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Chelae regeneration in European alien crayfish *Orconectes limosus* (Rafinesque 1817)

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ABSTRACT

Key-words:
non-indigenous,
crayfish,
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chelae,
regeneration,
growth

Crayfish chelae have important functions, including prey capture and manipulation, reproduction, defense against predators, and inter- and intra-specific interactions. Consequently a loss of chelae often occurs. Adult specimens of invasive spiny-cheek crayfish (*Orconectes limosus*) were reared from February to November 2008 to observe the ability to regenerate lost chelae. Percent molt increments (PMI) of postorbital carapace length (POCL), chela length (ChL) and width (ChW) were compared between crayfish with and without chelae. Regenerated chelae were found to grow faster (ChL, ChW) than initial chelae and POCL. This trend occurs in both reproductive and non-reproductive *O. limosus* males and females. Values of 55.6 ± 0.06% for ChL and 50.0 ± 0.06% for ChW, compared to the initial chelae, were obtained in regenerated chelae of adult *O. limosus* after one molt. After two molts regenerated chelae reached 67.6 ± 0.07% ChL and 69.7 ± 0.08% ChW of values for initial chelae. Growth rate of regenerated chelae decreased with an increase in chelae dimensions. Regenerated chelae were found to be more narrow (significantly lower ChW/ChL ratio) than initial chelae. Regenerating of chelae did not have any influence on POCL growth. This study presents evidence that the invasive *O. limosus* possesses the ability of rapid substitution of lost chelae.

RÉSUMÉ

Régénération des pinces chez l'écrevisse américaine *Orconectes limosus* (Rafinesque 1817)

Mots-clés :
non-indigène,
écrevisse,
invasive,
pince,
régénération,
croissance

Les pinces des écrevisses ont des fonctions importantes incluant la capture et la manipulation des proies, la reproduction, la défense contre les prédateurs et des relations inter- et intra-spécifiques. Il en résulte assez souvent la perte de ces pinces. Des spécimens adultes de l'écrevisse américaine (*Orconectes limosus*) ont été élevés de février à novembre 2008 pour observer la possibilité de régénération des pinces perdues. Les pourcentages d'incrément à la mue (PMI) de la longueur post-orbitale de la carapace (POCL), la longueur de la pince (ChL) et sa largeur (ChW) ont été comparés entre des écrevisses avec et sans pince. Les pinces régénérées grandissent plus vite (ChL, ChW) que les pinces initiales et POCL. Cette tendance existe à la fois chez les écrevisses mâles et femelles matures ou non. Des valeurs de 55,6 ± 0,06 % pour ChL et 50,0 ± 0,06 % pour ChW, par rapport aux pinces initiales, ont été obtenues pour les pinces régénérées d'adultes

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d'O. limosus après une mue. Après deux mues, les pinces régénérées atteignent 67,6 ± 0,07 % ChL et 69,7 ± 0,08 % ChW des valeurs des pinces initiales. Le taux de croissance des pinces régénérées décroît avec un accroissement des dimensions de la pince. Les pinces régénérées sont plus étroites (rapport ChW/ChL significativement plus faible) que les pinces initiales. Les pinces en cours de régénération n'ont pas d'influence sur la croissance de POCL. Cette étude montre bien que *O. limosus* possède une capacité de substitution rapide des pinces perdues.

INTRODUCTION

A spread of non-indigenous crayfish (NIC) populations is one of the most critical threats for indigenous crayfish (IC) stocks (Füreder *et al.*, 2006). One of the most established NIC in European inland waters is spiny-cheek crayfish, *Orconectes limosus* (Rafinesque, 1817), with an occurrence in at least 20 European countries (Pöckl *et al.*, 2006). *Orconectes limosus*, as the other members of North American NIC, is known as a vector of the crayfish plague pathogen, *Aphanomyces astaci* (Schikora, 1906), which has caused a radical decrease of IC stocks in Europe (Evans and Edgerton, 2002). Furthermore, rapid establishment in new habitats and further spreading of *O. limosus* are enhance due to, among others, high resistance to unfavorable conditions (Holdich *et al.*, 2006), early maturation (Hamr, 2002), high fecundity (Chybowski, 2007), short egg incubation (Kozák *et al.*, 2006) fast growth (Kozák *et al.*, 2007), high activity (Musil *et al.*, 2009), aggressiveness (Holdich and Black, 2007), and high migration ability (Buřič *et al.*, 2009a).

Orconectes limosus belongs to family Cambaridae (Hobbs, 1981), with a life cycle different from that of European native crayfish from the family Astacidae (Stucki, 2002). The differences of *O. limosus* are presented by two mating periods (Hamr, 2002), spring spawning and a short incubation period (Kozák *et al.*, 2006), and more frequent molting with form alternation in both, adult males (Buřič *et al.*, 2009b) and females (Buřič *et al.*, 2009c). The typical life cycle of male *O. limosus*, as reported by Hobbs (2001) in North America, is characterized by periodic form alternation throughout life, associated with two molts per year (spring – May/June; summer – August), and periodic changes in chelae dimensions. Despite some exceptions (only one or no one molt in large males), similar characteristics have been confirmed under European conditions (Buřič *et al.*, 2009b). In addition, females of *O. limosus* also undergo cyclic dimorphism, alternating between sexually active and sexually inactive stages, with a majority of females molting twice per year (Buřič *et al.*, 2009c). Chelae are essential for crayfish (Holdich, 2002), with numerous crucial functions, including prey capture and manipulation, defense against predators, inter- and intra-specific interactions, and reproduction (Stein, 1976). Chelae loss decrease the ability to compete for limited resources, and present a disadvantage in intra- and inter-specific interactions and defense against predators (Gherardi, 2002). Despite of this, form alternation, associated with two molts per year, involves effective use of resources to increase the potential for growth and regeneration of lost appendages *i.e.* increase fitness and appearance (Buřič *et al.*, 2009b, 2009c). Regeneration of lost chelae imposes an additional energy demand, altering energy allocation for somatic and/or reproductive processes (Mariappan *et al.*, 2000). The growth of injured crayfish is therefore negatively affected (Figiel and Miller, 1995). A requirement to improve competitiveness is a high ability to regenerate lost chelae.

The main objective of present study was to evaluate if the invasive *O. limosus*, which presents a high molting frequency, is capable to rapid substitution of loss chelae. Particular aims were to compare molt increments of the forms I and II between crayfish with and without regenerating chelae.

MATERIAL AND METHODS

> ANIMALS

Specimens of *O. limosus* (both sexes) were captured (n = 1157) in the Černovický brook (South Bohemia, Czech Republic) in August 2007. They were acclimated to laboratory conditions during autumn and winter, and placed in experimental tanks in February 2008. In overall, the experimental period lasted for 10 months (February–November). Each crayfish was identified according to sex and reproductive state by external appearance. Males were classified as sexually active (form I) or inactive (form II) using the criteria of Pieplow (1938) and Hobbs (1989), and females after Buřič *et al.* (2009c). Crayfish were kept in mixed sex groups (females:males 2:1) where visual, chemical and tactile contact was possible. Individual crayfish were identified with VI Alpha tags (Northwest Marine Technology^{TM}, Shaw Island, USA) following Isely and Stockett (2001) and Buřič *et al.* (2008). Regenerating (one chelae lost or regenerated) and control (both chelae in appropriate size) groups were kept under similar conditions, respectively.

> EXPERIMENTAL CONDITIONS

Crayfish were stocked in circular tanks (0.6 m diameter, volume 0.18 m^3) and supplied with ~3 shelters per crayfish. Photoperiod and water temperature was natural ambient, from natural daylight and a flow through water supply. Tanks were cleaned regularly, and dissolved oxygen was measured twice daily using an oximeter (Oxi 315i, WTW GmbH, Weilheim, Germany). Water temperature was measured every 3 h using data loggers (RT-F53, Qi Analytical, Prague, Czech Republic), with pH measured daily (pH 315i, WTW GmbH, Weilheim, Germany). Crayfish were fed in excess with fish pellets, frozen chironomid larvae, and carrots (two to five times per week, depending on season and amount of uneaten feed).

> DATA COLLECTION

Measurements were done before and after molting (when the exoskeleton was fully hardened). Postorbital carapace length (POCL, from the edge of eye socket to the posterior edge of the cephalothorax), chela length (ChL, from the tip of propodus to carpal joint), and chela width (ChW, at the widest part) were measured to the nearest 0.01 mm with digital callipers (Schut Geometrical Metrology, Groningen, The Netherlands). Wet body weight was determined to the nearest 0.01 g with an electronic balance (Kern & Sohn GmbH, Balingen, Germany). Molting and form alternation were recorded. Percent growth increment was calculated for each molted crayfish according to Brewis and Bowler (1982):

$$L_i = (L_a - L_b) \times 100/L_b \,[\%] \tag{1}$$

where L_i = length increment, L_a = length after molt, and L_b = length before molt.

> DATA ANALYSIS

Data were analyzed using Statistica 8.0 (StatSoft., Inc.). All values were examined for normality (Kolmogorov-Smirnov test) and homoscedaticity (Levene test). Paired t-tests were used to compare thickness of initial and regenerated chelae; ANCOVA with Tukey's *post hoc* test for a comparison of molt increments between particular groups and between sexes with POCL as covariate; and Spearman rank correlation analysis for evaluation of the relation between percent molt increments and body size. The null hypothesis was rejected at α = 0.05. Data are presented as mean ± SE.

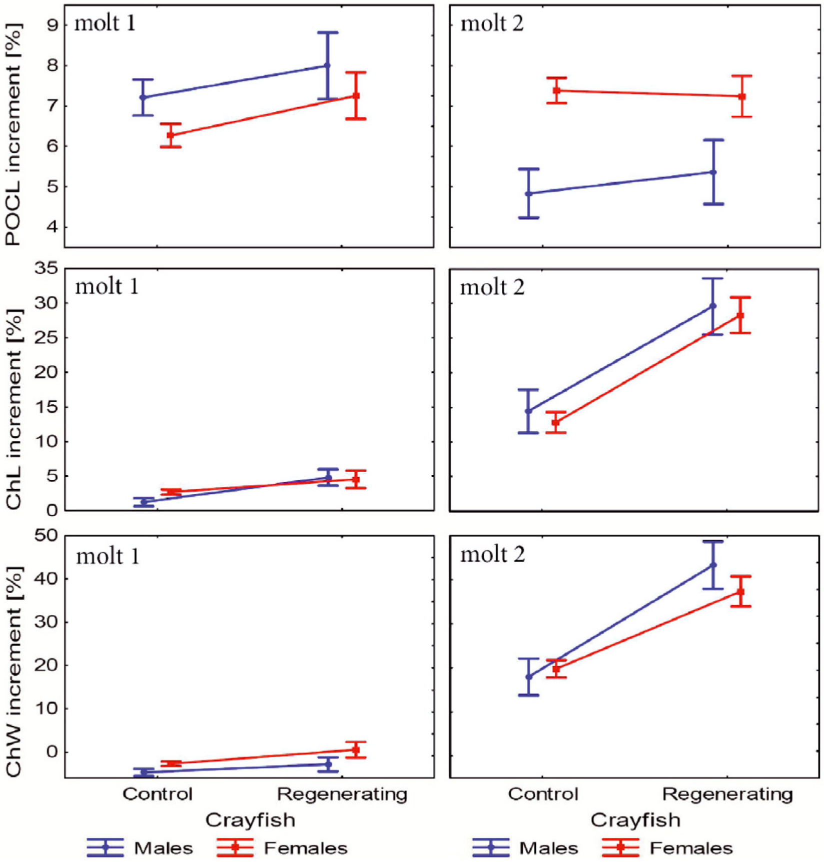

Figure 1
Comparison of percent molt increments of postorbital carapace length (POCL), chelae length (ChL) and chelae width (ChW) between control and regenerating crayfish. Increments after 1st molt (to form II) presented on left side; after 2nd molt (back to form I) presented on right side. Data are presented as mean ± SE.

Figure 1
Comparaison des pourcentages d'incrément à la mue de la longueur post-orbitale de la carapace (POCL), de la longueur de la pince (ChL) et de sa largeur (ChW) entre des écrevisses témoins et régénérées. Les incréments après la première mue sont sur la gauche et ceux après la seconde mue sur la droite de la figure. Les données sont des moyennes ± SE.

RESULTS

> *MOLT INCREMENTS OF CONTROL AND REGENERATING CRAYFISH*

In general, the 1st molt could be characterized by a low increment of ChL and a decrease of ChW. In contrast, the 2nd molt presented a higher growth of both ChL and ChW. Except for a higher POCL PMI at the 2nd molt ($F = 14.13$; $P = 0.0004$) in females, no other differences in PMI (POCL, ChL, ChW) were obtained between males and females. As expected, significant differences were observed in ChL and ChW PMI's between control and regenerating crayfish at both the 1st ($F_{ChL} = 8.43$; $P_{ChL} = 0.0047$; $F_{ChW} = 3.98$; $P_{ChW} = 0.0489$) and 2nd molt ($F_{ChL} = 26.48$; $P_{ChL} < 10^{-5}$; $F_{ChW} = 29.62$; $P_{ChW} < 10^{-5}$). The pattern of increasing ChL and ChW growth and invariant POCL growth between control and regenerating crayfish was similar for males and females without significant differences. These results are graphically demonstrated in Figure 1.

Two distinctive groups were identified within males and females: individuals that molted twice with form alternation (FI → FII → FI), and individuals presenting a single molt (41% and 8%

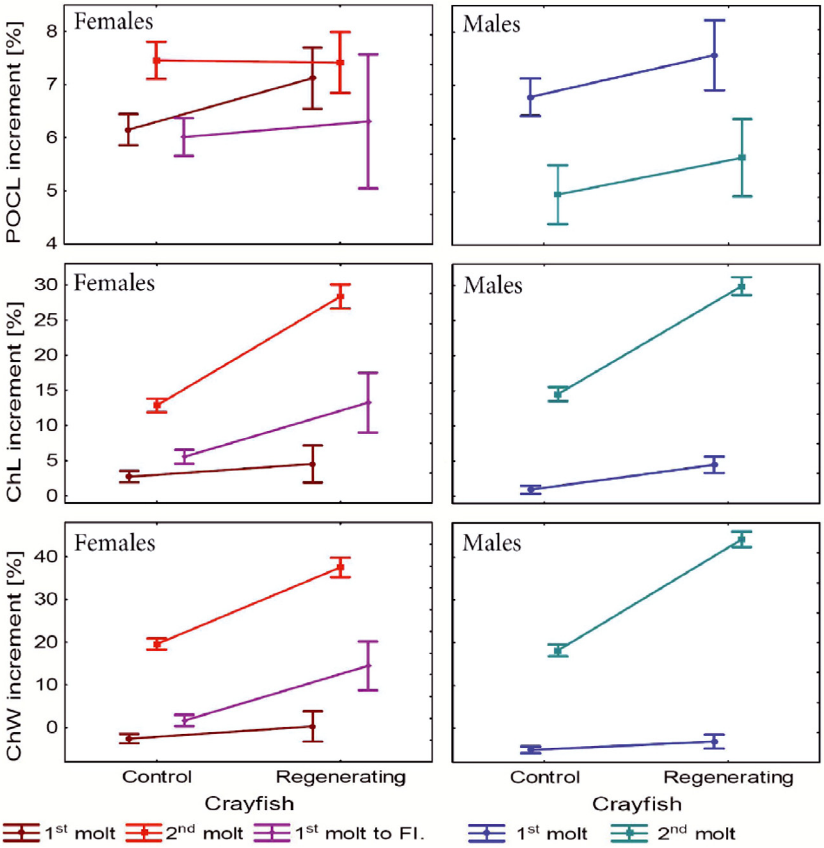

Figure 2
Comparison of percent molt increments of postorbital carapace length (POCL), chelae length (ChL) and chelae width (ChW) between control and regenerating crayfish in particular molts. Data are presented as mean ± SE.

Figure 2
Comparaison des pourcentages d'incrément à la mue de la longueur post-orbitale de la carapace (POCL), de la longueur de la pince (ChL) et de sa largeur (ChW) entre des écrevisses témoins et régénérées pour des mues individuelles. Les données sont des moyennes ± SE.

of females and males, respectively) without form alternation (FI → FI). Unfortunately a limited number of once molted males prevented their inclusion in the data analysis. The comparison of PMI at particular molts in males and females is shown in Figure 2. No differences were detected in POCL PMI between individual molts in females. In contrast, males reached higher values at the 1st molt ($F = 9.70$; $P = 0.0033$). Control and regenerating crayfish did not differ in POCL PMI, and the pattern of invariant POCL growth between control and regenerating crayfish was similar within all molts. Despite the above, significant differences in ChL and ChW PMI's were found between particular molts. The highest PMI of ChL and ChW in females was obtained at the 2nd molt, followed by values reached with molting without form alternation, and the lowest PMI's occurred at the 1st molt to FII ($F_{ChL} = 51.77$; $P_{ChL} < 10^{-5}$; $F_{ChW} = 89.19$; $P_{ChW} < 10^{-5}$). Similarly, higher ChL and ChW PMI's were reached at the 2nd molt in males ($F_{ChL} = 336.11$; $P_{ChL} < 10^{-5}$; $F_{ChW} = 563.27$; $P_{ChW} < 10^{-5}$). A higher PMI was observed in regenerating chelae in both females ($F_{ChL} = 20.61$; $P_{ChL} < 10^{-5}$; $F_{ChW} = 20.69$; $P_{ChW} < 10^{-5}$) and males ($F_{ChL} = 84.85$; $P_{ChL} < 10^{-5}$; $F_{ChW} = 94.65$; $P_{ChW} < 10^{-5}$) at all times. The strongest pattern of increasing ChL and ChW growth between control and regenerating crayfish was reached at the 2nd molt for males ($F_{ChL} = 32.86$; $P_{ChL} < 10^{-5}$; $F_{ChW} = 69.16$; $P_{ChW} < 10^{-5}$) and females. The stronger pattern at molt without form alternation was detectable compared to the 1st molt ($F_{ChL} = 8.45$; $P_{ChL} = 0.0003$; $F_{ChW} = 5.53$; $P_{ChW} = 0.0048$).

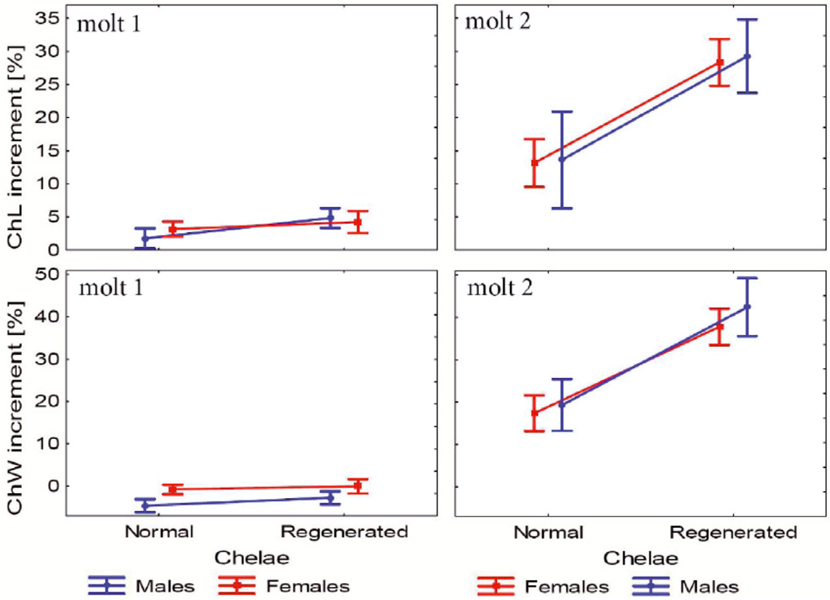

Figure 3
Comparison of percent molt increments in chelae dimensions (chelae length – ChL and chelae width – ChW) between normal and regenerated chelae in particular crayfish. Increments after 1st molt (to form II) presented on left side; after 2nd molt (back to form I) presented on right side. Data are presented as mean ± SE.

Figure 3
Comparaison des pourcentages d'incrément à la mue des dimensions de la pince (ChL et ChW) entre des écrevisses témoins et régénérées pour des mues individuelles. Les incréments après la première mue sont sur la gauche et ceux après la seconde mue sur la droite de la figure. Les données sont des moyennes ± SE.

> *MOLT INCREMENTS IN REGENERATING CRAYFISH*

The comparison of males and females PMI's after 1st and 2nd molt are shown in Figure 3. The only difference in PMI (ChL, ChW) between males and females was a higher ChW PMI for females at the 1st molt ($F = 4.68$; $P = 0.0412$). Significant differences were found in ChL and ChW PMI's between initial and regenerated chelae at the 2nd molt ($F_{ChL} = 9.15$; $P_{ChL} = 0.0054$; $F_{ChW} = 16.06$; $P_{ChW} = 0.0004$). However, no differences have occurred at the 1st molt. The pattern of increasing ChL and ChW growth between original and regenerated crayfish was similar for males and females without significant differences.

Figure 4 presented a comparison of PMI at particular molts in males and females. Significant differences in ChL and ChW PMI's were found between individual molts. The highest PMI of ChL and ChW in females was obtained at the 2nd molt, with the lowest PMI occurring at the 1st molt to FII ($F_{ChL} = 11.05$; $P_{ChL} = 0.0002$; $F_{ChW} = 16.25$; $P_{ChW} < 10^{-5}$). A similar tendency was found in males, with higher ChL and ChW PMI's reached at the 2nd molt ($F_{ChL} = 82.79$; $P_{ChL} < 10^{-5}$; $F_{ChW} = 329.63$; $P_{ChW} < 10^{-5}$). In both females ($F_{ChL} = 5.54$; $P_{ChL} = 0.0046$; $F_{ChW} = 5.62$; $P_{ChW} = 0.0037$) and males ($F_{ChL} = 21.12$; $P_{ChL} = 0.0004$; $F_{ChW} = 79.72$; $P_{ChW} < 10^{-5}$) a higher PMI was obtained in regenerating chelae. The strongest pattern of increasing ChL and ChW growth between control and regenerating crayfish was reached at the 2nd molt for females ($F_{ChL} = 4.19$; $P_{ChL} = 0.0408$; $F_{ChW} = 4.69$; $P_{ChW} = 0.0399$) as well as for males ($F_{ChL} = 9.07$; $P_{ChL} = 0.0088$; $F_{ChW} = 55.32$; $P_{ChW} < 10^{-5}$).

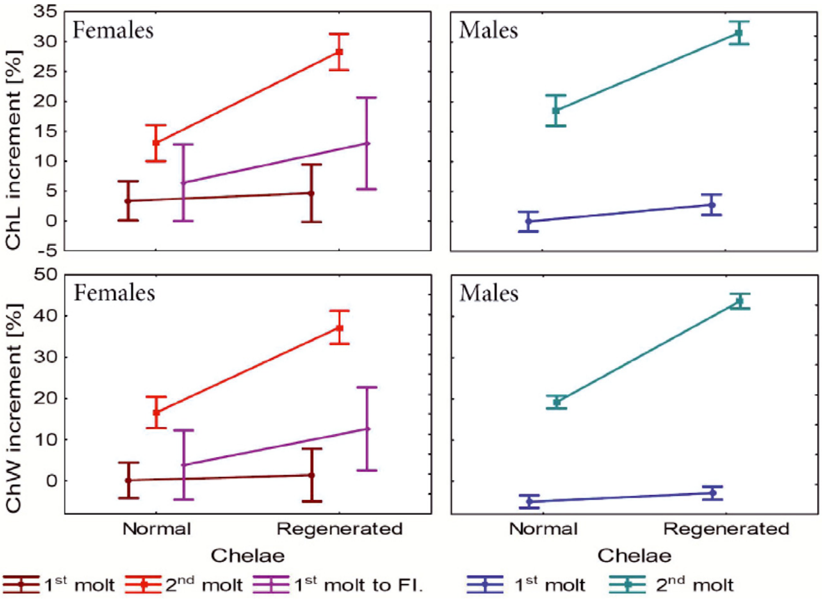

Figure 4
Comparison of percent molt increments in chelae dimensions (chelae length – ChL and chelae width – ChW) between normal and regenerated chelae of particular crayfish in particular molts. Data are presented as mean ± SE.

Figure 4
Comparaison des pourcentages d'incrément à la mue des dimensions de la pince (ChL et ChW) entre des écrevisses témoins et régénérées pour des mues particulières. Les données sont des moyennes ± SE.

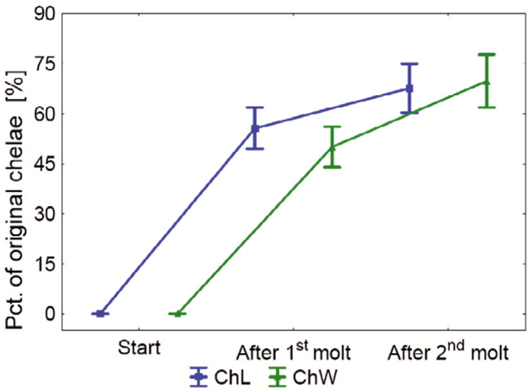

Figure 5
Size of regenerated chelae expressed as percentage of initial chelae [%] after 1st and 2nd molt. Data are presented as mean ± SE.

Figure 5
Tailles des pinces régénérées exprimées en pourcentage de la pince initiale [%] après la première et la seconde mue. Les données sont des moyennes ± SE.

> SUBSTITUTION OF LOST CHELAE

Within two molts regenerated chelae have reached two-thirds of the size of original chelae (Figure 5). However, growth of regenerating chelae gradually decreased with as size increased (Table I). Regenerated chelae were narrower than initial chelae (Table II).

Table I
Spearman rank correlations between growth (percent molt increment of chelae length – ChL PMI, and width – ChW PMI) and relative size (ChL/POCL and ChW/POCL) of regenerated chelae. Values indicated in italics are significant at $\alpha = 0.05$.

Tableau I
Corrélation de rangs de Spearman entre paramètres de croissance (pourcentage d'incrément à la mue de la longueur de la pince – ChL PMI, et de sa largueur – ChW PMI) de la pince régénérée. Les valeurs en italique sont significatives au risque $\alpha = 0,05$.

Spearman rank correlation	Males	Females
ChL/POCL *vs.* regenerated ChL PMI	*−0.893*	*−0.768*
ChL/POCL *vs.* original ChL PMI	−0.108	−0.183
ChW/POCL *vs.* regenerated ChW PMI	*−0.890*	*−0.851*
ChW/POCL *vs.* original ChW PMI	−0.596	−0.617

Table II
Comparison of the chelae width (ChW) and length (ChL) ratio between initial and regenerated chelae in particular crayfish. Data are presented as mean ± SE. Different superscripts in the same row differ at $\alpha = 0.05$ *(t-test, t).*

Tableau II
Comparaison du rapport entre valeurs initiale et régénérée de la largeur (ChW) et de la longueur (ChL) des pinces des écrevisses. Les données sont des moyennes ± SE. Les différentes lettres dans la même ligne indiquent des valeurs différentes au risque $\alpha = 0,05$ (*t*-test, *t*).

	ChW/ChL			
Sex	Original	Regenerated	*t*	*P*
Males	0.35 ± 0.00^{a}	0.32 ± 0.00^{b}	3.03	0.005
Females	0.42 ± 0.003^{a}	0.40 ± 0.006^{b}	2.37	0.020

DISCUSSION

The typical life cycle of male *Orconectes* in North America is periodic form alternation throughout life, associated with two molts per year (Hobbs, 2001). That was confirmed with some exceptions also in European conditions (Buřič *et al.*, 2009b) and even for females (Buřič *et al.*, 2009c). Morphological changes in chelae are associated with different molt increments at the particular molts (Buřič *et al.*, 2009b, 2009c). Chelae injuries and loss are usual in crayfish populations as a function of frequent social interactions between crayfish or predatory impact (Nyström, 2002). *Orconectes limosus* is highly active (Lozan, 2000; Musil *et al.*, 2009) and aggressive species (Holdich and Black, 2007) and therefore can be expected high frequency of chelae loss in this species. On the other hand, there is a need to substitute or repair the damages as soon as possible because of important functions of chelae, including prey capture and manipulation, defense against predators, inter- and intra-specific interactions, and reproduction (Stein, 1976; Holdich, 2002). In addition, it is necessary to built again functionally complete large chelae because the large chelae are advantageous in competition for limited resources and in aggressive behavior (Söderbäck, 1991; Nakata and Goshima, 2003). The characteristic two molts per year enables *O. limosus* great predisposition for substitution of lost chelae.

Orconectes limosus has two opportunities to substitute lost chelae. However, the present study indicates that the regeneration process is more effective at the molt to the reproductive active form I, and in specimens that are molting twice. These findings are in accordance to Buřič *et al.* (2009b, 2009c), which found low chelae growth at molt to the reproductive inactive form II. The above might be attributed to the absence of breeding behavior when form II occurs (Payne, 1978). Form II males spent significantly more time in shelters, displayed fewer agonistic acts, and spent less time fighting than did form I males (Tierney *et al.*, 2008). Males in the form II stage do not require large chelae due to minimal predatory risk, sexually inactivity, and less exposure to agonistic encounters. Priority of the 1st molt is therefore to increase body size while conserving energy for high chelae growth at the 2nd molt (Buřič *et al.*, 2009b, 2009c). The ability to increase chelae dimensions at the 2nd molt is increased in regenerating chelae. The only difference between initial and regenerated chelae was narrowing of the latter. Long but narrower regenerated chelae accentuate the importance of ChL (Bovbjerg, 1956).

It is expected that chelae regeneration would required high energy costs (Powell *et al.*, 1998; Reynolds, 2002), called "regeneration load" (Mariappan *et al.*, 2000). As a result body size increments should decrease (*e.g.* Figiel and Miller, 1995; Mariappan *et al.*, 2000). However, the present study did not reveal any differences in body size growth between regenerating and control crayfish, which could be explained by hypothetical higher foraging behavior in regenerating crayfish. Despite of this, *O. limosus* is able of rapid regeneration and growth of loss chelae, which confirms the strong adaptability and viability of *O. limosus*. The form alternation clearly plays an important role in the growth patterns, including chelae restoration, as an effective use of resources to increase fitness and appearance (Buřič *et al.*, 2009b, 2009c). The priority of the 1st molt is to increase body size (chelae growth is depressed) while retaining energy for considerable chelae growth at the succeeding molt.

Rapid chelae regeneration restores the capability of competitiveness within a population in this aggressive crayfish species. A high ability to achieve the original appearance and competitiveness can be added to the list of *O. limosus* advantages compared to IC.

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