     | F   | 17 | 12 | 13  | 166 |
| waren     | М   | 16 | 21 | 4   | 260 |
| April     | F   |    |    | 3   | 14  |
|           | М   | 5  | 11 | 8   | 130 |
| Max       | F   | 6  | 2  |     | 152 |
| May       | М   | 6  | 7  | 7   | 87  |
| from a    | F   | 13 | 13 | 30  | 85  |
| June      | М   | 26 | 15 | 34  | 93  |

Μ

5

0.13

| ARC, abnormality in right cheliped; ABC, abnormality in both chelipeds). |      |       |     |      |     |      |     |      |  |  |
|--|------|-------|-----|------|-----|------|-----|------|--|--|
| Sex  | Ν    | %     | ALC | %    | ARC | %    | ABC | %    |  |  |
| F  | 2593 | 40.24 | 21  | 0.81 | 19  | 0.73 | 9   | 0.35 |  |  |

0.83

32

 Table 4. The number and percentage of abnormalities in both sexes for chelipeds from the *Pontastacus leptodactylus* population sampled in Atikhisar Reservoir, Çanakkale, Turkey (F, female; M, male; N, number of individuals, ALC, abnormality in left cheliped; ARC, abnormality in both chelipeds).

The abnormalities (right cheliped, left cheliped and both chelipeds in the same individual) frequently observed in the sample population are illustrated in Figs. 2–4.

59.76

32

3851

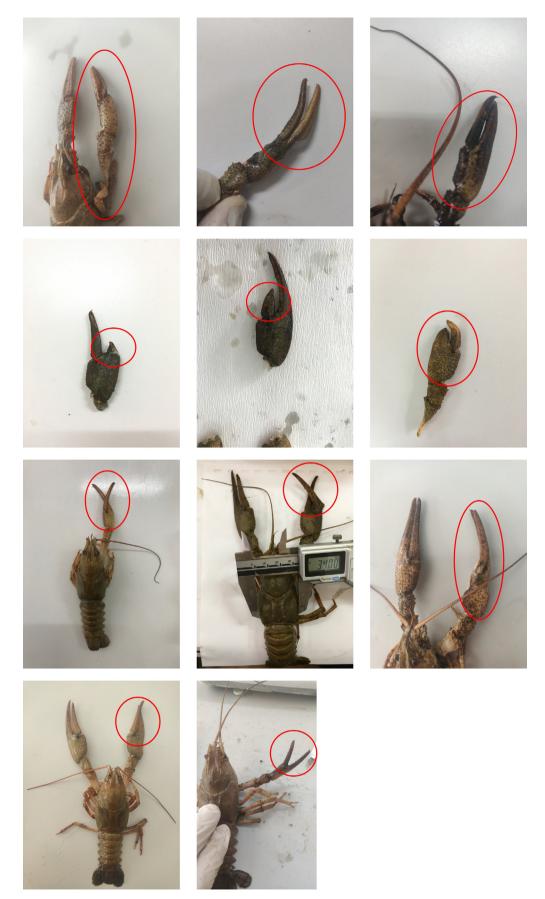
#### DISCUSSION

It was determined that 10.69% of the sampled individuals had a single cheliped (10.03% female, 11.14 male), 10.37% had no cheliped, and 78.94% had both chelipeds. The percentages of female and male individuals with no chelipeds were similar (9.83% for F; 10.73% for M) as were those with both chelipeds (80.14% for F; 78.14% for M). Likewise, the proportions of male and female individuals without a right cheliped were relatively similar. There was a statistical difference between cheliped loss and size groups (p < 0.05). Analyses of cheliped loss among carapace length groups revealed that the most intense loss occurred in the 40.0-49.9 and 50.0-59.9 mm size classes. For the length size classes between 10-29 mm and again for 70-80 mm, the cheliped loss was insignificant. These results suggest that individuals in both the 40.0–49.9 and 50.0–59.9 mm size groups may show more intense agonistic behaviors. Although Bovbjerg (1956) stated that larger crayfish typically hold an advantage over their smaller counterparts in aggressive encounters, Skurdal et al. (1988) noted that crayfish in the small size group did not lose more chelipeds than individuals in the larger size group. Nakata and Goshima (2003) emphasized that the outcome is strongly influenced by the advantage of body size when it comes to shelter competition between Cambaroides japonicus (De Haan, 1841) and Pacifastacus leniusculus (Dana, 1852). Similarly, Nakata and Goshima (2006) reported chelae loss was mainly from smaller individuals. However, on the other hand, Skurdal et al. (1988) reported that the loss of chelipeds occurs more commonly among crayfish of equal sizes, and small-sized crayfish tend to avoid conflicts with the larger ones.

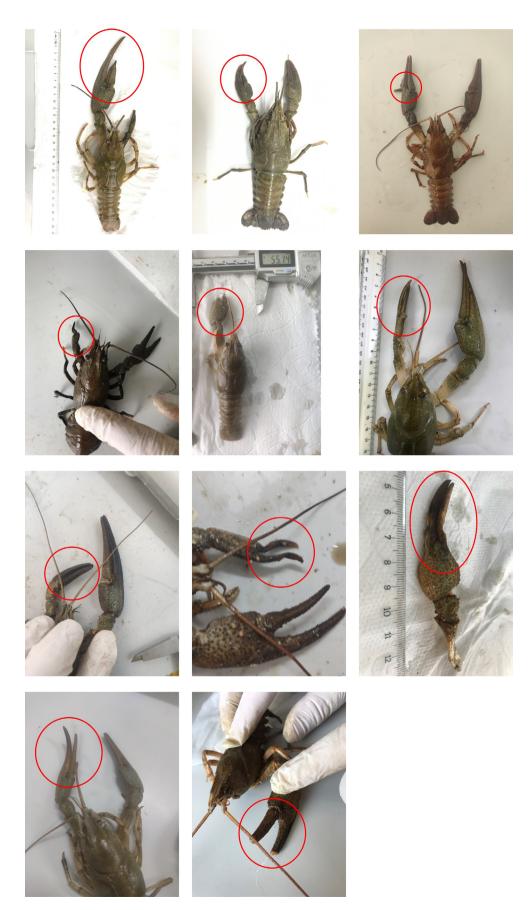
0.83

Many decapod crustaceans, particularly Astacidea, Anomura, and Brachyura, have large claws on the anterior pair of pereopods. Chelipeds are unique structures used in many important actions such as defending, attacking, feeding, and mating (Brown et al., 1979; Lee, 1995). Therefore, it is assumed that if a freshwater crayfish loses its chelipeds for any reason, or cannot use them functionally (abnormality), it will adversely affect its crucial activities. Gherardi et al. (2000) noted that chelipeds serve as the primary focal points of aggressive interactions, and crayfish primarily experience the loss of chelipeds and other appendages during confrontations with other crayfish, resulting in scars found predominantly on the chelipeds. In contrast to Skurdal et al. (1988), the absence of chelipeds in 10.37% of the sampled crayfish was found to be relatively high in the present study. If we add the proportions of crayfishes with either right or left lost chelipeds, the percentage reaches approximately 21%. Similarly, high cheliped loss in Lake Væleren and Lake Maridal (Maridalsvannet) where hunting is restricted, is reported to decrease rather than increase under continuous fishing (Skurdal et al., 1988).

The fact that males are more aggressive and exhibit more agonistic behaviors, means that a higher cheliped loss rate would have been expected when compared to females. Our results suggest that the difference between the sexes was significantly high ( $\chi^2 = 102.5$ , p < 0.05). This discrepancy could be explained by the fact that cheliped loss between sexes can be balanced because female individuals show more cheliped loss, especially during mating periods, and males are able to regenerate more frequently because of the higher molting frequency than females, after sexual maturity (Skurdal et al., 1988).



**Figure 2.** Right cheliped abnormalities observed in *Pontastacus leptodactylus* individuals collected from the Atikhisar Reservoir in Çanakkale, Turkey. Red circles indicate the abnormalities on the right chelipeds.



**Figure 3.** Left cheliped abnormalities observed in *Pontastacus leptodactylus* individuals collected from the Atikhisar Reservoir in Çanakkale, Turkey. Red circles indicate the abnormalities on the left chelipeds



**Figure 4.** Both right and left cheliped abnormalities observed in *Pontastacus leptodactylus* individuals collected from the Atikhisar Reservoir in Çanakkale, Turkey. Red circles indicate the abnormalities on both right and left chelipeds.

Capelli and Hamilton (1984) noted that the aggressive behavior of crayfish may decrease as the availability of both shelter and food increases. In the present study, cheliped losses increased in July, August, and September when self-feeding in the natural environment was comparatively intense. In the field study carried out in September 2020, the water level of the reservoir was relatively low (48 m) due to a drought period. The higher population density of crayfish in a more confined space in this period, due to the lower water level, may have caused an increase in agonistic interactions. Thus, resulting in an increase in the cheliped loss rate ( $\chi^2 = 880$ , p < 0.05).

Cheliped loss is a common occurrence among crustacean species in their natural habitats (Juanes and Smith, 1995; Mariappan et al., 2000). The loss of chelipeds can be attributed to various factors, including mating, digging, transportation, as well as aggression between individuals of the same or different species (Skurdal et al., 1988; Nyström, 2002). In male individuals, cheliped loss primarily stems from defensive encounters with predators and competition for mates and territorial defense (Vannini et al., 1983; Hunter and Naylor, 1993). Studies have indicated that the presence and variety of predators contribute to variations in cheliped loss (Berber et al., 2023). Reports have shown the existence of multiple species in reservoirs that potentially compete with crayfish for food and/or habitat (Kale et al., 2021a; 2022; 2023). Recently, Kale et al. (2021a) reported the occurrence of the freshwater turtle, Mauremys rivulata and Kale et al. (2022) noted the presence of the fish, Atherina boyeri in the study reservoir. It has been claimed that major crayfish predators such as European perch (Perca fluviatilis) and American mink (Mustela vison) can influence the variation in cheliped loss (Skurdal et al., 1988). The fact that northern pike (Esox lucius) is caught in large numbers by recreational fishermen in Atikhisar Reservoir indicates that its abundance is relatively high. It is assumed that one of the causes of crayfish limb loss, determined in this study, may be due to the presence of pike. As frequently exhibited by many other decapod crustaceans, crayfishes are capable of shedding their own appendages (autotomy) to escape from a predator or a trap (McVean, 1982).

Decapod chelipeds are usually larger in males than in females, and males win the competition for females by having larger chelipeds (Lee, 1995; Mariappan et al., 2000). Crayfish from the sampled population with right, left, or both chelipeds lost that resulted presumably from different factors were able to survive. However, ruptured chelipeds reduce the crustacean's ability to perform various functions effectively. For example, loss of chelipeds reduces its ability to compete for the limited resources and creates a disadvantage in intraspecific and interspecific interactions and defense against predators (Gherardi, 2002). Individuals with lost limbs have lower foraging efficiency compared to individuals with intact chelipeds in species that use their chelipeds to capture and handle their prey (Elner, 1980; Smith and Hines, 1991; Figiel and Miller, 1995; Flynn et al., 2015; Tummon et al., 2015). In a study conducted in Steinsfjorden Lake, it was suggested that the significant difference (3%) in the crayfish with defective chelipeds sampled by trap or by diving was a result of their foraging behavior change due to the loss of a cheliped, and therefore their physical ability to enter the sampling traps decreased. The reduced efficiency of the sampling gear was attributed to a decline in foraging and searching behavior among crayfish that have lost their chelipeds (Skurdal, 1988). Reproductive success in crayfish may also be negatively impacted by cheliped loss. This is due to the fact that individuals with missing chelipeds experience a decrease in mating success. Male crayfish with lost chelipeds face challenges in both finding a mating partner and defending the female. In trials between males with both chelipeds present and a cheliped absent under laboratory conditions, it has been demonstrated that loss of a cheliped reduces male copulatory success by an equivalent proportion of carapace size of 7–8 mm (Smith and Hines, 1991; Claverie and Smith, 2010). Male Orconectes propinguus (Girard, 1852) without fully functioning chelipeds failed to copulate successfully when coupled with females (Levenbach and Hazlett, 1996). Males with intact chelipeds are more likely to acquire mates in intrasexual competition and also in sexual selection by females.

Since limb loss (and especially cheliped loss) has important outcomes for growing and surviving, most crustaceans are able to regenerate lost limbs (Juanes and Smith, 1995). Crustaceans can replace their lost appendages through regeneration, closely coupled with molting (Skinner, 1985). Although the regenerated limb is structurally functional, like the undamaged contralateral pristine structure, it is not a perfect replica, particularly, compared to the initial chelipeds. Both smaller size and weaker pinching forces of the regenerated chelipeds have been reported (Buřič et al., 2009; McLain et al., 2010; Bywater et al., 2015). In addition, the regeneration process of lost chelipeds demands additional energy and changes the energy allocation for reproduction and/or somatic growth (Mariappan et al., 2000; Reynolds, 2002). Therefore, the growth of injured crayfish is negatively affected. That is, loss of chelipeds results in a shorter intermolt period and growth is delayed to promote cheliped regeneration.

Right and left asymmetry in chelipeds (heterochely) is commonly observed in decapods (Crane, 1975; Lee, 1995; Mariappan et al., 2000). Heterochely is mainly initiated at an early development period, either due to the regeneration process of a lost cheliped or by the differential usage between right and left chelipeds (Govind and Pearce, 1989; Young et al., 1994; Goldstein and Tlusty, 2003). Heterochely could be regarded as a significant morphological feature in crustaceans resulting from an ontogenic mechanism associated with the functional importance of a specific cheliped shape (Claverie and Smith, 2010). Although crayfish chelipeds are slightly unequal in size, they are typically homogeneous in shape (Maguire and Klobučar, 2011; Lele and Parvulescu, 2019).

The Fisher's exact test indicated that 52.79% of the samples had right and left chelipeds of equal size within 5,087 (2,078 F, 3,009 M) freshwater crayfish with both chelipeds present. While this ratio was 49.5% in females, it was estimated as 55.0% in males. The proportion of individuals with larger right (22.59%) or left (24.65%) chelipeds was found to be relatively close to each other. Lele and Pârvulescu (2019) reported that there was no significant difference in right and left cheliped size between sexes when relatively larger and equal in size groups of P. leptodactylus where compared. The proportion of individuals with cheliped size equal on both sides were reported to be 32.9% for females and 37.3% for males, lower than our results for both genders (50% F; 55% M). Lele and Pârvulescu (2019) also suggested that relatively small differences in cheliped size do not affect vital living activities of crayfish. In addition, chelipeds were found to be more likely to exhibit heterochely in smaller individuals when compared with relatively larger size groups. In the studied crayfish sample, homochely was very low in frequency in the size groups up to 40 mm, while individuals that possessed homochelous chelipeds were encountered considerably more frequent in the size groups between 40–70 mm carapace length. This indicates that larger crayfish have increased heterochely due to the greater frequency of aggressive encounters during their lifetime, and therefore they are more likely to lose both chelipeds and then regenerate them (Brewis and Bowler, 1982; Figiel and Miller, 1995). The heterochelous morphology in chelipeds may have developed because of highly complex evolutionary factors for certain decapods that show an advantage in agonistic behavior, as well as in other vital activities such as foraging and feeding (Lee, 1995; Baeza and Asorey, 2012). Brachyuran crabs commonly exhibit heterochely. Families such as Calappidae, Cancridae, Portunidae, and Xanthidae are known to display heterochely. In these populations, the right cheliped is usually a crusher and the left cheliped is a cutter (Lewis, 1969; Yamada and Boulding, 1998; Schenk and Wainwright, 2001).

Out of the 6,444 freshwater crayfish examined, 53 individuals had a left cheliped abnormality, 51 individuals had a right cheliped abnormality, and 14 individuals had abnormalities on both chelipeds. The fact that the individuals were obtained alive, was an indication that the abnormalities did not significantly affect their vital activities directly. However, morphological abnormalities in appendages, especially in the chelipeds, may impair their functional use compared to a healthy cheliped. This abnormal condition is assumed to carry the same disadvantageous limitations experienced by the absence of a cheliped. Many assumptions and controlled laboratory studies have been conducted on various anomalies in decapod species. Pârvulescu (2009) stated that the abnormalities probably occur after molting, due to aggression experienced during shell formation, and can be observed mostly in the rostrum and carapace of the spiny-cheek crayfish Orconectes limosus.

Cheliped abnormalities are also caused by abnormal healing of a wound, especially one that

follows a damaged propodus (Okomoto, 1991; Nakatani et al., 1992; Chokki and Ishihara, 1994; Murayama et al., 1994; Okada et al., 1997; Nakatani and Kitahara, 1999). Pârvulescu et al. (2009) reported that approximately 1/3 of the sampled spiny-cheek crayfish *Orconectes limosus* showed abnormal body shape or appearance, and the authors suggested that the possible reasons for these abnormalities may be not only natural variability, but also aggression between individuals and minor natural accidents.

Chelipeds of decapod crustaceans have attracted the attention of scientists for a long time and their functional use in the vital activities of the individual has been thoroughly investigated. Having especially large chelipeds is one of the effective factors in occupying a higher social status in the hierarchical order. In addition, these individuals are more inclined to have conflict with other individuals, causing physical damage, loss of limbs, and increased mortality rates. Although it varies between species, cheliped weights can constitute between 10 and 26% of the total body weight (Simonson and Steele, 1981; Lee and Seed, 1992; Mariappan and Balasundaram, 1999). An individual that has lost its limbs as a result of a conspecific attack, is pacified, lowering its social rank in the hierarchical order. The individual is then exposed to limitations in many aspects of his vital activities, such as foraging ability, predator avoidance, reproductive success, shelter competition, and defending territory (Smith and Hines, 1991). In some crustacean species culturing, ablation or immobilization of chelipeds plays an active role in preventing cannibalism. However, due to removal of the limbs, and the following regeneration, more metabolic energy expenditure will be required, which will result in the delay of molting and in restrictions on reproductive output. Therefore, the use of these methods in crustacean culturing is limited (Mariappan et al., 2000).

In conclusion, several factors might be the cause of the cheliped loss and abnormalities of the narrowclawed crayfish, *P. leptodactylus*. The development of cheliped may be influenced by biotic and abiotic factors. Functional and structural changes in chelipeds may be affected by species-specific needs and environmental conditions in addition to feeding and movement patterns. Therefore, monitoring studies should be continued to ensure the sustainability of a healthy crayfish population due to the commercial values of abnormal crayfish being lower. Further studies should investigate the contribution of genetic factors or potential environmental stressors such as diet, temperature and salinity on the emergence of cheliped loss and abnormalities.

#### ACKNOWLEDGEMENTS

The authors thank their colleagues for their support in the laboratory and the field.

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# ADDITIONAL INFORMATION AND DECLARATIONS

## **Author Contributions**

Conceptualization and Design: SB. Performed research: SB, SK, DA. Acquisition of data: SB, SK, DA. Analysis and interpretation of data: SB, SK, DA. Preparation of figures/tables/maps: SB, SK. Writing - original draft: SB, SK. Writing - critical review & editing: SB, SK.

## **Consent for publication**

All authors declare that they have reviewed the content of the manuscript and gave their consent to submit the document.

## **Competing interests**

The authors declare that they have no conflict of interest.

## Data availability

All study data are included in the article.

# **Ethical approval**

For this type of study, formal consent is not required.

## Funding and grant disclosures

This study was financially supported by Çanakkale Onsekiz Mart University The Scientific Research Projects Coordination Unit (ÇOMÜBAP) projects under FBA-2020-3250 (granted to Dr. Selçuk Berber). We would also like to thank all researchers for their contributions.

## **Study association**

Not applicable.

## **Study permits**

Field collection and transportation of specimens were made under the Republic of Türkiye, Ministry of Agriculture and Forestry, General Directorate of Fisheries and Aquaculture permit number 67852565-140.03.03-E.1811532 issued to S. Berber.