

# Impacts of leg loss and regeneration on body condition, growth, and development time in the wolf spider *Schizocosa ocreata*

K.M. Wrinn and G.W. Uetz

**Abstract:** Autotomy (self-amputation) of appendages and subsequent regeneration is common to many taxa. These processes are known to affect foraging abilities, growth, and development time in many taxa. However, little is known about their effects in arachnids. We addressed the effects of autotomy and regeneration on body condition, growth (size and mass), and development time (molt interval) for the wolf spider *Schizocosa ocreata* (Hentz, 1844) in the field and laboratory. Frequency of autotomy in the field was high (11%–19%). Field-caught individuals with missing or regenerating legs had significantly lower body size, mass, and condition. To test the effects of regeneration on size, mass, and molt interval in the laboratory, we induced autotomy of one or both forelegs. Spiders regenerating two legs had reduced molt intervals, were smaller, and weighed less than spiders that were intact or regenerating one leg. Field-caught spiders that had undergone autotomy and regeneration in the laboratory exhibited reduced size, mass, and molt interval. In contrast, laboratory-reared spiders exhibited increased molt intervals but no difference in mass after regeneration. These results reveal that limb loss via autotomy is common (but potentially costly) in *S. ocreata*, and that environmentally mediated trade-offs between growth and development time may occur during regeneration.

**Résumé :** L'autotomie (amputation autoprovocquée) des appendices et leur régénération subséquente sont des phénomènes communs à plusieurs taxons. Ces processus affectent, on le sait, les capacités de recherche de nourriture, la croissance et la durée du développement chez de nombreux organismes. On connaît mal, cependant, leurs effets chez les arachnides. Nous étudions les effets de l'autotomie et de la régénération sur la condition corporelle, la croissance (en taille et en masse) et la durée du développement (intervalle entre les mues) chez des araignées-loups *Schizocosa ocreata* (Hentz, 1844) en nature et au laboratoire. La fréquence de l'autotomie en nature est élevée (11 % – 19 %). Les individus capturés en nature avec des pattes manquantes ou en régénération ont une taille corporelle, une masse et une condition significativement inférieures. Afin de vérifier les effets de la régénération sur la taille, la masse et l'intervalle entre les mues en laboratoire, nous avons provoqué l'autotomie de l'une ou des deux pattes antérieures. Les araignées avec deux pattes en régénération ont des intervalles entre les mues plus courts ainsi qu'une taille et une masse inférieures par comparaison aux araignées intactes ou celles qui ont une seule patte en régénération. Les araignées capturées en nature qui ont subi l'autotomie et la régénération en laboratoire ont une taille, une masse et un intervalle entre les mues réduits. En revanche, après la régénération, les araignées élevées en laboratoire ont des intervalles entre les mues plus longs, mais pas de différence de masse. Ces résultats montrent que la perte de membres par autotomie est commune (mais potentiellement coûteuse) chez *S. ocreata* et qu'il peut y avoir au cours de la régénération des compromis conditionnés par l'environnement entre la croissance et la durée du développement.

[Traduit par la Rédaction]

## Introduction

The ability to autotomize (self-amputate) appendages occurs in many animals, including echinoderms (Ramsay et al. 2001), vertebrates (Arnold 1984), crustaceans (Juanes and Smith 1995), and arachnids (Roth and Roth 1984). Autotomy provides direct fitness benefits, including avoidance of being killed (Formanowicz 1990; Klawinski and Formanowicz 1994; Punzo 1997) and avoidance of being poisoned

by venomous prey (Eisner and Camazine 1983). However, autotomy may also lower competitive ability (Mariappan et al. 2000; Taylor and Jackson 2003; Dodson and Beck 1993), reduce speed (Formanowicz 1990; Bateman and Fleming 2005; Apontes and Brown 2006), decrease foraging capacity (Vollrath 1990; Brock and Smith 1998; Stoks 1999; Ramsey et al. 2001), and negatively affect mating behavior (Taylor et al. 2006).

Many animals can regenerate autotomized appendages, which may compensate for some of the costs of missing an appendage, but regeneration itself has costs. These include developmental impacts, such as reduced growth and longer duration between growth periods (Goss 1969; Vitt et al. 1977; Juanes and Smith 1995; Ramsay et al. 2001), and reduced function, which may affect mating, competition, locomotion, and foraging (Uetz et al. 1996; Brock and Smith 1998).

Autotomy and regeneration may be particularly important

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for spiders, because they use their legs for multiple functions that include locomotion, prey capture, and communication. Autotomy is a frequent occurrence for many spider taxa, as evidenced by field collections that often contain 5%–20% of individuals with legs missing (Foelix 1996; Uetz et al. 1996). Many (but not all) spiders that autotomize can also regenerate lost legs (Vollrath 1990; Johnson and Jakob 1999), with regeneration appearing to be an ancestral trait (Goss 1969). Therefore, the loss of the ability to regenerate by some spider groups over evolutionary time suggests that the costs of regeneration may differ among groups (Vollrath 1990; Maginnis 2006). To maintain regeneration, its costs must be outweighed by its benefits over the autotomized condition.

Few laboratory studies have addressed the effects of autotomy or regeneration on foraging in adult spiders (Amaya et al. 2001; Brueseke et al. 2001; Vollrath 1995), and no studies have focused on these processes in juveniles, whose foraging patterns may differ from adults (Beck and Connor 1992; Persons 1999). Furthermore, no study has looked at the effects of autotomy and regeneration on growth and development time in spiders. Reduced foraging as a juvenile may affect size and mass as an adult (Beck and Connor 1992; Uetz et al. 1996). A reduction in growth owing to regeneration has the potential to do the same. This may be important, since size and mass are both correlated with fitness in spiders (Wise 1975; Beck and Connor 1992; Simpson 1993; Spence et al. 1996).

We studied *Schizocosa ocreata* (Hentz, 1844), a wolf spider for which there is considerable background information. These spiders are commonly found in the leaf litter of eastern deciduous forests (Cady 1984), and individuals are often found exhibiting autotomy in the field (Uetz et al. 1996). It is easy to induce autotomy in the laboratory for this species; they are abundant, easy to maintain, and have short growth periods, and frequent molts until adulthood.

This study has two objectives. The first was to observe the frequency of autotomy in *S. ocreata* in the field and to compare size, mass, and body condition between individuals with intact or missing legs. The second objective was to compare under laboratory conditions the growth (change in size and mass) and development time (molt interval) of spiders regenerating one or both forelegs with intact controls. Our first hypothesis was that losing and regenerating a leg affects fitness because of increased developmental costs or decreased foraging ability. Based on this hypothesis we predicted that field-caught intact spiders would be larger and have higher body condition than spiders with missing or regenerated legs. Secondly, we hypothesized that regeneration affects growth and (or) development. Based on this we predicted that regenerating spiders would have longer intervals between molts and (or) be smaller in size and (or) gain less mass during a molt than intact spiders.

## Materials and methods

### General methods

Spiders were collected by hand from forest-floor leaf litter at the Cincinnati Nature Center, Rowe Woods, in Clermont County, Ohio. The life cycle of *S. ocreata* results in two different generations during each calendar year, with the fall

population being the offspring of the previous spring population. Spiders are hatched during the summer, overwinter as juveniles, and mature the following spring. Spring collecting (for both juveniles and adults) occurred during April–June (2003, 2004, 2005) and fall collecting (for juveniles only) occurred during September–October (2003, 2004). All spiders collected from the field (juveniles and adults) were housed individually in circular opaque plastic containers (9 cm diameter × 6 cm high) with clear lids. All spiders were maintained under the following controlled laboratory conditions: temperature between 21 and 24 °C, approximately 65%–80% relative humidity, and a 11 h dark : 13 h light photoperiod. The spiders were fed 10-day-old crickets (*Acheta domesticus* L., 1758) twice a week and provided water ad libitum through a dental wick connected to a reservoir in a container below. Adult females were checked daily for egg production and hatching. Upon hatching, spiderlings were left with the mother until dispersal (7–10 days). After this time period, spiderlings were transferred to separate 120 mL specimen cups with damp dental wicks for water and were fed Collembola (mixed species) or fruit flies (*Drosophila melanogaster* Meigen, 1830) twice a week. After reaching their fourth instar, spiderlings were placed in plastic containers (as above) and fed crickets of appropriate size twice weekly (cricket size varied depending on spider size, but in general crickets were approximately 75% of the spider size).

### Study 1: Frequency of leg loss in the field and its impact on size, mass, and body condition

*Schizocosa ocreata* juveniles collected from the field were examined for leg loss; side (left or right) and position (legs I–IV) of any leg loss or regeneration were recorded for spring and fall 2003, spring and fall 2004, and spring 2005. The phenology of *S. ocreata* created some overlap in generations across seasons (see General methods). Two cohorts of spiders that spanned two seasons (fall 2003 and spring 2004 and fall 2004 and spring 2005) were examined to tease apart some of the effects of season and year on size (measured by cephalothorax width), mass, and body condition in a biologically meaningful way.

All spiders missing or regenerating legs, and an equal number of randomly selected intact spiders, were weighed to the nearest milligram within 48 h of collection (prior to being fed) and digitally photographed using a Pixera 1.2 megapixel digital camera through a Wild M5 microscope. For each picture, the spider was placed in a small petri dish and allowed to settle so that the cephalothorax was flat against the substratum and the spider's legs were stretched out in a resting position. From these pictures, measurements were taken of the cephalothorax width (CW, which is the distance across the widest point of the spider's carapace measured in millimetres) using ImageTool version 2.00 (Wilcox et al. 1998). In addition to comparing mass and CW between groups, we used a body-condition index (BCI) as a measure of fitness in this study (Jakob et al. 1996; Danielson-Francois et al. 2002; Uetz et al. 2002). To create a BCI, log-transformed mass (mg) was regressed against log-transformed CW and the residuals of these regressions were used for analyses (as in Jakob et al. 1996).

A  $2 \times 2 \chi^2$  contingency table was used to test whether

the number of spiders missing legs each year (2003, 2004, 2005) was independent of the year of collection. Using goodness-of-fit  $\chi^2$  tests, we tested for differences in leg loss by side and by position.

Three-factor nested ANOVAs, with the factors being injury status (autotomized or intact), season (fall or spring), and week (1 or 2) (season), were used to test for differences in the response variables mass, CW, and body condition.

## Study 2: The growth and developmental costs of leg regeneration

Regeneration has the potential to affect spider size and mass through two mechanisms: (1) by impairing foraging abilities and (2) by causing a reallocation of energy to the regenerating part, thus decreasing general growth and (or) affecting development time of the individual. Laboratory studies were performed in an attempt to distinguish between these two mechanisms. Mass and molt interval data were compared for laboratory-reared spiders in the fall of 2003. These individuals were the offspring of females that had been collected as adults in the field the previous spring. As a control for possible egg-sac effects, spiderlings were taken from 11 egg sacs, each produced by a different female. Because of differences in initial egg-sac size and survivorship, 6–15 spiderlings were used from each egg sac. Spiders were checked twice a week for molts until they reached their fourth molt and were checked daily thereafter. When spiders reached their fourth molt, approximately half of them were chosen at random and induced to autotomize an arbitrarily selected foreleg by restraining the femur with a pair of forceps. The rest of the spiders served as a control group. The number of days directly after manipulation between the fourth and fifth molts and between the fifth and sixth molts were compared between control and manipulated spiders. Additionally, spiders were weighed within 48 h of each molt (prior to being fed). Mass gains between molts were compared between manipulated and control spiders. It should be noted that in laboratory tests the loss of a leg did not measurably change a spider's overall mass, therefore any mass differences between intact and autotomized spiders were due to other factors.

In spiders, the CW changes only with molting, whereas mass varies within instar based on feeding and water intake (Anderson 1974). Therefore, we conducted an experiment to measure CW in fall 2004 using spiders captured in the field as juveniles in the third to fourth instar and then raised in the laboratory. Within two molts after autotomy, most individuals of *S. ocreata* regenerate a normal-sized leg (K.M. Wrinn, personal observation). To compare individuals of the same age under different conditions, 60 individuals were induced to autotomize immediately upon reaching the laboratory, 60 were induced to autotomize after molting once in the laboratory, and 60 were left intact. Comparisons between groups were made for the molt interval (days) needed for the first group to regenerate a full-sized leg from a partial one, the second group to partially regenerate a leg, and the third group (intact) to undergo normal growth. Masses and CWs were compared for a subset of these spiders ( $n = 30$  spiders per group for a total of  $n = 90$ ; see study 1 above for details on weighing and measuring).

According to field data from this study, multiple leg

losses for *S. ocreata* are not uncommon (about 20% of the spiders collected with autotomized limbs were missing more than one leg). In other arthropods the effects of multiple leg losses on molt intervals and size gain are often different from those caused by a single autotomy (Goss 1969; Juanes and Smith 1995). Based on this we were interested in determining if spiders with multiple leg losses also had higher fitness costs. To test this we compared molt intervals, mass gains, and CW gains as above between a subset of spiders that were intact, had autotomy of one foreleg, or had autotomy of both forelegs. We conducted this experiment later in the spring, so the spiders we used entered the experiment at the fifth instar, a molt interval older than those in previous experiments.

For data from fall 2003, two-sample *t* tests were run to compare both molt duration and mass gain between control and manipulated spiders, as there were only two groups (autotomy or intact). For data from fall 2004, separate one-way ANOVAs were used to compare mass gain, molt interval, and gain in CW between the three groups for the first experiment (single autotomy) and for the second experiment (multiple autotomy). All data analyses for studies 1 and 2 were performed using JMP<sup>®</sup> release 4.0.2 (SAS Institute Inc. 2000). For all ANOVAs and post hoc tests, we accepted a priori  $p = 0.05$ .

## Results

### Study 1: Frequency of leg loss in the field and its impact on size, mass, and body condition

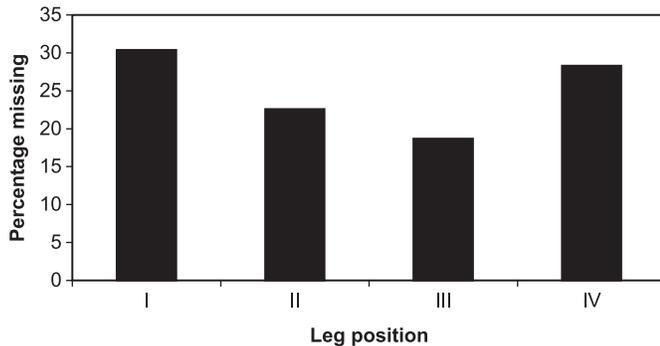
#### Frequency of leg loss

Frequency of leg loss within the population was considerable for all periods measured, ranging from 10.9% to 19.3% (spring 2003:  $n = 693$ ; fall 2003:  $n = 450$ ; spring 2004:  $n = 938$ ; fall 2004:  $n = 656$ ; spring 2005:  $n = 796$ ). The proportion of spiders collected with missing legs for 2004 did not differ between seasons (spring and fall) ( $\chi^2_{[1]} = 0.113$ ,  $p < 0.75$ ). However, in 2003 a significantly larger proportion of spiders were missing legs in fall than in spring field collections ( $\chi^2_{[1]} = 9.56$ ,  $p < 0.005$ ). There were no differences between collecting periods for the side (right and left) or position (legs I–IV) at which leg loss occurred, so data were pooled for all periods ( $\chi^2_{[3]} = 3.09$ ,  $p < 0.10$ , and  $\chi^2_{[12]} = 5.244$ ,  $p < 0.9$ , respectively). A goodness-of-fit  $\chi^2$  test of the pooled data showed no overall difference in leg loss between sides ( $\chi^2_{[1]} = 2.34$ ,  $p < 0.10$ ). However, a significant difference in leg loss by position did occur for the pooled data ( $n = 517$ ), with legs being lost most often at the first and fourth positions (Fig. 1).

#### Size, mass, and body condition

Spiders with autotomized and (or) regenerating legs tended to be smaller, weigh less, and have lower body condition than intact spiders, although these measures were not significant for all of the periods considered (Table 1, Figs. 2, 3). These spiders overwinter as immatures and mature in the spring, so spiders were larger and weighed more in the spring, as expected from normal growth and development (Table 1, Fig. 3). Body condition did not differ significantly between seasons (spring vs. fall), but there were some differences

**Fig. 1.** Percentage of legs lost or regenerated according to position for field-caught *Schizocosa ocreata* ( $\chi^2_{[3]} = 19.9, p < 0.001$ ). Data were pooled for the spring of 2003, 2004, 2005 and fall of 2003, 2004 ( $n = 517$ ).



within season, depending when (early vs. middle or late) the spiders were captured (Table 1). Autotomized spiders measured in mid- to late fall 2003 had significantly lower body condition than intact spiders (residuals  $-0.42$  and  $0.42$ , respectively; ANOVA:  $p < 0.001$ ). By early spring 2004, all spiders showed a marked decrease in condition from the previous fall and differences between groups were no longer significant. Both groups increased in condition by mid-spring, but the increase was greater for intact spiders, leaving autotomized spiders in significantly lower condition (Table 1, Fig. 2A). Because of changes in the body condition of spiders from early to mid-spring 2004, early- and middle-period weeks were examined for fall 2004 and spring 2005 as well. For both fall 2004 and spring 2005, there was a trend for intact spiders to have higher body condition than autotomized spiders towards the middle of the season (Figs. 2B, 2C).

## Study 2: The growth and developmental costs of leg regeneration

As spiders were randomly selected from the population, it was assumed that all groups of spiders began the study in equal body condition, and they were fed equally to control food availability. In fall 2004, body condition (a measure of foraging success) did not differ significantly between groups at the end of the experiment, indicating that all changes in size and mass were due to changes in growth and development (ANOVA:  $F_{[2,73]} = 0.2540, p = 0.7764$ ).

### Autotomy of a single leg

#### Laboratory-reared spiders (2003)

Regenerating spiders had longer initial molt intervals compared with intact control spiders (34.22 vs. 30.56 days) and this difference was significant ( $t_{[112]} = 2.266, p = 0.026, n = 114$ ). Regenerating spiders actually molted more quickly than controls during the second interval following autotomy (33.26 vs. 34.89 days), although this difference was not significant ( $t_{[104]} = -1.042, p = 0.300, n = 106$ ). Groups did not significantly differ in number of days between molts when both intervals were considered together ( $t_{[104]} = 0.625, p = 0.5331$ ). Finally, mass change did not differ between the groups for either molt interval (molt 1:  $t_{[108]} = 0.283, p = 0.7778$ ; molt 2:  $t_{[76]} = 0.583, p = 0.5179$ ).

### Field-caught, laboratory-maintained spiders (2004)

For these spiders, CW was marginally significantly different between groups (ANOVA:  $F_{[2,77]} = 3.0197, p = 0.0548$ ; Fig. 4A). Tukey post hoc tests showed that spiders had the greatest CW at the second molt after autotomy (full regeneration) (Fig. 4A). Mass also differed significantly between groups (ANOVA:  $F_{[2,133]} = 7.1403, p = 0.0011$ ; Fig. 4B). Spiders that had just undergone a second molt after autotomy weighed the most, followed by unmanipulated intact spiders and spiders having undergone the first molt after autotomy (Fig. 4B). Groups did not differ in the number of days between molts (ANOVA:  $F_{[2,83]} = 1.0499, p = 0.3546, n = 60$  spiders per treatment).

### Autotomy of two legs

Groups that lost one or two legs differed significantly in molt interval (ANOVA:  $F_{[2,47]} = 7.2824, p = 0.0018$ ; Fig. 5A). Spiders that had undergone autotomy of a single leg weighed significantly more than spiders that had undergone autotomy of two legs after the first period of regeneration ( $F_{[1,29]} = 5.5457, p = 0.0255$ ; Fig. 5B). Groups also differed significantly in CW (ANOVA:  $F_{[2,45]} = 5.8329, p = 0.0056$ ; Fig. 5C). Tukey–Kramer post hoc tests showed that intact spiders were the largest but took the longest to molt, whereas spiders that were regenerating two legs were the smallest but molted the fastest (Figs. 5A–5C). The molt interval differed significantly between groups for the second period of regeneration (ANOVA:  $F_{[2,47]} = 5.6461, p = 0.0063$ ), with intact spiders once again having the longest intervals. However, for this period neither mass nor CW was significantly different between groups (ANOVA — mass:  $F_{[1,30]} = 1.6282, p = 0.2118$ ; CW:  $F_{[2,43]} = 0.3504, p = 0.7064$ ; Figs. 5B, 5C).

## Discussion

### Study 1: Frequency of leg loss in the field and its impact on size, mass, and body condition

Limb loss via autotomy is common in *S. ocreata*. The frequency of leg loss in *S. ocreata* fell within the upper part of the range of values reported in the literature for other species (5%–20%; Foelix 1996), and was comparable with a previous study on this species (15%; Uetz et al. 1996). Since spiders that have molted more than twice after autotomy are visually indistinguishable from intact spiders (K.M. Wrinn, personal observation), these percentages may actually underestimate the true amount of autotomy occurring in the field. There were significant differences in the percentage of leg loss between spring and fall of 2004, but not 2003. This suggests that if predation is the major cause of leg loss in the field, then predation pressures may differ by season and (or) year. It would be interesting to examine whether differences in environmental factors, e.g., temperature and precipitation, correlate with frequency of leg loss and with the density of potential predators such as toads and other spiders.

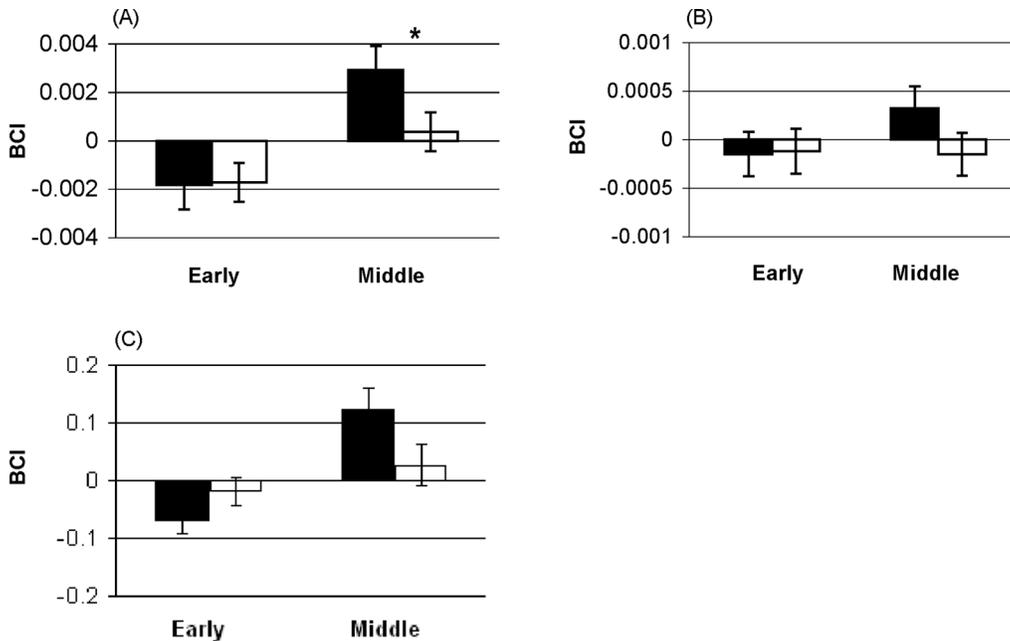
*Schizocosa ocreata* were found most frequently with legs missing at the first and fourth positions, both in this study and a previous one (Uetz et al. 1996). Predation or cannibalism may cause *S. ocreata* to lose their first and fourth legs more often because they are longer and (or) used in threat

**Table 1.** Results of three-factor ANOVA (injury status, season, and collecting period (early or late) [season]) comparisons within generations of *Schizocosa ocreata*.

|  | Fall 2003 – Spring 2004 |                   | Fall 2004 – Spring 2005 |               |
|--|-------------------------|-------------------|-------------------------|---------------|
|  | <i>F</i>                | <i>p</i>          | <i>F</i>                | <i>p</i>      |
| <b>Cephalothorax width</b>                 |                         |                   |                         |               |
| Injury status                              | 10.5326                 | <b>0.0121</b>     | 5.4846                  | <b>0.0201</b> |
| Season                                     | 4.3989                  | <b>0.0084</b>     | 536.74                  | <b>0.0001</b> |
| Collecting period [season]                 | 6.7182                  | <b>0.0336</b>     | 18.6061                 | <b>0.0001</b> |
| Injury status × season                     | 4.559                   | 0.5118            | 0.0328                  | 0.8564        |
| Collecting period [season] × injury status | 0.2307                  | 0.8631            | 0.1613                  | 0.8512        |
| <b>Mass</b>                                |                         |                   |                         |               |
| Injury status                              | 23.6629                 | <b>&lt;0.0001</b> | 4.7446                  | <b>0.0305</b> |
| Season                                     | 760.251                 | <b>&lt;0.0001</b> | 351.18                  | <b>0.0001</b> |
| Collecting period [season]                 | 23.4628                 | <b>&lt;0.0001</b> | 32.339                  | <b>0.0001</b> |
| Injury status × season                     | 11.2403                 | <b>0.0009</b>     | 2.119                   | 0.3688        |
| Collecting period [season] × injury status | 0.3172                  | 0.7284            | 0.811                   | 0.1227        |
| <b>Body condition</b>                      |                         |                   |                         |               |
| Injury status                              | 5.8512                  | <b>0.0162</b>     | 1.6649                  | 0.1984        |
| Season                                     | 1.0706                  | 0.3017            | 0.442                   | 0.5069        |
| Collecting period [season]                 | 20.9663                 | <b>&lt;0.0001</b> | 12.0709                 | <b>0.0001</b> |
| Injury status × season                     | 0.0383                  | 0.8451            | 0.3377                  | 0.5618        |
| Collecting period [season] × injury status | 1.647                   | 0.1945            | 9.7                     | <b>0.0021</b> |

**Note:** Significant *p* values are in boldface type.

**Fig. 2.** Body-condition index (BCI; residuals of mass × cephalothorax width) comparisons between intact (solid) and autotomized (open) *S. ocreata* in early vs. middle season for (A) spring 2004, (B) fall 2004, and (C) spring 2005. The asterisk indicates significant differences between autotomized and intact spiders at *p* < 0.05.

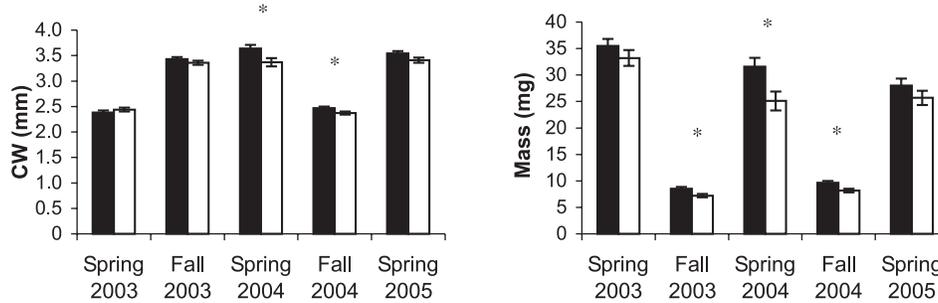


displays (Aspey 1976), which makes them more exposed. This is supported by research with crabs which demonstrates that autotomy occurs most frequently in the two longest most exposed limbs (Spivak and Politis 1989).

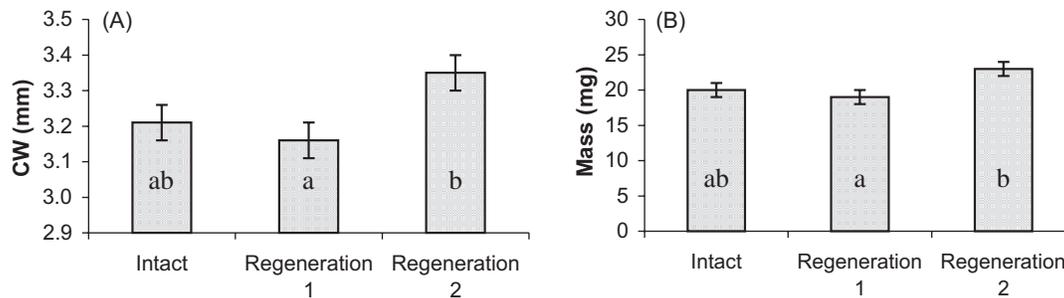
Loss and regeneration of limbs appear to incur fitness costs in the field, as size, mass, and body condition were, on average, lower in field-caught spiders that were missing or regenerating legs. This was most likely due to reduced

foraging ability, as foraging success has the potential to affect all three of these measurements. Cephalothorax width is fixed between molts, whereas mass varies with food and water intake (Anderson 1974). Thus, these measures can indicate past and recent feeding history, respectively. Evidence of this can be seen in several studies (Uetz et al. 1996; Uetz et al. 2002) showing that underfed juvenile *S. ocreata* had smaller CWs as adults when compared with well-fed indi-

**Fig. 3.** Comparisons of mean ( $\pm$ SE) cephalothorax widths (CW) and masses (Mass) for autotomized (open) and intact (solid) *S. ocreata*. Asterisks indicate significant differences between intact and autotomized or regenerating spiders at  $p < 0.05$ .



**Fig. 4.** Mean ( $\pm$ SE) cephalothorax widths (CW; A) and masses (Mass; B) of *S. ocreata* of the same age that were caught in the field and then underwent autotomy in the laboratory, according to treatment (fall 2004). Intact spiders were unmanipulated, regeneration 1 spiders molted once after autotomy, and regeneration 2 spiders molted twice before measurements were made ( $n = 90$  total, with 30 spiders per treatment). Tukey post hoc tests were used to compare means ( $p < 0.05$ ). Identical letters indicate groups that are not significantly different.



viduals. Likewise, Beck and Connor (1992) found that CW and mass differences in juvenile crab spiders (*Misumenoides formosipes* (Walckenaer, 1837)) in the field were related to varying prey-capture success. Body-condition indices (BCI: the residuals of a mass  $\times$  CW regression) measure both past and recent foraging histories. Studies have shown that feeding spiders different diets (high or low quantity) can affect body condition (Jakob et al. 1996; Uetz et al. 1996; Uetz et al. 2002).

Interestingly, autotomy did not significantly affect foraging of three wolf spider species studied in the laboratory (Amaya et al. 2001; Brueske et al. 2001). However, the effect of autotomy on foraging has not often been tested for spiders under field conditions, where results may differ. Studies of other arthropods have demonstrated reduced foraging in autotomized or regenerating individuals using simulated field conditions and mark-recapture techniques, which measure individual body condition before and after autotomy (Juanes and Smith 1995; Stoks 1999).

Regeneration of limbs may create energetic costs that affect growth and development time (Goss 1969). In the field, it is difficult to tease apart whether regenerating spiders that are smaller have undergone reduced foraging or increased energetic costs. In some cases there may even be an interaction between the two as shown by Ramsay et al. (2001) in *Asterias rubens* L., 1758. Further experiments crossing leg autotomy with a high and low feeding treatments in *S. ocreata* are needed to determine what type of interactions between growth and foraging may be occurring in the field.

It is possible that rather than autotomy affecting foraging and (or) growth, spiders in the field that were smaller and in lower condition in the first place were more prone to preda-

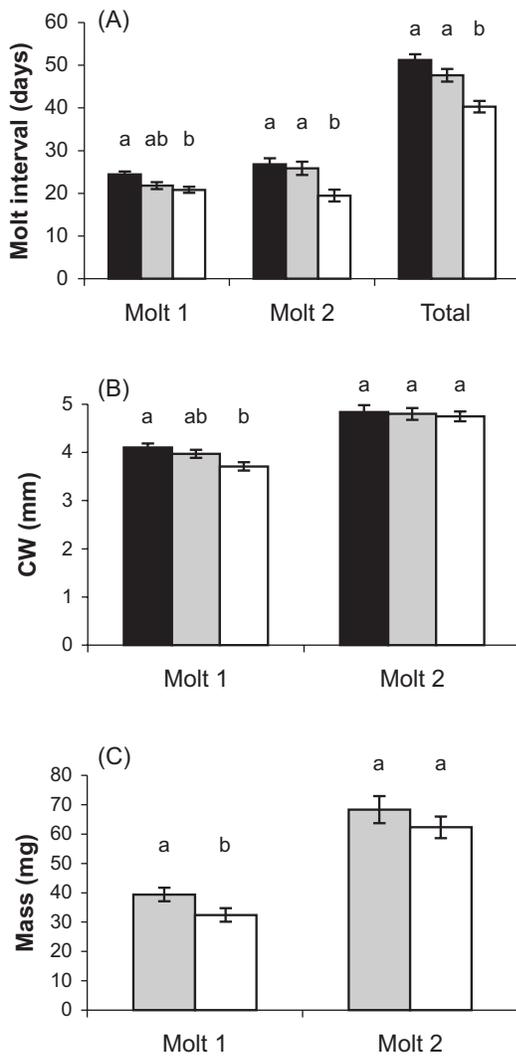
tion and leg loss. However, the pattern of changes in body condition of autotomized spiders across the season would indicate otherwise. Early in the season body condition did not differ between intact and autotomized spiders, but towards the middle of the season condition of autotomized spiders became lower. This indicates that the negative effects of autotomy and regeneration may be cumulative over time.

### Study 2: The growth and developmental costs of leg regeneration

Results from laboratory studies suggest that there may be environmentally mediated trade-offs between growth and development time occurring during regeneration. Laboratory-reared spiders that were regenerating a leg took an average of 3.7 days (10.8%) longer to molt than intact spiders, although these groups did not differ significantly in mass after molting. In contrast, field-caught spiders that were regenerating a leg showed no difference in molt interval between groups, but were, on average, smaller in size and weighed significantly less after molting compared with intact spiders. Lower increases in size at molting and (or) reduced molt intervals have also been shown for both crustaceans and insects during regeneration of an appendage (Kunkel 1981; Waddy et al. 1995).

Although spiders appear to show costs of regeneration, the differences in molt interval, size, and mass between intact and regenerating spiders were only true for the first molt after autotomy. During the second molt after autotomy, regenerating spiders were able to compensate for previous costs by either shortening their molt interval or increasing their growth. This indicates that these spiders may minimize the physiological costs of regeneration by limiting them to

**Fig. 5.** Differences were measured between *S. ocreata* that were intact (solid;  $n = 17$ ), with one foreleg regenerating (shaded;  $n = 16$ ), or both forelegs regenerating (open;  $n = 17$ ). Identical letters indicate groups that are not significantly different according to Tukey post hoc tests ( $p < 0.05$ ). (A) Mean ( $\pm$ SE) molt intervals of spiders were measured as follows — molt 1: days until partial regeneration; molt 2: days between partial and full regeneration; total: days for both intervals. (B) Mean ( $\pm$ SE) cephalothorax widths (CW) of spiders were measured initially before manipulation and after the following two molts. (C) Mean ( $\pm$ SE) masses of spiders were measured initially before manipulation and within 24 h after the two molts. Masses of intact spiders were not included because they were not obtained within 24 h of molting and would thus have been inaccurate.



only one instar. However, the resulting ecological costs may vary depending on the instar in which the spider loses a leg. For example, spiders undergoing autotomy in a penultimate stage may have developmental costs that last into adulthood, as the spiders can only molt one more time. This has the potential of affecting fitness, because decreases in condition, size, and mass as juveniles have the potential to affect reproductive success as adults (Wise 1975; Beck and Connor 1992; Simpson 1993; Spence et al. 1996; Uetz et al. 1996). Autotomy in a penultimate stage also has reproductive im-

plications for this species unrelated to size or mass. For example, in a previous study, Uetz et al. (1996) found that male *S. ocreata* with regenerated forelegs exhibited asymmetry in decorative leg tufts, which serve as a criterion for female mate choice in this species (Uetz and Smith 1999).

Regeneration of a single leg in *S. ocreata* led to either a longer molt interval or smaller size and lower mass after molting, but not both. A trade-off appeared to be occurring between size and development time, i.e., the spiders either took longer to molt to reach full size or developed at the normal rate but ended up at a smaller size. Field-caught spiders subjected to autotomy and treated the same as laboratory-reared spiders after reaching the laboratory were still affected differently by regeneration. These results indicate that these spiders may have some developmental flexibility to moderate the fitness effects of regeneration based on external conditions. Some of the external conditions that differed between laboratory-reared and field-caught spiders include nutrition, light levels, and temperature. Spiders in the field are often starved (Anderson 1974; Kreiter and Wise 2001), whereas spiders in the laboratory are not limited by food quantity, although spiders in the laboratory have a more monotypic diet that can also negatively affect growth (Uetz et al. 1992). If food quantity was the most important variable, it is possible that regenerating laboratory-reared spiders, having been reared on a constant diet their entire lives, would take the extra few days to develop to full size. However, for field-caught spiders that had been raised on fluctuating diets, likely with periods of starvation, molting at a smaller size and mass but more quickly may have been a better strategy. Light levels and temperature are another set of factors that affect the molt cycle in spiders (Schaefer 1987). *Schizocosa ocreata* in the field were subjected to naturally varying light levels and temperatures, whereas both of these factors remained constant throughout the lives of the laboratory-reared spiders. Further tests could be done to address these variables, any of which could have led to the differences in growth vs. development time between the two groups of regenerating spiders.

Regeneration of two legs had a greater effect on growth and development time in *S. ocreata* than regeneration of a single leg. In some cases, molt interval may be lengthened because of multiple autotomy, e.g., as in the cockroach (Kunkel 1981). However, in *S. ocreata*, loss of two legs significantly decreased the molt interval for both of the molts following autotomy. It is possible that missing multiple legs in this species is so costly that it is advantageous to molt early and grow them back as quickly as possible, even if this leads to a large decrease in size and mass (as discussed below). Multiple autotomy causes decreased regeneration time and molt interval in a number of other animals, including echinoderms, crustaceans, and chilopods, although these taxa differ in the number of regenerating appendages necessary to cause this decrease (Zeleny 1905; Skinner and Graham 1972; Cameron 1927). In addition to decreased molt interval, spiders regenerating two legs were smaller than intact spiders. Similar results have been shown for the crab *Cancer productus* J.W. Randall, 1840 (Brock and Smith 1998). Spiders regenerating two legs also weighed significantly less than those that were regenerating one leg. As

with regeneration of a single leg, growth costs (both size and mass) of spiders regenerating two legs were limited to the first molt after autotomy.

Overall, results of these studies reveal that limb loss via autotomy incurs costs in terms of growth and development time for *S. ocreata*, but that environmentally mediated trade-offs may occur during regeneration and affect outcome. Differences between field-collected and laboratory-reared spiders suggest that impacts of autotomy and regeneration are complex and likely to be affected by as yet unstudied variables such as feeding rate and environmental conditions.

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