

## RESEARCH ARTICLE

# Autotomy-induced effects on the locomotor performance of the ghost crab *Ocypode quadrata*

Janne A. Pfeiffenberger\* and S. Tonia Hsieh

## ABSTRACT

The voluntary amputation of an appendage, or autotomy, is an effective defensive mechanism that allows an animal to escape aggressive interactions. However, animals may suffer long-term costs that can reduce their overall fitness. Atlantic ghost crabs (*Ocypode quadrata*) are one of the fastest terrestrial invertebrates, and regularly lose one or more limbs in response to an antagonist encounter. When running laterally at fast speeds, they adopt a quadrupedal gait using their first and second pairs of legs while raising their fourth, and sometimes the third, pair of legs off the ground. This suggests that some limbs may be more important for achieving maximal locomotor performance than others. The goal of this study was to determine whether the loss of certain limbs would affect running performance more than others, and what compensatory strategies were used. Crabs were assigned to four different paired limb removal treatments or the control group and run on an enclosed trackway in their natural habitat. Ghost crabs were found to adjust stride kinematics in response to limb loss. Loss of the second or third limb pairs caused a reduction in running speed by about 25%, suggesting that the remaining intact limbs were unable to compensate for the loss of either limb, either due to a lack of propulsive forces produced by these limbs or issues stemming from re-coupling limb arrangements. Loss of any of the other limbs had no detectable effect on running speed. We conclude that compensatory ability varies depending on the limb that is lost.

**KEY WORDS:** Stability, Limb pairing, *Ocypode*, Limb loss, Differential limb function, Locomotor biomechanics

## INTRODUCTION

Autotomy is a defense mechanism during which the animal voluntarily sacrifices an appendage in response to aggressive inter- and intraspecific events, such as predation and competition (Maginnis, 2006). Although autotomy may facilitate survival during these encounters, there are potential long-term costs associated with autotomy, which can reduce the animal's fitness. For example, autotomy of an appendage can cause decrements in locomotor performance, survivorship, foraging success and defense capabilities (Amaya et al., 2001; Bateman and Fleming, 2006; Brautigam and Persons, 2003; Fleming et al., 2007; Guffey, 1999; Juanes and Smith, 1995; Maginnis, 2006). Yet, this behavior is relatively common, and has independently evolved in numerous vertebrate and invertebrate taxa, such as reptiles, amphibians, arthropods and mollusks (Fleming et al., 2007; Juanes and Smith,

1995; Maginnis, 2006), suggesting that the benefits outweigh the potential costs.

Effective locomotor performance is crucial to the survival of many animals (Arnold, 1983; Husak, 2006; Irschick and Garland, 2001; Jagnandan and Higham, 2018). Autotomy can impede locomotor performance as animals must adjust their locomotor kinetics, kinematics and behavior in order to compensate for the lost appendage. For example, lizards can autotomize their tails, even though they play a crucial role for stabilization during jumping (Gillis et al., 2009) and climbing (Jusufi et al., 2008). During running, anole lizards can compensate kinematically for tail loss, but such compensation had a negative impact on stability when navigating on increasingly narrow surface breadths (Hsieh, 2016). Similarly, dogs must move tripedally after a single limb amputation, which requires redistribution of loads to the remaining limbs as well as other kinetic and kinematic changes (Fuchs et al., 2014; Goldner et al., 2015; Kirpensteijn et al., 2000).

It has been hypothesized that multi-legged animals possess redundant limb functions, which make these animals more resilient to the negative effects of limb loss (Guffey, 1999). Cockroaches, which use six limbs in locomotion, have been found to alter limb phasing and placement, as well as increase their stride lengths, after losing a limb (Delcomyn, 1991a,b; Hughes, 1957). Among arachnids, limb loss can result in decreased locomotor performance (Amaya et al., 2001; Brown and Formanowicz, 2012; Domínguez et al., 2016; Lutzky and Morse, 2008), reduced reproductive success (Brautigam and Persons, 2003) and lower rates of prey capture and predator evasion (Brautigam and Persons, 2003; Wrinn and Uetz, 2008). These outcomes may be synergistic as a study on crab spiders found indirect negative effects on locomotor performance due to poor body condition after limb loss (Lutzky and Morse, 2008).

Whereas most studies have focused on the effects of limb loss on anteriorly moving animals, little is known about how laterally moving animals, such as crabs, are affected by limb loss. Crabs typically lose one limb at a time; however, previous limb loss does not exempt them from losing additional limbs (Juanes and Smith, 1995). For instance, Davis et al. (2005) found that ~25% of Asian shore crabs (*Hemigrapsus sanguineus*) were missing two or more limbs, and another study on the swimming crab (*Portunus trituberculatus*) found that around 55% of crabs observed with autotomized limbs were missing more than two limbs (He et al., 2016). Depending on the species, it can take between two (Smith, 1990) to seven molt cycles (Edwards, 1972) to fully regenerate a limb of comparable size and function to the original (Juanes and Smith, 1995).

The Atlantic ghost crab (*Ocypode quadrata*, Fabricius 1787), a semi-terrestrial decapod, lives in burrows on the beaches and dunes of the western Atlantic Ocean, where it scavenges and preys upon hatchling sea turtles and fledgling birds (Fowler, 1979). The ghost crab is one of the fastest terrestrial invertebrates and can stop and

Department of Biology, Temple University, Philadelphia, PA 19122, USA.

\*Author for correspondence (jpfeiff2@gmail.com)

 J.A.P., 0000-0003-0682-1558

Received 20 July 2020; Accepted 23 March 2021

change running directions within a few strides (Blickhan and Full, 1987; Blickhan et al., 1993; Burrows and Hoyle, 1973; Perry et al., 2009). A sister species of the Atlantic ghost crab, the horned ghost crab (*Ocypode ceratophthalmus*), runs quadrupedally on the first and second pairs of limbs at high velocities, while the third and fourth pairs of limbs are raised and do not contribute toward propulsion (Burrows and Hoyle, 1973; Hafemann and Hubbard, 1969). Atlantic ghost crabs have been shown to raise their fourth pair of limbs while running (Blickhan and Full, 1987); however, it is not known whether other limbs are raised during high-velocity runs similar to *O. ceratophthalmus*. Whether the first and second pairs of limbs are important for high-speed locomotion, in comparison to the third and fourth pairs of limbs, has not been previously tested.

In this study, we investigated the impacts of paired limb autotomy on the locomotor performance of the Atlantic ghost crab. While paired limb ablations do not necessarily reflect the most common natural limb loss pattern, we chose this approach to maximize the measurable impact of the loss of a particular limb, permitting quantification of what could otherwise be subtle control responses to a common survival strategy. The goals of this study were to elucidate compensatory control mechanisms by: (1) quantifying the impacts of paired limb loss on the locomotor performance of ghost crabs; and (2) investigating how the ghost crab compensates for paired limb loss in order to maintain locomotor performance. Based on the aforementioned running habits of *O. ceratophthalmus*, and assuming that *O. quadrata* behaves similarly, we hypothesized that the loss of limbs that are raised during high-speed runs (limbs three and four) would have less impact on the running speed of ghost crabs whereas the loss of limbs typically retained for high-speed runs (limbs one and two) would have the greatest effect on locomotor performance. We also hypothesized that crabs would compensate for limb loss by changing footfall timing characteristics and gait to counteract locomotor destabilization. We expected crabs to increase locomotor stability by shortening stride lengths while maintaining an alternating tripod gait with their remaining limbs.

## MATERIALS AND METHODS

### Animal collection

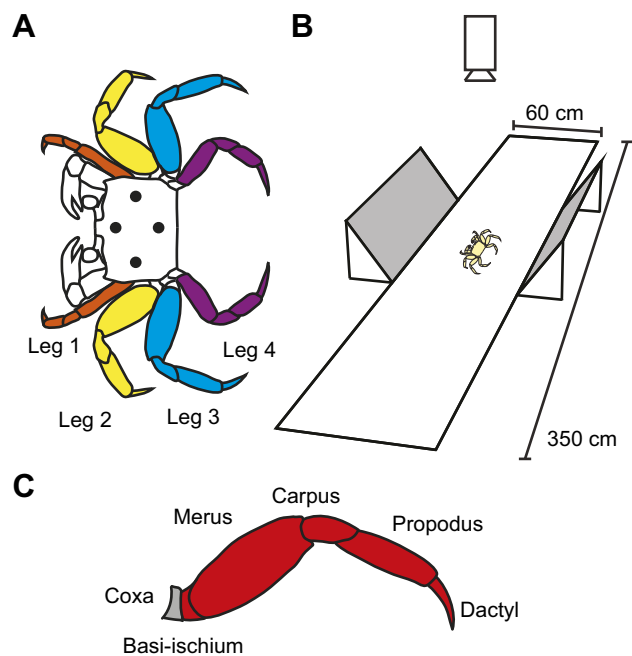
We collected 85 subadult ghost crabs, *O. quadrata*, of similar size (37 males, 48 females; mean carapace width $\pm$ s.e.m.: male 36.64 $\pm$ 0.54 mm, female 36.50 $\pm$ 0.52 mm; mean body mass $\pm$ s.e.m.: male 34.35 $\pm$ 1.46 g, female 30.77 $\pm$ 1.14 g), at the Two Mile Beach unit of the Cape May National Wildlife Refuge in New Jersey, USA (National Wildlife Refuge Special Use Permit #13007, New Jersey Division of Fish and Wildlife Permit #1355). Subadults were selected because larger individuals do not run as readily, preferring instead to defend themselves. No sex-related differences in size distribution were noted. Only those animals with all limbs intact and no visible injuries were retained for the study. Because recently regenerated limbs are smaller than the originals, we also eliminated any crabs that appeared to have recently regrown limbs. All crabs were housed individually in plastic containers until their release after data collection, within 18 h after capture. All data were collected in July and August 2013 while temperatures in the trackway ranged between 22°C and 27°C.

### Experimental set-up

Early trials in this study attempted to collect data on captive ghost crabs running in the laboratory along a small trackway (100 $\times$ 30 cm). Animals served as their own controls, and were run with their limbs intact, after which they had selected limbs autotomized. However, ghost crabs became increasingly reluctant

to run after prolonged handling and captivity. Their antagonistic attitude has been previously documented by other investigators as well (Blickhan and Full, 1987; Burrows and Hoyle, 1973). In those studies, which were conducted in laboratory settings, measured runs were slow (<0.7 m s<sup>-1</sup>) and no statistically meaningful comparison was possible between treatments due to the low number of completed trials. We decided to increase the trackway length, have a separate control group and minimize handling time by running animals within 24 h after capture at the site of capture.

Each ghost crab was randomly assigned to one of four symmetrical limb loss treatments (Fig. 1A) or the control group, which had all limbs intact. For the purposes of this study, any mention of ‘limb loss’ with relation to this study refers to paired limb removals. Limb autotomy was induced at the basi-ischium and coxa joint by holding the limb with forceps at the proximal base of the merus (Fig. 1C) until the crab voluntarily self-amputated the limb. On average, crabs would autotomize their limbs within 1 or 2 min. To facilitate motion tracking, we glued four 1 mm diameter black glass beads to the dorsal surface of the carapace using cyanoacrylate glue. The centroid of these four markers depicted the approximate location of the center of mass (see Fig. 1A). The center of mass was determined for a subset of ghost crabs ( $N=10$ ) that were not assigned to any of the trials. Using the suspension method, the crabs were first euthanized, and all limbs were removed. The carapace was hung from two different locations, with the intersection of the droplines indicating the center of mass. All crabs involved in the study were handled for similar periods of time for measurement and marking, although control crabs were not subjected to additional



**Fig. 1. Experimental set-up used in this study.** (A) Diagram of an Atlantic ghost crab, *O. quadrata*, with paired limb autotomy treatments color-coded and labeled for identification. Filled black circles indicate where black glass beads were attached for body motion tracking. (B) Schematic of the trackway, showing the position of the high-speed camera capturing a dorsal view and two lateral views through two mirrors positioned at 55 deg to the ground. (C) Anatomy of crab limb segments, with the proximal segments to the left and distal segments to the right. Limb autotomy occurred between the coxa and the basi-ischium. Segments in red are lost in autotomy whereas the gray segment remains attached.

handling necessary to induce limb autotomy. All crabs had a minimum of 12 h of rest after induced limb loss.

All crabs were run between 10:00 h and 15:00 h along a 350×60 cm enclosed trackway set up on the beach (Fig. 1B) while filming with a high-speed camera (Photron SA-3, Photron USA Inc., San Diego, CA, USA) at 500 frames s<sup>-1</sup> with a 1/2000 s shutter. The camera was positioned above the trackway to capture a dorsal view of the crab. Two simultaneous lateral views were obtained through two mirrors placed along the trackway at a 55 degree angle to the ground. We collected two to three constant-speed runs, defined as ≤10% fluctuations from the mean trial speed, during which crabs also did not touch the sides of the trackway. All runs that were not at constant speed, in which animals stopped during the run or during which they touched the side of the trackway, were excluded. Due to their antagonistic behavior, obtaining more than this number of runs per individual was not possible. Crabs were then released and the fastest running trial for each crab was retained for analysis.

### Video analysis

All videos were digitized and coordinates reconstructed into 3D using Digitizing Tools (Hedrick, 2008) in MATLAB (version R2014a, The MathWorks Inc., Natick, MA, USA). We quantified five variables to characterize the running performance of the crabs: running speed, stride length, stride frequency, duty factor, and static stability margin (SSM). Kinematic data were filtered using a fourth-order low-pass Butterworth filter (cut-off frequency 40 Hz). Instantaneous running speed was calculated as the derivative of position, which was averaged for each trial to determine mean running speed. Individual stride lengths were measured by digitizing the footfalls of the individual limbs and then averaged for each trial. Stride frequency for each trial was calculated by dividing the number of strides taken in a trial by the time it took to complete these strides. Duty factor was calculated as a ratio of stance period divided by stride period, and then averaged for each trial.

We calculated relative phasing differences between adjacent limbs to quantify changes of limb coupling after limb loss. The phasing difference between two limbs was defined according to when a limb made contact with the ground within the limb cycle of an adjacent leg. A limb cycle begins at the initial contact with the ground (0) and ends when the limb makes contact with the ground again (2 $\pi$ ). Limbs that moved in-phase had a relative limb phase difference of 0 or 2 $\pi$  whereas limbs that moved in antiphase had a relative limb phase difference of  $\pi$ .

We also used locomotor stability as a criterion for assessing running performance. SSM calculations are most accurate at quasi-static speeds (Ting et al., 1994). Animals moving at high speeds are best described using dynamic metrics, such as dynamic stability (Full et al., 2002; Pai and Patton, 1997). Quantifying dynamic stability, however, is difficult, especially in a field setting such as our study. The inherently complex and destabilizing characteristics of the natural environment in which the crabs are moving make defining state variables or stable limit cycles necessary for calculating dynamic stability metrics (Bruijn et al., 2013; Full et al., 2002; Hurmuzlu and Basdogan, 1994) impossible to achieve. Furthermore, many of these methods for calculating dynamic stability have not been thoroughly validated for multi-legged animals (Marghitu et al., 1996). Keeping this in mind, we used SSM to compare instantaneous stability in our study while acknowledging that it does not ideally represent the stability of our study animal, but nevertheless provides us with a useful metric for the crab's instantaneous stability.

The SSM was defined as the distance of the center of mass to the closest edge of the base of support. The base of support was designated by all of the limbs in contact with the ground at a given moment (Ting et al., 1994). If the center of mass fell within the base of support, the crab was considered statically stable and the SSM would have a value greater than zero. If the center of mass fell outside the base of support, the crab was considered statically unstable and the SSM would have a negative value. During these moments of negative static stability, if the crab continued along a predictable trajectory, then this implied that the crab was relying on dynamic stability mechanisms to maintain undisturbed locomotion (Koditschek et al., 2004).

In addition to instantaneous SSMs, we also calculated the mean SSM and duty factor when the crab had one, two, three or four legs on the ground. Coupled with the SSM data, this allowed us to quantify how much time ghost crabs spent in a statically stable configuration during the stance phase, as well as any effects limb loss had on limb use patterns during a stride.

For calculations of stability margin, we used the approximate location of the center of mass, which was indicated by the glass markers. Marker placement was informed on previously determined center of mass locations. Because we were using these calculations as a proxy for instantaneous stability, we did not account for slight shifts of the center of mass during locomotion due to locomotor limb swing.

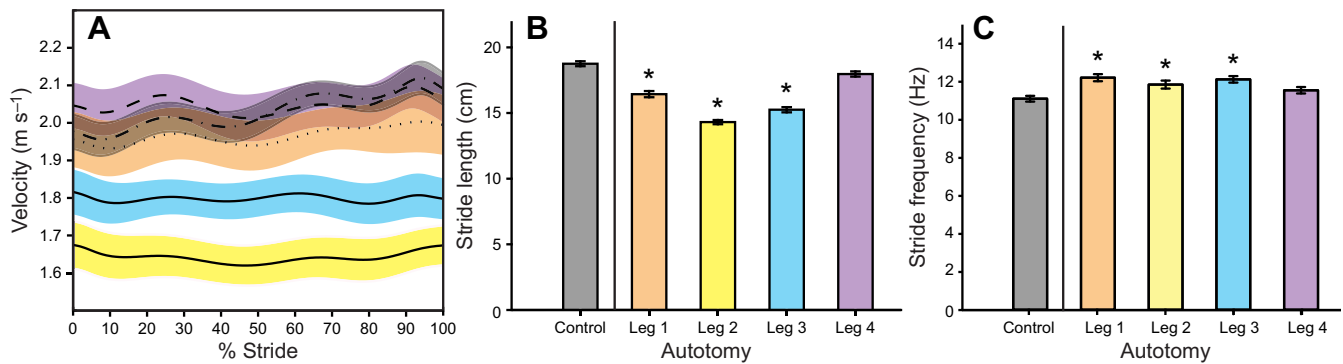
### Statistical analysis

To address whether limb loss affects locomotor performance, we compared intact and autotomized mean running speed using a mixed-model analysis of covariance (ANCOVA), with carapace width as a covariate, treatment as a factor and individual as a random blocking factor. Stride frequency, stride length and duty factor were tested with multiple mixed-model nested ANCOVAs with mean running speed as a covariate, treatment as a factor and individual as a random blocking factor with legs nested within the individual. To compare SSMs among different limb loss treatments, we analyzed the data using a mixed-model ANCOVA, with velocity as a covariate, treatment as a factor and individual as a random blocking factor, to test for differences in SSM between treatments. We then compared the frequency of occurrence during a stride for each of the limb ground contact categories between treatments using a mixed-model ANCOVA, with velocity as a covariate, treatment as a factor and individual as a random blocking factor. When applicable, a Tukey's honest significant difference *post hoc* test was performed to identify significant differences between treatments.

All statistical analyses were performed using JMP version 10.0.2 (SAS Institute Inc., Cary, NC, USA). As we conducted multiple statistical tests for comparison, we adjusted all *P*-values for multiple testing to reduce type I errors in hypothesis testing. We used the false discovery rate (FDR) method, which is more powerful and less conservative than its familywise error rate counterparts, such as the Bonferroni method (Benjamini and Hochberg, 1995). The FDR method estimates the proportion of falsely rejected null hypotheses from which adjusted *P*-values, also called adjusted FDR values, can be derived (Benjamini and Hochberg, 1995; Nathan, 2010). We converted our *P*-values using the FDR method (Benjamini and Hochberg, 1995) using the *p.adjust* function in R (R Development).

### RESULTS

There were no differences in the results due to sex ( $F_{1,80}=3.303$ ,  $P=0.085$ ), possibly due to the study being performed on subadult crabs. As a result, all kinematic data were pooled across sex. The



**Fig. 2. Mean instantaneous running velocity, stride length and stride frequency of four paired limb removal treatments compared against the control treatment.** Removal of the second and third legs (yellow and blue, respectively) resulted in significant decreases to (A) running speed when compared with the control treatment (gray). (B) Stride length and (C) stride frequency were significantly different from the control when the first (orange), second and third pairs of legs were removed. Removal of the fourth limbs (purple) had no impact on locomotor performance for any of these variables. Asterisks denote a significant difference compared with the control at  $P < 0.05$ .

final data set is composed of kinematic results from 85 ghost crabs ( $N=17$  per treatment). Intact crabs – which served as our control – ran at  $2.04 \pm 0.04 \text{ m s}^{-1}$  ( $\sim 60$  carapace lengths  $\text{s}^{-1}$ ). Whereas autotomy of limb pairs one and four had no detectable effect on running speed ( $P > 0.05$ ), autotomy of the second and third limb pairs was correlated with slower running speeds ( $F_{4,80}=9.488$ ,  $P < 0.001$ ; Fig. 2A, Table 1; Fig. S2A).

#### Novel limb couplings as result of autotomy of the middle limb pairs

As shown in Fig. 3A, when running at high speeds, intact crabs ran hexapedally – rather than octapedally – with an alternating tripod gait, because they raised their fourth limbs off from the ground. As a result, autotomy of the fourth limb pairs had no effect on the running gait or the proportion of the stride crabs ran with three legs in contact with the ground (Table 2). However, autotomy of any of the other limb pairs resulted in crabs running quadrupedally – the posterior-most fourth limbs continued to be raised or were used infrequently – using a diagonal couplet gait (Fig. 3B–D). This is most evident when the first limb pairs were autotomized, with three-limb contact periods dropping from 23.5% (intact) to 0.5% (autotomy) per stride (Table 2).

The alternating tripod gait typical of intact, control crabs was achieved by the simultaneous stepping of leading limbs one and three, and trailing limb two, followed by leading limb two and trailing limbs one and three (Fig. 3A). Autotomy of the first or fourth limb pairs did not disrupt this alternating pattern (Fig. 3B,E), permitting maintenance of the original couplings of the remaining limbs as a diagonal couplet or alternating tripod, respectively. However, autotomy of the second or third limb pairs required novel limb couplings to achieve the observed diagonal couplet gait. Calculations of relative limb phases between adjacent limbs revealed that on average, regardless of control or treatment condition, synchronized limb groups moved out-of-phase ( $\pi$ ) of

the other group, even when novel limb couplings were used (Fig. 3, right panels).

#### Limb loss results in decreased stride lengths and increased stride frequencies and duty factors

Although stride length and stride frequency did not differ among legs and were therefore pooled among limbs, duty factor was consistently higher in trailing limbs than leading limbs and were therefore analyzed separately (Table 3, Fig. 4). Autotomy of the fourth limb pairs had no detectable impact on general stride characteristics, such as stride length, stride frequency or duty factor ( $P > 0.05$ ). However, loss of any of the other limb pairs was correlated with decreased stride length (Fig. 2B, Tables 1 and 3; Fig. S1B), increased stride frequency (Fig. 2C, Tables 1 and 3) and increased duty factor (Fig. 4, Table 3). We excluded the fourth pair of limbs from this analysis due their infrequent use during locomotion (Fig. 3).

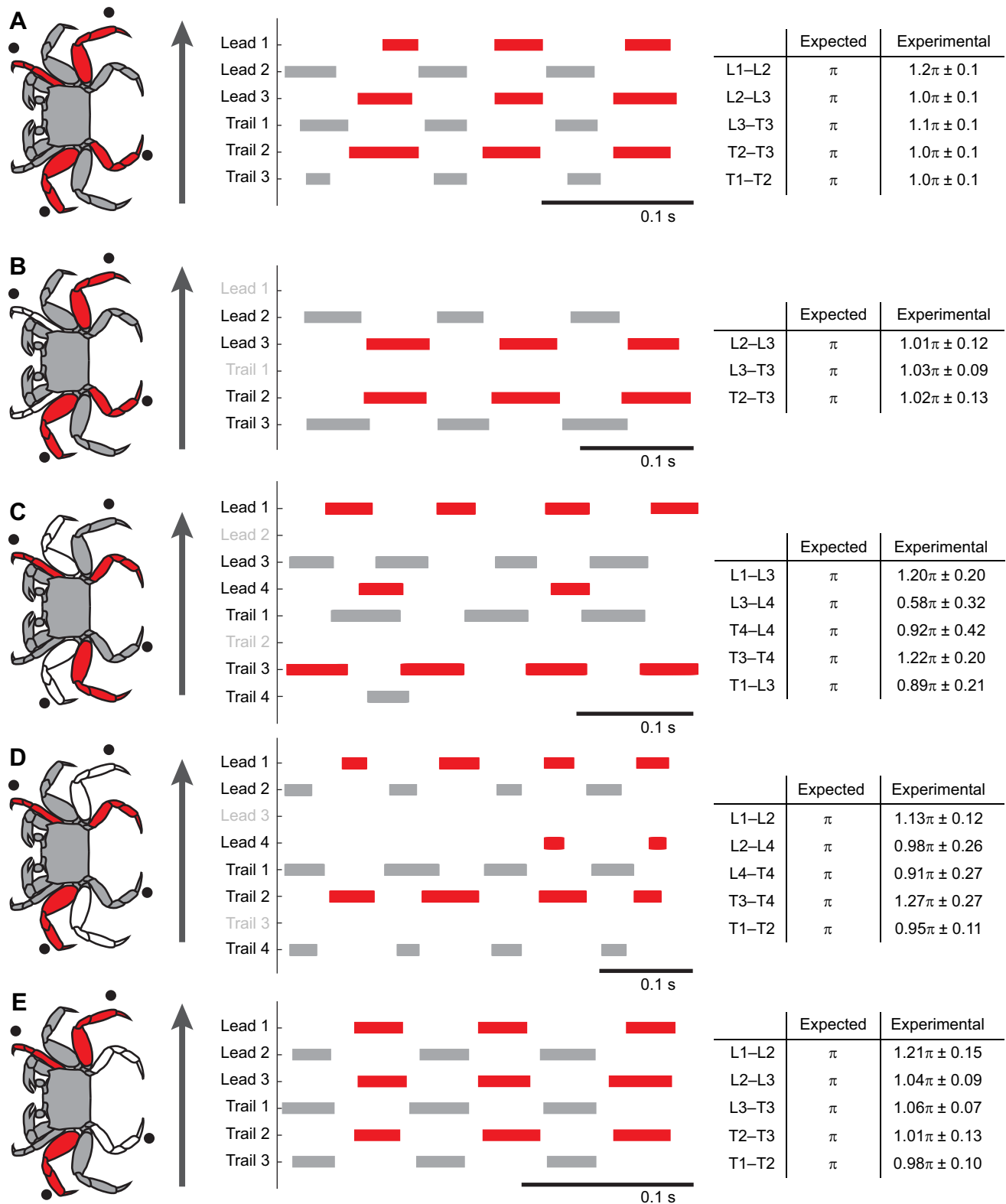
#### Limb loss induces instability due to a shift in patterns of limb contact during strides

Ghost crabs altered the amount of time different numbers of limbs are in contact with the ground when the first, second and third pairs of limbs were removed. Loss of the fourth pair of limbs was indistinct from the control runs and in both cases ghost crabs used three limbs on the ground more than two or one limbs on the ground (Fig. 5, Table 2). Loss of the second and third pairs of limbs resulted in increases in single-limb use compared with control runs, while three-limb contact is maintained at similar levels to those seen in control runs due to the infrequent use of the fourth pair of limbs (Fig. 5, Table 2). Without the infrequent use of the fourth limb pairs, three-limb contact would be drastically reduced to below 5% for the loss of the second and third pairs of limbs (Table 2). In these limb loss treatments, crabs very rarely placed four limbs simultaneously on the ground. Loss of the first pair of limbs resulted in a pattern of limb usage distinct from other limb loss treatments. In this treatment,

**Table 1. Mean velocity, stride frequency and stride length for control and four limb loss treatments**

Variable	Treatment				
	Control	Leg 1	Leg 2	Leg 3	Leg 4
Running speed ( $\text{m s}^{-1}$ )	$2.04 \pm 0.04$	$1.97 \pm 0.07$	<b><math>1.68 \pm 0.06^*</math></b>	<b><math>1.83 \pm 0.05^*</math></b>	$2.05 \pm 0.06$
Stride frequency (Hz)	$11.11 \pm 0.15$	<b><math>12.22 \pm 0.19^*</math></b>	<b><math>11.85 \pm 0.21^*</math></b>	<b><math>12.13 \pm 0.17^*</math></b>	$11.55 \pm 0.17$
Stride length (cm)	$18.76 \pm 0.189$	<b><math>16.44 \pm 0.24^*</math></b>	<b><math>14.31 \pm 0.16^*</math></b>	<b><math>15.25 \pm 0.2^*</math></b>	$17.97 \pm 0.2$

Values represent means  $\pm$  s.e.m.; 85 ghost crabs, 17 crabs per treatment. \*Significantly different from control indicated in bold (for ANCOVA results, see Table 2).



**Fig. 3. A comparison of limb couplings for intact and autotomized crabs.** (A) Although when running with all limbs, intact crabs will use an alternating tetrapod gait (red legs synchronized followed by gray legs), during high-speed runs, crabs often lift their fourth limb pair and run with an alternating tripod gait on only six limbs. (B–E) In autotomized crabs, the black circles correspond to the intact limb coupling pattern, to highlight changes in gait with limb loss (in white). Loss of the (B) first or (E) fourth pairs of limbs does not alter the original limb couplings, as shown by the red limbs corresponding to the positions of the black circles. In contrast, removal of the (C) second and (D) third pairs of limbs results in a novel limb coupling as well as the use of the fourth pair of limbs while running. Arrows indicate running direction and point towards the leading limbs. Middle plots show representative footfall diagrams with colors indicating coupled limbs for each control and autotomized condition. Pale gray text indicates autotomized legs (B–E). The black scale bar represents 0.1 s along the x-axis. Tables on the right show expected limb phases when an alternating gait is used and experimental means  $\pm$  s.e.m. limb phases. L, leading; T, trailing (with limbs numbered from one to four anterior to posterior). Kinematic data were collected for 85 crabs ( $N=17$  per group).

**Table 2. Mean contact period durations for different numbers of limbs on the ground and results of ANCOVAs comparing contact period durations within and between limb loss treatments in Atlantic ghost crabs**

Contact period (% stride)	Treatment				
	Control	First	Second	Third	Fourth
Single-limb contact	13.1±1.5	24.6±1.4	28.4±2.5 (38.9±8.9)	20.4±2.1 (32.5±8.6)	12.4±1.0
Two-limb contact	17.6±1.6	48.6±3.6	29.9±2.9 (47.4±17.9)	27.3±2.1 (53.6±8.2)	15.3±1.6
Three-limb contact	23.5±2.2	0.5±0.3	19.8±2.9 (3.8±8.7)	26.3±2.6 (1.0±3.0)	29.1±2.3
Four-limb contact	N/A	N/A	1.5±0.7 (N/A)	1.0±0.6 (N/A)	N/A
Within treatments					
<i>F</i>	40.677	132.571	22.510	33.766	53.211
d.f.	3,48	3,48	3,48	3,48	3,48
<i>P</i>	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>
Between treatments	Single-limb contact	Two-limb contact	Three-limb contact		
<i>F</i>	13.184	31.147	24.439		
d.f.	4,79	4,79	4,79		
<i>P</i>	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>	<b>&lt;0.001*</b>		

Values represent means±s.e.m. for all four limb pairs. Values in parentheses depict values where the fourth pair of limbs was not included in the analysis due to infrequent use. \*Significant *P*-values corrected for false discovery rate are indicated in bold. Results of Tukey's honest significant difference visible in Fig. 5. N/A indicates no instances of four-limb contact.

ghost crabs infrequently used three limbs on the ground, which is a stable configuration. Instead, they relied on statically less stable configurations by increasing the use of two limbs and a single limb on the ground (Fig. 5, Table 2).

Mean SSM for all treatments was negative (Table 4), indicating that crabs were not statically stable and relied on dynamic stability mechanisms for locomotion. When examining individual values for periods of each stride when different numbers of limbs were in contact with the ground, positive SSM was achieved only when three or four limbs were on the ground during autotomy of the third and fourth limb pairs. SSM differences among treatments with four legs on the ground were not analyzed because this was limited to a few observations ( $N=12$  out of 34) when the second or third pairs of limbs were removed.

## DISCUSSION

### Field experiments yield fast, consistent runs for Atlantic ghost crab

In this study, we observed the locomotor performance of Atlantic ghost crabs in the field, and investigated how ghost crabs were impacted by and compensated for paired limb loss. By reducing handling time, and allowing animals to run in their natural habitat on their natural substrate, we were able to measure some of the fastest running speeds for this genus that have been recorded in quantitative studies (maximum speed of  $2.5 \text{ m s}^{-1}$ ), except for a single observation of *O. ceratophthalmus* reported to be running at  $4 \text{ m s}^{-1}$  on the deck of a ship (Hafemann and Hubbard, 1969). Stride lengths and frequencies of our control runs fell into the same range as those measured for similar-sized *O. ceratophthalmus* (Burrows and Hoyle, 1973; see Table S1). Compared with other

studies looking at *O. quadrata*, our study found consistently higher running speeds and stride frequencies for similar-sized crabs (Blickhan and Full, 1987; Perry et al., 2009; Whittemore et al., 2015; see Table S1). To our knowledge, no other studies have observed locomotor performance after limb loss of ghost crabs in a naturalistic field setting. We encourage future studies interested in maximum performance to consider field experimentation, as evidenced by the consistently fast running speeds observed in this study.

### The fourth pair of limbs increases stability during fast locomotion

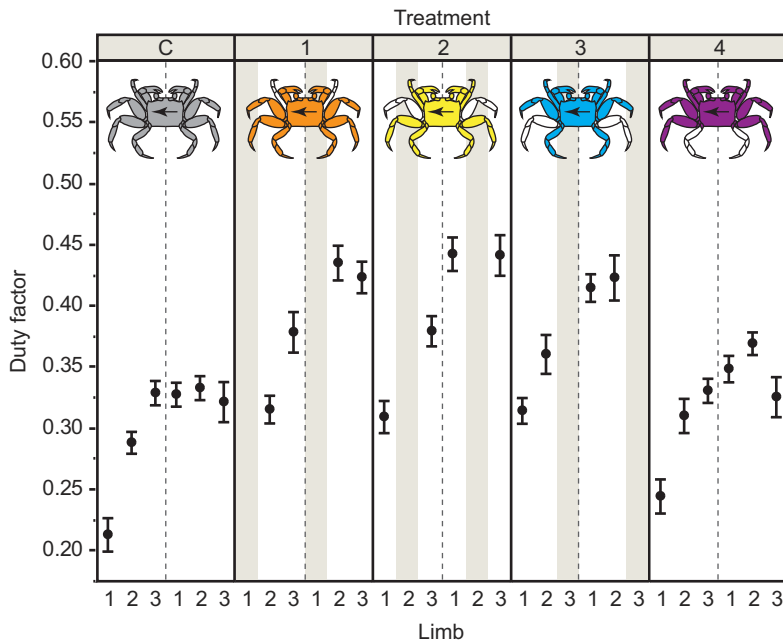
Although ghost crabs have four pairs of locomotor limbs, the fourth limb pair is usually only used during slow locomotion. During high-speed locomotion observed here and elsewhere in intact animals, these limbs are held up and no longer have an obvious locomotor role (Blickhan and Full, 1987; Burrows and Hoyle, 1973). In fact, their shorter length requires increased body pitch for these limbs to contact the ground during high-speed running – which could have a destabilizing effect if they had to be used. The fourth limb pair likely serves a diversity of roles, as exemplified among other eight-legged arthropods with reduced or specialized fourth limbs. For example, *Hemigrapsus nudus*, an intertidal crab of the family Grapsidae, uses its reduced fourth pair of limbs for climbing rocks (Maginnis et al., 2014). Likewise, among garden spiders, although different-sized limbs have been noted to result in slower flat running speeds when compared with spiders that have limbs of similar lengths, the short legs give the spider better climbing abilities (Foelix, 2011; Jacobi-Kleemann, 1953). Among portunid crabs (Hartnoll, 1971), the fourth pair of limbs has evolved into specialized swimming limbs. It is possible that the fourth limb pair in ghost crabs shares a similar functional distinction from the other locomotor limbs, and is used to support the body primarily during slow locomotion, as is seen in kangaroo tail use during pentapedal locomotion (O'Connor et al., 2014).

In contrast, during high-speed locomotion, the fourth limb pair would periodically contact the ground only when the second or third limb pairs were autotomized (Fig. 3). Arguably, the reduced fourth limb pair is unlikely to produce a lot of propulsive forces and, as mentioned above, may add instability to the animal during locomotion. This suggests a stabilizing role for this reduced limb pair, perhaps in an inertial manner much like lizard tails, or simply to catch the crab if it topples backwards. Periodic use of the fourth

**Table 3. Results of nested ANCOVAs examining the impacts of limb loss treatments on locomotor performance and differences among individual legs in ghost crabs**

Variable	Treatment			Individual leg		
	<i>P</i>	<i>F</i>	d.f.	<i>P</i>	<i>F</i>	d.f.
Stride frequency	<b>&lt;0.037*</b>	2.677	4,78	0.892	0.335	5,313
Stride length	<b>&lt;0.001*</b>	21.858	4,78	0.972	0.176	5,313
Duty factor	<b>&lt;0.001*</b>	28.361	4,78	<b>&lt;0.001*</b>	64.430	5,313

\*Significant *P*-values corrected for false discovery rate are indicated in bold.



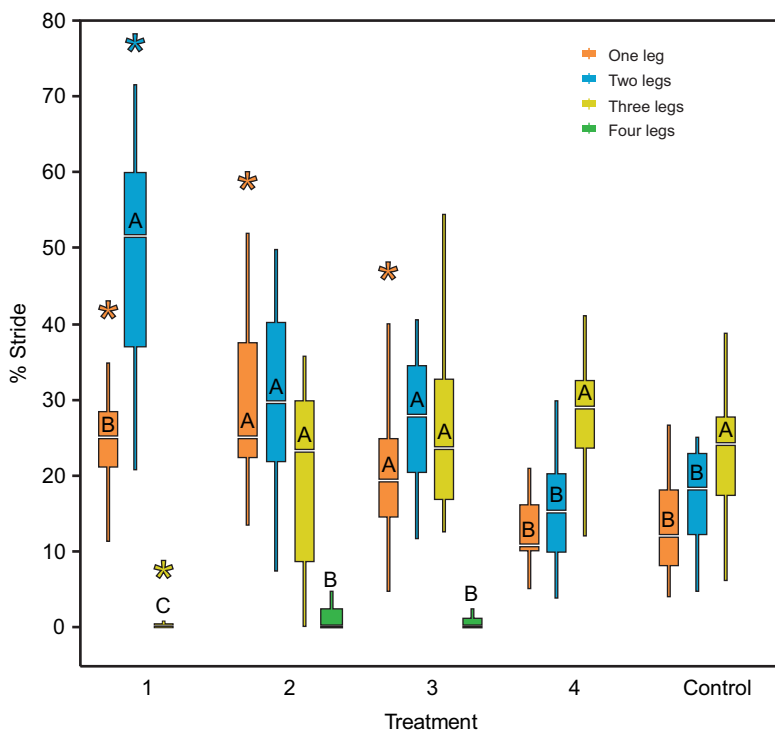
**Fig. 4. Mean duty factor for individual leading and trailing legs depicted for each of the four limb autotomy treatments and the control treatment.** Each box separated by solid lines represents a specific treatment. Leading and trailing limbs are separated for each treatment by the dashed line, with leading limbs on the left and trailing limbs on the right side. Gray bars indicate legs missing due to limb autotomy, depicted as white legs on the crab diagrams. Duty factors  $\pm$ s.e.m. are shown for each limb within a treatment. Trailing legs tended to exhibit significantly higher duty factors than leading legs ( $P < 0.001$ ). Removal of the first, second or third legs resulted in increased duty factors ( $P < 0.001$ ). The duty factor for the fourth legs was not quantified due to infrequent use during runs. C, control.

limb pair also increased the calculated SSM by increasing the number of limbs in contact with the ground (Table 2).

#### Ghost crabs alter stride kinematics to compensate for limb loss

Modulating locomotor kinematics and kinetics is an essential compensatory strategy, allowing animals to maintain stable locomotor dynamics when facing destabilizing events (e.g. Daley et al., 2006; Dickinson et al., 2000; Hsieh, 2016; Hsieh and Lauder, 2004; Libby et al., 2012). In our study, ghost crabs adjusted locomotor kinematics after limb loss to maintain a stable gait and fast running speeds: they shortened their strides and increased stride

frequency, while increasing relative ground contact time after limb loss (Figs 2 and 4). Similar locomotor adjustments have been observed in other animals as well. After limb loss, spiders increased the stance period of the remaining legs (Foelix, 2011) and increased their stride frequency (Wilshin et al., 2018; Wilson, 1967). Stick insects achieved stable gaits after limb loss by reducing walking speed and changing their stepping patterns (Graham, 1977). After tail loss, anole lizards increased stride frequency, decreased stride length and increased the duty factor of the hindlimbs (Hsieh, 2016). Even bipedal animals altered kinematics as a response to destabilizing events. Humans who encountered slippery conditions decreased stride lengths, which placed the center of mass closer to the base of



**Fig. 5. Percent of a stride during which different numbers of legs are in stance.** Control trials and fourth limb pair removal treatments mainly relied on the same three limbs to form alternating tripods. In contrast, loss of the first, second and third pairs of limbs resulted in changes from the pattern observed in the control group. Following loss of the first pair of limbs, ghost crabs ran primarily with two limbs on the ground. In the box plots, the white line in each box represents the median percent stride, with the top and bottom of the box indicating 25% and 75% quartiles, respectively. Whiskers represent the minimum and maximum of all the data. A colored asterisk indicates a significant difference from control at  $P < 0.05$  for that particular number of limbs on the ground. Differences within limb loss treatments are represented with different letters at  $P < 0.05$ . Data were collected for 85 crabs ( $N = 17$  per group).

**Table 4. Overall and mean static stability margins for different numbers of limbs on the ground during stance**

SSM	Treatment					Mixed-model ANCOVA		
	Control	First	Second	Third	Fourth	F	d.f.	P
Mean SSM	-14.01±1.58	-26.09±2.27	-24.07±3.71	-17.34±1.68	-15.69±1.84	3.105	4,75	<b>0.025*</b>
Single-limb contact	-45.11±2.47	-47.45±2.05	-46.93±3.54	-43.95±1.36	-46.33±2.26	0.336	4,80	0.942
Two-limb contact	-18.95±1.65	-14.89±2.64	-16.83±2.06	-17.22±1.97	-21.45±1.63	1.487	4,80	0.254
Three-limb contact	6.52±1.45	-13.94±7.42	-0.41±1.68	1.65±2.35	0.75±2.37	4.256	4,59	<b>0.005*</b>
Four-limb contact	N/A	N/A	-6.91±5.5	2.01±3.97	N/A	N/A	N/A	N/A

Values represent means±s.e.m.; 85 ghost crabs, 17 trials per treatment. \*Significant *P*-values corrected for false discovery rate are indicated in bold. SSM, static stability margin. N/A indicates no instances of four-limb contact.

support, in order to avoid falling (Myung and Smith, 1997; You et al., 2001). We believe that ghost crabs, like previous examples, reduced stride lengths and increased duty factors to counter instability induced by limb loss while increasing stride frequency to maintain high running speeds similar to the intact condition.

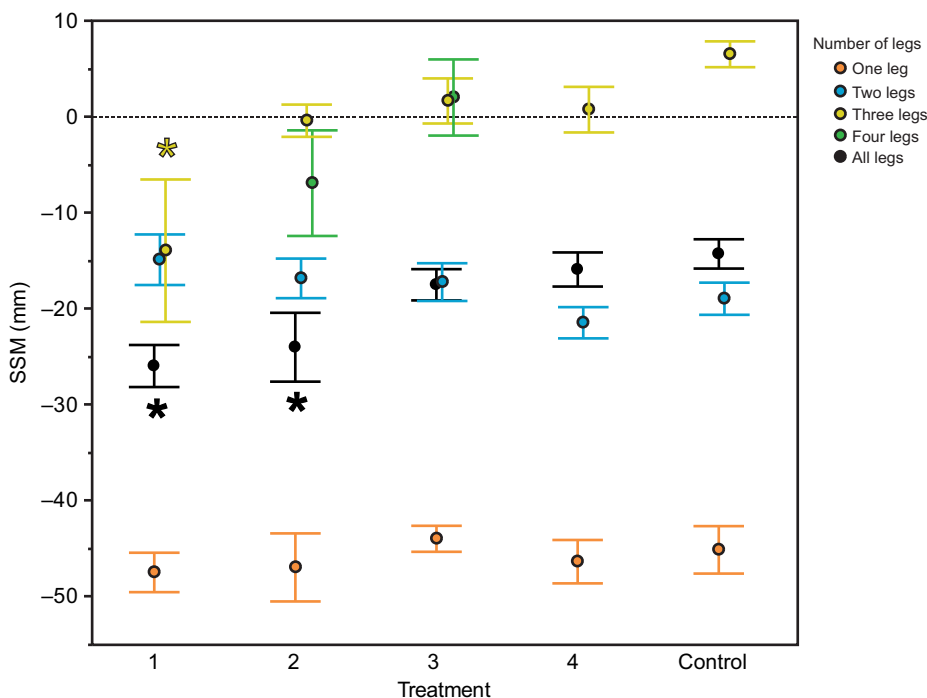
Animals moving at increasingly higher velocities experience reductions in static stability, relying on dynamic stability to bridge these moments of static instability (Ting et al., 1994). As ghost crabs compensated for limb loss, they also spent more time in statically less-stable states during stance periods (Figs 5 and 6). In fact, our results indicate that animals without their first pair of limbs had no statically stable periods in their stance periods at all, suggesting a reliance on dynamic stability, and corroborating many studies in other animals (Daley et al., 2007; Full et al., 2002; Sponberg and Full, 2008; Ting et al., 1994), including other ghost crabs (Blickhan et al., 1993; Full et al., 2002).

#### Differential limb functions determine impact of autotomy on locomotor performance

During locomotion, limbs often have different functions depending on their position with respect to the direction of motion.

Quadrupedal animals, such as horses (Heglund et al., 1982), dogs (Lee et al., 1999) and lizards (Chen et al., 2006; Foster and Higham, 2012), use their hindlimbs to generate propulsive forces, while the forelimbs decelerate and aid in maneuvering (Chen et al., 2006; Demes et al., 1994; Foster and Higham, 2012; Lee et al., 1999; Sullivan and Armstrong, 1978). Crustaceans and spiders also use their individual limbs differently during locomotion (Blickhan et al., 1993; Clemente and Federle, 2008; Goldman et al., 2006). For instance, the main propulsive forces are provided by the posterior limbs in spiders (Ehlers, 1939; Foelix, 2011) and by the trailing limbs in crabs (Blickhan and Full, 1987; Clarac et al., 1987). Crayfish use different sets of limbs when traveling on land and in water, which demonstrate that limb functions can change depending on the locomotor substrate (Pond, 1975). Cellar spiders use their anterior pair of legs in a sensory capacity, feeling for disturbances ahead as well as for stable footholds, while using the remaining limbs for propulsion (Pinto-da-Rocha et al., 2007).

In spite of these known differences in function, the spare leg hypothesis proposed that among harvestmen – and by extension among other arachnids and animals with eight or more legs – there is enough redundancy in limb function such that other limbs can



**Fig. 6. Effects of limb loss on mean static stability margins (SSMs) for different numbers of limbs in stance.** There was a significant decrease in SSM compared with the control with the loss of the first and second pairs of limbs for all legs in stance. Loss of the first pair of limbs resulted in a significantly lower SSM compared with the other treatments and the control group with three legs on the ground. There were no changes in SSM due to limb loss for one and two limbs on the ground. As contact of four limbs on the ground only occurred with the loss of the second and third pairs of limbs, no statistical analysis was conducted on this category. The mean SSM±s.e.m. is shown for each limb loss treatment. The asterisk denotes significance at  $P < 0.01$ . Data were collected for 85 crabs ( $N = 17$  per group).



partially or wholly offset costs associated with autotomy (Brautigam and Persons, 2003; Guffey, 1998; 1999). For example, terrestrial crabs can use their pincers (which are mainly used in defense and to manipulate food) to assist in locomotion (Barnes, 1975; Herreid and Full, 1986). Another study on the wolf spider *Pardosa milvina* found that prey capture and locomotor behavior were not affected by limb loss, although they did prefer smaller prey items after limb loss (Brueseke et al., 2001).

In this study, ghost crabs were able to maintain running speed following the loss of the first and fourth limb pairs, in support of the predictions of the spare leg hypothesis, but with detectable kinematic adjustments. In contrast, they were unable to fully compensate for the loss of the second and third pairs of limbs, as evidenced by lower running speeds. We propose two non-mutually exclusive explanations for this performance decrement.

First, these two middle limbs are the largest locomotor limbs and therefore house proportionately larger flexor and extensor muscles in the merus (Biewener and Corning, 2005; Perry et al., 2009), which are crucial for the production of propulsive forces. Because the fourth limbs are raised during running (Blickhan and Full, 1987; Burrows and Hoyle, 1973), autotomy of these limbs likely requires body repositioning due to a shift in weight distribution (see next paragraph); but this would not affect propulsive force production. In contrast, because of their active involvement during locomotion, loss of the first limb pair could negatively impact propulsive force production. However, the ability of the crabs to maintain running speeds during this treatment is likely due to the second and third limb pairs being able to produce sufficient force to counteract autotomy costs of losing the first limb pair (Fig. 2A). The running speed decrements observed with the loss of the second or third limb pairs may simply be due to the remaining limbs being unable to produce enough propulsive force to make up for the autotomy event.

Appendage loss is known to impact weight distribution and thereby affect body position and even locomotor performance. For instance, lizards that undergo tail loss can lose a significant proportion of its mass (e.g. 10–22% of body mass), which can shift the center of mass anteriorly between 5.6% in anoles (Hsieh, 2016) and 13% in geckos (Jagnandan et al., 2014). Lizards have been shown to sometimes increase or decrease locomotor performance as a result of these changes in weight distribution, depending on the ability of the animals to adjust their locomotor strategies (Jagnandan and Higham, 2018). It is possible that the loss of the two biggest limbs (second and third pairs of limbs) could shift the center of mass enough to destabilize the crab such that locomotor performance is reduced. However, considering that crabs were subject to symmetrical, paired limb removals, and that these limbs were located medially, we suspect that removal of these pairs of limbs may have actually reduced limb swing-induced center of mass movement following autotomy.

Second, another potential cause for the observed performance decrements upon loss of the second and third leg pairs may be due to a requisite realignment of limb couplings. When running at high speeds, crabs tended to alternate the phasing of adjacent limbs to achieve a diagonal couplet, alternating tripod or alternating tetrapod gait, depending on whether four, six or eight legs were being used for locomotion, respectively. Whereas removal of the first and fourth leg pairs permitting continued use of the same phase patterns as when intact, removal of the second and third leg pairs required novel limb couplings (Fig. 3). Similar rearrangements of limb couplings have been observed in other six- and eight-legged animals when middle legs are autotomized (Delcomyn, 1991a,b;

Herreid and Full, 1986; Hughes, 1957). The removal of the middle legs has been applied for decades as a method to challenge the motor system of walking insects (Delcomyn, 1991b). For example, cockroaches that had their middle legs removed switched their limb coupling such that the hind leg moved in-phase with the forelimb on the contralateral side, forming a diagonal gait (Hughes, 1957). Similar rearrangements have also been observed among tarantulas (Wilson, 1967) and wolf spiders (Wilshin et al., 2018). When wolf spiders maintained their original limb configuration following autotomy, they limped to increase their mean locomotor stability (Wilshin et al., 2018).

Realignment of limb couplings is thought to occur due to the lack of proprioceptive feedback from missing or damaged limbs (Delcomyn, 1991a,b; Evoy and Fournier, 1973; Graham, 1977; Herreid and Full, 1986; Spirito et al., 1973); yet we know very little about how rearranging limb couplings or altering the phasing of limbs affects locomotor performance. It is possible that the loss of the middle legs affects the muscle activity of the remaining limbs. For example, the muscle activity of legs neighboring autotomized legs in walking cockroaches altered frequency and timing of motor bursts, and phasing of motor bursts at slow speeds. Cockroaches running at higher speeds, however, did not exhibit these changes, with muscle activity patterns staying identical to those measured in intact cockroaches (Delcomyn, 1991a,b). There are no studies that address whether muscle activity of ghost crab limbs is affected by limb loss and remains a topic for future exploration.

## Conclusion

Our hypothesis that the removal of the third and fourth pairs of limbs would have the least effect on locomotor performance was partially supported by our findings. As predicted, the loss of the fourth pair of limbs had no effect on locomotor performance. However, the loss of the third pair of limbs significantly reduced running performance in ghost crabs. Ghost crabs are remarkably robust to limb loss, suffering a maximum running speed decrement of only ~25%. Faced with the loss of a pair of walking limbs, ghost crabs compensated by adjusting their stepping and limb cycling patterns. The second and third pairs of limbs, however, appear to be the most crucial for maintaining overall running performance. Upon losing either of these limb pairs, we observed dramatic changes in limb timing characteristics and decrements in running speed whereas the impacts were far more modest following the loss of any of the other limbs.

We present two, mutually non-exclusive possibilities as to why the second and third pairs of limbs are important to locomotor performance. (1) These two limbs may serve locomotor roles that cannot be replicated by the remaining limbs, due to their larger size and potentially greater muscle volume. In that case, the remaining limbs may not be able to produce similar propulsive forces, resulting in slower running speeds. (2) The removal of the second and third pairs of limbs results in novel limb couplings, which likely requires adjustments to neurological coordination. This new stepping pattern may also impact the muscle activity of the other limbs, as seen in cockroaches, exacerbating the observed decrease in locomotor performance.

Lastly, the findings of our study can potentially be incorporated in future developments of robots by including kinematic adjustments to different limb loss scenarios. Considering the difficulty of the retrieval of some remotely operated vehicles and their repairs, improving robustness to otherwise debilitating damage would significantly improve their utility in search and rescue or exploration.

**Acknowledgements**

Thank you to Heidi Hanlon at the Cape May National Wildlife Refuge for help with permissions and set-up of the study site, to Nicole Mazouchova, Kyle Mara, Carlos Gomez, Elizabeth Szablya, Michael Fath, Alina Gawlinski, Lawrence Gardner, Emily Dabashinsky and Joe Bondi with assisting in animal and data collection, and to Edward Gruberg, Andrew Spence, Amy Freestone, Erik Cordes and Michel Lemay for discussions related to this topic.

**Competing interests**

The authors declare no competing or financial interests.

**Author contributions**

Conceptualization: J.A.P., S.H.; Methodology: J.A.P., S.H.; Software: S.H.; Formal analysis: J.A.P., S.H.; Investigation: J.A.P., S.H.; Data curation: J.A.P., S.H.; Writing - original draft: J.A.P., S.H.; Writing - review & editing: J.A.P., S.H.; Visualization: J.A.P.; Supervision: S.H.; Funding acquisition: S.H.

**Funding**

This study was supported by Temple University start-up funds and a National Science Foundation CAREER grant IOS-1453106 to S.T.H.

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