BIOMECHANICAL CONTROL MECHANISMS AND MORPHOLOGICAL ADAPTATIONS FOR Locomotion IN CHALLENGING SCENARIOS

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ABSTRACT

Everyday ecologically relevant tasks that affect organismal fitness, such as foraging, reproduction, predator avoidance, and escape responses, rely upon successful locomotion. The effectiveness of animal locomotion depends on many underlying factors, such as the morphology of the locomotor limbs, which evolved to fulfill specific locomotor tasks. Besides morphology, the material properties of the limbs also play a crucial role in locomotion. The skeletal structures of locomotor limbs must be able to withstand the repeated stresses that come with locomotion, either on land or underwater, as they use their limbs to generate propulsive forces.

The natural environment animals move in is complex and dynamic, as various conditions crucial to locomotor performance can change unexpectedly. Perturbations to locomotor stability can take different forms, such as elevation changes, obstacles, substrate changes, and slipping. To maintain stable locomotor performance in these environments, animals rely on locomotor control mechanisms to counteract destabilizing effects of locomotor perturbations. In this Dissertation, I investigated the biomechanical control mechanisms and morphological adaptations during locomotion in challenging locomotor scenarios. Over the course of three chapters, the goals were to: 1) explore the effects of limb loss on a side-ways running sprint specialist, the Atlantic ghost crab, 2) determine the response and control mechanisms that allow ghost crabs overcome slip perturbations, and 3) to describe the pelvic morphology of bottom-walking
Antarctic plunderfish and compare the pelvic morphologies among multiple species of nothenioids that do not bottom-walk.

This study demonstrates the robustness of Atlantic ghost crabs to limb loss and slip perturbations. Paired limb removals resulted in a pattern of kinematic adjustments, which reduced locomotor performance by up to 25%, which was dependent on specific limbs being lost. I suggest that these limbs serve more important limb functions that can’t be replaced by the remaining limbs, however the loss of these particular limbs also results in re-patterning of limb relationships, which may reveal a neural component that may be the cause of decreased locomotor performance. Slip perturbations on the other hand were found to not have any significant effects on the locomotor performance of ghost crabs. Kinematics remained constant as ghost crabs traversed the slip surface, suggesting that ghost crabs may rely on feedforward control to overcome slip perturbations, however further studies measuring neural activity are required to confirm our finding. Most importantly though this chapter demonstrates and corroborates the role of momentum and how it allows animals to overcome perturbations. The last chapter investigated the pelvic morphology and material properties of fin rays in bottom walking fish. The Antarctic plunderfish was found to possess high flexural stiffness in its pelvic fin rays, which likely facilitate the bottom walking behavior in this species. Other, non-bottom walking notothenioids did not have fin rays of similar stiffness. Pelvic plate morphology was not different between species, however there were stark differences in mineralization. The bottom-walking fish had higher bone mineral density compared to the other species analyzed in this
chapter. I also found mineralization patterns which seem to align with muscle fiber alignment of the major pelvic muscles, suggesting that these regionalized increases in stiffness provide stability while allowing for a lightweight pelvic plate.
Dedicated to the memory of my grandfather,

Dr. Franklin H. Pfeiffenberger.

His love and support made all of this possible.
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# TABLE OF CONTENTS

<table>
<thead>
<tr>
<th>Section</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>ABSTRACT</td>
<td>III</td>
</tr>
<tr>
<td>DEDICATION</td>
<td>VI</td>
</tr>
<tr>
<td>ACKNOWLEDGMENTS</td>
<td>VII</td>
</tr>
<tr>
<td>LIST OF TABLES</td>
<td>XV</td>
</tr>
<tr>
<td>LIST OF FIGURES</td>
<td>XVII</td>
</tr>
<tr>
<td>CHAPTER</td>
<td></td>
</tr>
<tr>
<td>1 INTRODUCTION</td>
<td>1</td>
</tr>
<tr>
<td>1.1 Locomotion</td>
<td>1</td>
</tr>
<tr>
<td>1.1.1 Terrestrial locomotion</td>
<td>1</td>
</tr>
<tr>
<td>1.1.2 Aquatic locomotion</td>
<td>2</td>
</tr>
<tr>
<td>1.2 Morphology - Form and function</td>
<td>3</td>
</tr>
<tr>
<td>1.3 Material properties and biomechanics</td>
<td>4</td>
</tr>
<tr>
<td>1.4 Locomotor challenges and control strategies</td>
<td>5</td>
</tr>
<tr>
<td>1.5 Objectives and hypotheses</td>
<td>7</td>
</tr>
</tbody>
</table>
AUTOTOMY INDUCED EFFECTS ON
THE LOCOMOTOR PERFORMANCE OF THE GHOST CRAB,
*OCYPODE QUADRATA* ................................................................. 9

2.1 Abstract .............................................................................................. 9

2.2 Introduction .......................................................................................... 10

2.3 MATERIALS AND METHODS ............................................................... 13

  2.3.1 Animal Collection ........................................................................... 13

  2.3.2 Experimental Setup .......................................................................... 14

  2.3.3 Video Analysis .................................................................................. 14

  2.3.4 Statistical Analysis ............................................................................ 17

2.4 RESULTS .................................................................................................. 18

  2.4.1 Limb loss results in decreased stride lengths and
       increased stride frequencies and duty factors .................................. 20

  2.4.2 Novel limb couplings as result of autotomy
       of the median limbs ........................................................................... 24

  2.4.3 Limb loss induces static instability due to a shift
       in patterns of limb contact during strides ........................................ 26

x
2.5 DISCUSSION

2.5.1 Ghost crabs alter stride kinematics to compensate for limb loss

2.5.2 Some limbs are costly to autotomize due to differential limb functions

2.5.3 Realignment of limb couplings correlates with decreased performance

2.5.4 Conclusion

3 FEED-FORWARD CONTROL STRATEGIES ENABLE SIDEWAYS-RUNNING ANIMALS TO OVERCOME LOCOMOTOR PERTURBATIONS

3.1 Abstract

3.2 Introduction

3.3 MATERIALS AND METHODS

3.3.1 Animal Collection and Experimental Setup

3.3.2 Data Analysis

3.3.3 Statistical Analysis
3.4 RESULTS .................................................................................................................. 48

3.4.1 Running speed remained constant

despite large slip distances ................................................................. 48

3.4.2 Perturbation had no effect

on kinematic timing variables .......................................................... 51

3.5 DISCUSSION ........................................................................................................... 56

3.5.1 Constant cycling of limbs provides

immediate stabilization .................................................................. 56

3.5.2 Momentum enables ghost crabs to overcome

slip perturbations .............................................................................. 59

3.5.3 Limb phasing different between leading

and trailing limbs ........................................................................... 60

3.5.4 Conclusion ....................................................................................................... 62

4 COMPARISON OF PELVIC FIN MORPHOLOGY ACROSS

WALKING AND NON-WALKING

ANTARCTIC NOTOTHENIOIDS ................................................................. 63

4.1 Abstract .................................................................................................................. 63

4.2 Introduction ............................................................................................................ 64
4.1 Materials and methods ................................................................. 70

4.1.1 Specimens ........................................................................... 70

4.1.2 Video recording and analysis .............................................. 70

4.1.3 CT imaging and reconstruction .......................................... 71

4.1.4 Bone mineral density conversion ....................................... 72

4.1.5 Segmentation of CT scans .................................................. 72

4.1.6 Fin ray material properties .................................................. 73

4.1.7 Second moment of area and density calculations

of fin rays .................................................................................. 74

4.1.8 Calculations of flexural stiffness and

apparent material stiffness ...................................................... 74

4.1.9 3D geometric morphometric analysis of pelvis shape .......... 75

4.2 Results .................................................................................... 76

4.2.1 Video analysis ................................................................. 76

4.2.2 Pelvic volumes and densities ............................................ 77

4.2.3 Pelvic shape differences ................................................... 81
4.2.4 Densities and volumes of the pelvic fins ........................................... 83

4.2.5 Proximo-distal reduction of CSA, BMD, and $I_{NA}$

  in individual fin rays ................................................................. 83

4.2.6 Fin ray MTS and flexural stiffness ................................................. 88

4.3 Discussion .......................................................................................... 91

  4.3.1 Locomotor kinematics resemble those of other

    bottom-walkers ........................................................................ 91

  4.3.2 Fin flexural stiffness is highest in Histiodraco velifer .............. 93

  4.3.3 Pelvic shape increased density for support ............................ 95

  4.3.4 Proximo-distal changes in fin rays suggest

    regionalized functions ................................................................ 97

  4.3.5 Conclusion .................................................................................. 98

5 CONCLUSION .................................................................................... 100

REFERENCES CITED ............................................................................. 103
# LIST OF TABLES

<table>
<thead>
<tr>
<th>Table</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.1: Average velocity, stride frequency, and stride length for four limb loss treatments and the control treatment in 85 ghost crabs (n=17)</td>
<td>20</td>
</tr>
<tr>
<td>2.2: Results of ANCOVAs examining impacts of limb loss treatments on locomotor performance and differences among individual legs in ghost crabs</td>
<td>22</td>
</tr>
<tr>
<td>2.3: Average 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</td>
<td>29</td>
</tr>
<tr>
<td>2.4: Average Static stability margins for different numbers of limbs on the ground during stance as well as overall SSM including all limbs and results of ANCOVAs determining differences in Static stability margins between Treatments</td>
<td>30</td>
</tr>
<tr>
<td>3.1: Results of ANCOVA analyses on average running speed, stride frequency, stride length, and duty factor for three strides in 13 Atlantic ghost crabs</td>
<td>49</td>
</tr>
<tr>
<td>3.2: Average limb phases ± s.e.m. for three strides in 13 Atlantic ghost crabs, using the third leading limb as the reference</td>
<td>52</td>
</tr>
</tbody>
</table>
3.3: Results of mixed-model ANCOVA’s comparing limb phasing within and between strides of single and double contact runs for 13 Atlantic ghost crabs. ..................................................................................................................53

4.1: Volumes (mm$^3$) and bone mineral densities (g/cm$^3$) for the right pelvic plate and the right pelvic fins of five Nototheniid species ..............................................79

4.2: Averages ± standard deviation for cross-sectional area (CSA), bone mineral density (BMD), and second moment of area (I$_{NA}$), and the force required to reach 0.5 mm deflection for each fin ray among five species of Notothenioids ..........................................................................................90
LIST OF FIGURES

Figure 2.1: Experimental set-up used in this study .................................................................16

2.2: Average instantaneous running velocity, stride length, and stride frequency of four paired limb removal treatments compared against the control treatment in 85 Atlantic ghost crabs (n=17) ........................................19

2.3: Graph showing the percentage of trials which used the 4th leg during stance phase for 78 Atlantic ghost crabs.................................................................21

2.4: Average duty factor for individual leading and trailing legs depicted for each of the four limb autotomy treatments and the control treatment .....................23

2.5: A comparison of limb couplings (red) during running for intact and autotomized treatments.................................................................................................24

2.6: Box plot comparing between and within limb loss treatment differences of % stride for different number of limbs in stance............................................25

2.7: Effects of limb loss on average Static Stability Margins (SSM) for all limbs and different numbers of limbs on the ground..............................................27

3.1: Relationship of pattern generators in eight-legged locomotion .................................44

3.2: Experimental trackway (A) and illustration of Atlantic ghost crab (B) ..............47
3.3: Average individual limb slippage for each initial limb condition. ................................51

3.4: Average running speed of Atlantic ghost crabs for three strides. ..........................50

3.5: Average stride frequency, stride length, and duty factor

of 13 ghost crabs over 13 Atlantic ghost crabs ......................................................54

3.6: Polarplots with the average limb phases ± s.e.m. for three strides

in the Atlantic ghost crab indicating differences in limb coupling

of leading and trialing limbs ...........................................................................55

4.1: Picture of Antarctic plunderfish, Histiodraco velifer .........................................69

4.2: Phylogeny of the Suborder Notothenioidei, down to the family level. ..................71

4.3: Reconstructed model of the right pelvic plate of Histiodraco velifer

with 15 landmark used in 3D geometric morphometric analysis .......................76

4.5: Reconstructed right pelvic plates for (A) Histiodraco velifer,

(B) Akarotaxis nudiceps, (C) Gymnodraco acuticeps,

(D) Trematomus bernacchii, and (E) Chaenodraco wilsoni. ...............................80

4.6: Volume rendering depicting bone mineral densities (BMD)

for the pelvic plates of five Notothenioid species ...........................................81

4.7: Principal component analysis depicting shape differences

in the right pelvis of five Notothenioid species ..............................................82
4.8: Pelvic fin rays volumes and material properties

for *Histiodraco velifer* ...........................................................................................................84

4.9: Pelvic fin rays volumes and material properties

for *Akarotaxis nudiceps* ...........................................................................................................104

4.10: Pelvic fin rays volumes and material properties

for *Gymnodraco acuticeps* ...........................................................................................................86

4.11: Pelvic fin rays volumes and material properties

for *Trematomus bernacchii* ...........................................................................................................87

4.12: Pelvic fin rays volumes and material properties

for *Chaenodraco wilsoni* .............................................................................................................88

4.13: Graph showing the (A) Flexural stiffness and (B) Young’s modulus

for the 1st fin ray (1), 2nd dorsal (2D), and 3rd ventral (2V)

among five Notothenioid species .................................................................................................91
1.1 Locomotion

Locomotor performance is crucial to the overall fitness of animals, as everyday tasks, such as foraging, reproduction, predator avoidance, and escape responses, rely upon successful locomotion (Arnold, 1983; Huey and Stevenson, 1979). How effectively animals move depend on many factors such as the mode of locomotion, the skeletal support structures, the morphology and material properties of these skeletal structures, as well as the control mechanisms that govern locomotor dynamics (Alexander, 1984; Blickhan, 1989; Dickinson et al., 2000; Ennos, 2012; Jindrich and Full, 2002; Nishikawa et al., 2007; Vogel, 2003).

1.1.1 Terrestrial locomotion

Arthropods were the first animals to invade the land, as evidence of terrestrial, eight-legged arthropods date back to 450 million years ago (Jeram et al., 1990). Early tetrapods evolved around 350 million years ago as fish began transitioning onto land (Pierce et al., 2013; Shubin et al., 2006). On land, animals must use their appendages or their body to generate propulsive forces, on top of supporting their bodies against the gravity acting on them (Shubin et al., 2006). The underlying principles governing propulsive force production in terrestrial animals is the same, regardless of the number of limbs (Dickinson et al., 2000). All terrestrial legged locomotion can be modeled by an inverted pendulum at slow speeds or an spring loaded inverted pendulum at higher speeds.
(Blickhan, 1989; Seyfarth et al., 2002). In the inverted pendulum, animals vault over their legs, which are modeled as stiff components, and they do not compress. In the spring-mass model, that leg is modeled with a spring, which compresses at foot down and decompresses at foot off, which allows for some energy recovery through tendons (Blickhan, 1989). Once a threshold velocity has been reached and certain kinematic parameters have been met, the spring-mass model predicts self-stabilization in the locomotor system (Seyfarth et al., 2002).

1.1.2 Aquatic locomotion

The viscosity of water is much higher than air. Aquatic animals therefore experience a lot of drag forces on their bodies when moving (Vogel, 1994). The viscosity of water however also allows aquatic animals to swim in their environment, which they do by oscillating appendages or undulating their body, essentially pushing of the medium they are surrounded by (Drucker and Lauder, 1999; Tytell and Lauder, 2004) Aquatic animals can improve swimming performance by adopting a streamlined body shape, which reduces the drag the animal experiences (Webb, 1984).

On top of swimming, a large number of aquatic animals also interact directly with the substrate to propel themselves forward. Many turtles, such as snapping turtles and red-eared sliders have been described to use bottom-walking in addition to regular swimming (Willey and Blob, 2004). Other vertebrates, such as salamanders (Azizi and Horton, 2004), newts (Ashley-Ross et al., 2009), pinnipeds (Fish et al., 1988), and hippopotamuses (Coughlin and Fish, 2009) have also been described to walk on the
bottom. Invertebrates, for example octopi and crabs (Martinez et al., 1998), also use their appendages to bottom-walk. It has been hypothesized that bottom-walking played a crucial intermediary step in the evolutionary water-land-transition of animals (King et al., 2011).

1.2 Morphology - Form and function

Animal limbs have evolved to fulfill specific functions, for locomotion and other ecologically relevant tasks, such as mating, defense, and predation (Brautigam and Persons, 2003; Fleming et al., 2007; Juanes and Smith, 1995). The function of animal limbs depends on the morphology of the limb, both the external and internal features, such as muscles, tendons, bones, and cartilage (Ennos, 2012; Vogel, 1987). Several animals have evolved specific limb shapes to serve specialized functions. For example, swimming crabs within the Portunidae evolved a morphologically distinct, flattened 4th limb that serves as a swimming limb (Plotnick, 1985). The pincers in crabs that feed on hard-shelled prey, such as stone crabs, evolved specific structures that increase their mechanical advantage, making it easier to peel back the shells of their prey (Brown et al., 1979). Prominent examples are the attachment structures which animals have on their limbs, such as tarsi in cockroaches and toe pads in geckos (Autumn et al., 2006; Clemente and Federle, 2008; Irschick et al., 2006). These structures allow them to climb vertically and even stay attached to the substrate while upside down. Bottom-walking fish, like the searobin, use the first three pectoral fin rays to walk on the substrate (Jamon et al., 2007).
1.3 Material properties and biomechanics

Skeletal elements heavily depend on their material properties to function (Currey, 2002; Ennos, 2012; Vogel, 2003). As the animal’s moves along, they exert forces onto the substrate or medium around them. These forces result in ground reaction forces, which are opposite and equal to the force applied by the animal (Dickinson, 1996; Vogel, 2003). In terrestrial locomotion, the horizontal vector derived from the ground reaction force are the forward propulsive forces (Dickinson et al., 2000). In aquatic animals, because of their three dimensional environment, movement can occur forward, up, down, and backwards because of thrust vectors generated by the interaction of the limbs with water (Drucker and Lauder, 1999; Lauder, 2015). The skeleton of terrestrial and aquatic animals must withstand the stresses generated by locomotion. These stresses can be in the form of compression, torsion, tension, shear, and bending (Ennos, 2012; Vogel, 2003). Fish fins, for instance, experience bending forces as they move through the water (Alben et al., 2007; Flammang et al., 2013; Lauder et al., 2011; Taft, 2011). Another aspect of locomotion is the repeated loading of the locomotor limbs. Cyclical loading of skeletal elements over time will result in fatigue of the material, which can change its material properties (Ennos, 2012; Vogel, 2003). Failure to withstand these forces can cause a catastrophic failure in the structure, i.e. breaking, or deforming the structure to a point where it does not return to its original shape (Biewener and Corning, 2005; Currey, 2002; Ennos, 2012; Vogel, 2003). The skeleton for that matter must be resilient to cyclical loading and also have mechanisms of material maintenance in place. Vertebrate bone undergoes constant remodeling, where bone is absorbed and deposited through osteoclasts and osteoblasts (Cohen et al., 2012; Horton and Summers, 2009). This
mechanism repairs bone that has experienced micro-cracks or fractures, and can reinforce areas of bone according to loading stresses it experiences (Cohen et al., 2012; Horton and Summers, 2009). Crustaceans however do not have this ability, as their exoskeleton is shed and replaced by a new one periodically (Juanes and Smith, 1995; Parle et al., 2015). Damages incurred to limbs therefore are semi-permanent, until the exoskeleton is replaced in the next shedding cycle (Juanes and Smith, 1995).

Variables that describe how a material responds to bending stresses are the Young’s modulus, the second moment of area, and flexural stiffness. The Young’s modulus, or elastic modulus, describes the material stiffness and is based on the relationship of stress and strain in solid materials. The second moment of area measures is a geometric measure and describes a shapes resistance to deformations. It is determined by the distribution of material in relationship to a neutral axis around which bending occurs (Ennos, 2012; Vogel, 2003). Flexural stiffness is the product of the Young’s modulus and the second moment of area and describes how resistant a structure is to bending (Ennos, 2012; Vogel, 2003).

1.4 Locomotor challenges and control strategies

The natural environment animal’s move in is complex and dynamic, as various conditions crucial to locomotor performance can change unexpectedly (Müller et al., 2014). Changes, such as uneven terrain (Daley et al., 2006; Sponberg and Full, 2008), changes in substrate compliance (Mazouchova et al., 2010; Moritz and Farley, 2004; Spence et al., 2010), inclines and declines (Birn-Jeffery and Higham, 2014; Goldman et
al., 2006; Higham and Jayne, 2004a), obstacles (Olberding et al., 2012; Tucker and McBrayer, 2012), and slipping (Clark and Higham, 2011; Myung and Smith, 1997; You et al., 2001). The animal can become inherently unstable due to these perturbations, and if no recovery measures are undertaken, these perturbations can ultimately lead to falls. Animals have evolved a variety of active and passive control mechanisms that allow the animal to respond to the perturbation and maintain or return to stable locomotor mechanics (Daley et al., 2007; Koditschek et al., 2004; Nishikawa et al., 2007).

Legged locomotion is controlled by a central pattern generator which coordinates limb movements (Marder and Bucher, 2001). The central pattern generator is constantly sending feed-forward signals to the limbs (Full and Koditschek, 1999; Nishikawa et al., 2007). Using neural feedback, these feed-forward patterns are altered based on sensory input, such as visual cues (i.e. change in elevation) or sensors in the limbs, resulting in changes to limb movement or activity (Nishikawa et al., 2007). However, when traveling at fast speeds, the neural response time to perturbations is decreased due to increases in stride frequency and shorter stance periods (Brown and Loeb, 2000; Full and Koditschek, 1999). In these scenarios, mechanical feedback and feedforward control can maintain stability until the neural response can take effect (Jindrich and Full, 2002). Mechanical feedback depends on the viscoelastic, nonlinear properties of the musculoskeletal system, and can stabilize the animal momentarily. Feed-forward control uses the constant cycling of limbs to maintain stability (Brown and Loeb, 2000).

Locomotor stability can be challenged by other means than perturbations. For instance, the loss of a locomotor appendage can have severe effects on the locomotor
performance of animals and affect its fitness (Juanes and Smith, 1995; Maginnis, 2006). Animals may have to compensate for limb loss by altering stride kinematics in order to maintain a stable gait (Wilson, 1967). It has been hypothesized that an increased redundancy of limbs can alleviate negative effect of limb loss. For instance, bipedal and quadrupedal animals have to alter their gait and exert a lot of energy to move after the loss of a locomotor appendage. In contrast, animals with six or more limbs have been shown to be less severely impacted by limb loss, with some animals exhibiting no negative impacts on their locomotor performance (Guffey, 1999).

1.5 Objectives and hypotheses

In the following chapters, I investigated the locomotor biomechanics and control mechanisms of animals experiencing challenging scenarios while moving in their habitat. In Chapter 1, I investigated changes in the locomotor performance of the Atlantic ghost crab, *Ocypode quadrata*, in response to limb autotomy, as well as adaptive locomotor strategies to maintain stable locomotion after limb loss. I hypothesized that observed natural limb loss frequencies were reflecting the locomotor function of the limb, with limbs not important to locomotion more frequently autotomized.

Chapter 2 investigated the control mechanisms and recovery strategies of ghost crabs overcoming slip perturbations. At their fast running velocities, it was hypothesized that feed-forward control is an integral component of their strategy to overcome slippery surfaces, which would be exhibited by constant cycling of locomotor kinematics.
In Chapter 3, I incorporated material testing, micro CT-scanning, and biomechanics to describe the differences in the pelvic girdle among five species of nototheniod fishes to explore which morphological and biomechanical differences allow the Antarctic plunderfish, *Histiodraco velifer*, to bottom-walk. I hypothesized that *H. velifer* has increased flexural stiffness in its pelvic girdles compared to other notothenioid fishes, similar to what has been found in other bottom-walking animals.
CHAPTER 2

AUTOTOMY INDUCED EFFECTS ON THE LOCOMOTOR PERFORMANCE OF THE GHOST CRAB, OCEPYODE QUADRATA

2.1 Abstract

The voluntary amputation of a limb, or autotomy, is an effective defensive mechanism that allows an animal to escape aggressive interactions. However, animals may suffer longer-term costs that can affect their overall fitness. We were interested in exploring whether large numbers of limbs could mediate the impacts of limb loss. Atlantic ghost crabs (*Ocypode quadrata*) are one of the fastest terrestrial invertebrates and adopt a quadrupedal gait using their 1\textsuperscript{st} and 2\textsuperscript{nd} pair of legs while raising their 3\textsuperscript{rd} and 4\textsuperscript{th} pair of legs off the ground when running at fast speeds. This suggests that some limbs may be more important for achieving maximal locomotor performance than others. The goal of this study was to quantify the effects of limb loss on maximum locomotor performance and to determine whether crabs adjusted their gait in response to limb loss. Crabs were assigned to four different paired limb removal treatments or the control group and run in a fenced in trackway in their natural habitat. Ghost crabs were found to adjust stride kinematics in response to limb loss. We conclude that compensatory ability varies depending on the autotomized limb. For example, running speed was reduced by ~25 % with the loss of the 2\textsuperscript{nd} or 3\textsuperscript{rd} limbs. The remaining limbs were unable to compensate for the loss of either limb, either due to lack of propulsive forces produced by these limbs or issues stemming from re-coupling limb arrangements. In contrast, removal of the other limbs had no detectable effect on running speed.
2.2 Introduction

Autotomy is a defense mechanism during which the animal voluntarily sacrifices a limb in response to aggressive inter- and intraspecific events, such as predation and competition. Although autotomy may facilitate survival during these encounters, there are potential long-term costs associated with autotomy which can affect the animal’s fitness. For example, limb loss 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 an animal’s survival (Arnold, 1983; Husak, 2006; Irschick and Garland, 2001). The loss of a limb can impede locomotor performance as they must adjust their locomotor kinetics, kinematics and behavior in order to compensate for the lost limb. Several studies investigating the impacts of limb loss on the locomotor performance of animals have found that the effects vary greatly from species to species. For example, quadrupedal animals, such as dogs, must move tripodally after 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). Another quadruped, lizards, can autotomize their tails which have been shown to play a critical role for stabilization
during jumping (Gillis et al., 2009), and climbing (Jusufi et al., 2008). A recent study showed that anole lizards are capable of compensating for the tail loss kinematically; however this did negatively impact their stability when navigating on increasingly narrow surface breadths (Hsieh, 2016). The impacts of tail autotomy vary among species and may decrease (Martin and Avery, 1998; McElroy and Bergmann, 2013), increase (Brown et al., 1995; Hsieh, 2016), or have no effect on running speed (Bateman and Fleming, 2009).

It has long been hypothesized that the more limbs animals possess, the more resilient they are to limb loss (Guffey, 1999). Cockroaches, which use six limbs in locomotion, have been found to alter limb phasing, placement, as well as increase their stride lengths when losing a limb (Delcomyn, 1991a; Delcomyn, 1991b; Hughes, 1957). Several studies have investigated the effects of limb loss in arachnids. Limb loss can result in decreases in locomotor performance (Amaya et al., 2001; Brown and Formanowicz, 2012; Domínguez et al., 2016; Lutzy and Morse, 2008), reduced reproductive success (Brautigam and Persons, 2003), and lower success rates of prey capture and prey evasion (Brautigam and Persons, 2003; Wrinn and Uetz, 2008). All of these effects may be interconnected, as a study on crab spiders found indirect negative effects on locomotor performance due to poor body condition after limb loss (Lutzy and Morse, 2008). Interestingly, 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.
Anteriorly and laterally-moving animals use their limbs in different ways. For example, in anteriorly-moving animals, the contralateral hind limbs generate propulsive forces (Autumn et al., 2006; Chen et al., 2006; Demes et al., 1994; Lee et al., 1999; Sullivan and Armstrong, 1978) whereas in laterally-moving animals, the ipsilateral trailing limbs generate forces (Blickhan et al., 1993; Ting et al., 1994). Despite the different force production patterns, limbs of anterior and sideways moving animals have similar neurological coupling: ipsilateral limbs are coupled more tightly compared to contralateral limbs (Cruse, 1990; Cruse et al., 1995). As a result of these considerations, it seems reasonable to expect that these animals would respond differently to limb loss.

In this study, we investigated the impacts of limb autotomy on the locomotor performance of a semi-terrestrial decapod, the Atlantic ghost crab, *Ocypode quadrata* (Fabricius, 1787). This sideways running crab 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 change running directions within a few strides (Blickhan and Full, 1987; Blickhan et al., 1993; Burrows and Hoyle, 1973; Perry et al., 2009).

In a preliminary survey on the natural limb loss frequencies of a population of ghost crabs in Brevard County, Florida, we found that the pincher, the 3rd, and the 4th pair of limbs were the most commonly autotomized limbs, whereas the 1st and 2nd pairs of limbs where the least frequently autotomized. When running at high velocities, ghost crabs run quadrupedally on the 1st and 2nd pair of limbs while the 3rd and 4th pair of limbs are raised and do not contribute towards propulsion (Blickhan and Full, 1987; Burrows...
and Hoyle, 1973; Hafemann and Hubbard, 1969). This implies that the observed patterns of limb loss are correlated with the use of limbs during high speed locomotion, such that those most important for high-speed locomotion are less frequently autotomized. However, this has not been directly tested, nor is it known how the crabs compensate for the loss of limbs.

The goals of this study were to (1) quantify the impacts of limb loss on the locomotor performance of ghost crabs; and (2) investigate how the ghost crab compensates for limb loss in order to maintain locomotor performance. We hypothesized that limbs that are lost more frequently in nature (limbs 3 and 4) would have less impact on the running speed of ghost crabs, whereas the least frequently lost limbs (limbs 1 and 2) would have the greatest effect on locomotor performance.

2.3 MATERIALS AND METHODS

2.3.1 Animal Collection

We collected 186 ghost crabs (carapace width: 30 – 40 mm), 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). Only those animals with all limbs intact and no visible injuries were retained for the study. All crabs were housed individually in plastic containers until their release after data collection, within 18 hours after capture. All data were collected in July and August 2013 while temperatures in the trackway ranged between 22 – 27 °C.
2.3.2 Experimental Setup

Each ghost crab was randomly-assigned to one of four symmetrical limb loss treatments (Fig. 2.1A) or the control group, which had all limbs intact. 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. 2.1C). To facilitate motion tracking, we glued 1 mm diameter black glass beads to the body using cyanoacrylate glue.

All crabs were run between 10:00 and 15:00 hours along a 350 x 60 cm enclosed trackway set up on the beach (Fig. 2.1B) while filming with a high-speed camera (Photron SA-3, Photron USA Inc., San Diego, CA, USA) at 500 frames per second with a 1/2000 s shutter. The camera was positioned above the trackway to capture a dorsal view. Two simultaneous lateral views were obtained through two mirrors placed along the trackway at a 55° angle to the ground. Crabs were released once three constant-speed runs (≤ 10% fluctuations from the mean trial speed) were collected, during which they did not touch the sides of the trackway. Only the fastest running trial for each crab was retained for analysis.

2.3.3 Video Analysis

All videos were digitized using Digitizing Tools (Hedrick, 2008) in MATLAB (version R2014a, The Mathworks Inc., Natick, MA, USA), which reconstructed two-dimensional tracked points into three-dimensional coordinates using direct linear transformation algorithms. We quantified five variables to characterize the crabs’ running performance: running speed, stride length, stride frequency, duty factor, and static
stability margin. Kinematic data were filtered using a 4th order low pass Butterworth filter (cutoff frequency 40 Hz). Individual stride lengths were measured by digitizing the footfalls of the individual limbs. Mean stride frequencies were determined by dividing the number of strides by the time it took to complete these strides. Duty factor was calculated as a ratio of stance period divided by stride period.

We calculated static stability margins (SSM) to quantify the instantaneous stability of the animals during runs. We also calculated a mean SSM for frames in which one, two, three, or four feet were in contact with the ground. The SSM is the distance of the center of mass to the closest edge of the base of support, which is defined by all the limbs in contact with the ground at a given moment (Ting et al., 1994). If the center of mass falls within the base of support, the crab is considered statically stable and the SSM was assigned a value greater than zero. If the center of mass falls outside the base of support, the crab is considered statically unstable and the SSM was assigned a negative value. During these moments of static instability, if the crab continued along a predictable trajectory, then this implies the crab was relying on dynamic stability mechanisms to maintain undisturbed locomotion (Koditschek et al., 2004).
Figure 2.1: Experimental set-up used in this study. (A) A diagram of an Atlantic ghost crab, *Ocypode quadrata* (Fabricius, 1787), with paired limb autotomy treatments color-coded and labeled for identification. (B) A 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° 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 grey segment remains attached.

SSM calculations are more 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 similarity is difficult, especially in a field setting such as our study, in which stable limit cycles necessary for calculating some more established dynamic stability metrics are
impossible to achieve (Bruijn et al., 2013; Hurmuzlu and Basdogan, 1994). 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 as a metric of stability, acknowledging that it does not ideally represent the stability of our study animal, but nevertheless provides us with a useful reference for the crab’s instantaneous stability. In addition to SSM’s, we also calculated the duty factor for which 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.

2.3.4 Statistical Analysis

To address whether limb loss affects locomotor performance, we compared intact and autotomized average running speed using a mixed-model ANCOVA, with carapace width as covariate, treatment as factor, and individual as random blocking factor. Stride frequency, stride length, and duty factor were tested with multiple mixed-model ANCOVAs with average running speed as covariate, treatment and individual legs as factors, and individual as a random factor. To compare SSM’s among different limb loss treatments, we analyzed the data using a mixed model ANCOVA, with velocity as covariate, treatment as factor, and individual as 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 covariate, treatment as factor, and individual as random blocking factor. When applicable, a Tukey HSD post-hoc test was performed to determine significant differences between treatments.

All statistical analyses were performed using JMP version 10.0.2 (SAS Institute Inc., Cary, NC, USA). P-values were adjusted for multiple testing using the False Discovery Rate (Benjamini and Hochberg, 1995).

2.4 RESULTS

Of the 186 captured crabs, the final dataset comprises kinematic results from 85 ghost crabs (n=17 per treatment). Intact crabs—which served as our control—ran at 2.04 ± 0.04 m/s (~60 carapace lengths per second). Autotomy of the 2nd and 3rd limb pairs resulted in slower running speeds ($F_{4, 80} = 9.488, p < 0.001$) when compared to the control treatment (see Fig. 2.2A and Table 2.1). Running velocities in ghost crabs with the 1st and 4th pair of limbs autotomized did not differ statistically from control groups ($p > 0.05$).
**Figure 2.2:** Average instantaneous running velocity, stride length, and stride frequency of four paired limb removal treatments compared against the control treatment in 85 Atlantic ghost crabs (n=17). Removal of the 2nd and 3rd legs (yellow and blue, respectively) resulted in significant decreases to (A) running velocity when compared to the control treatment (grey). (B) Stride length and (C) stride frequency was significantly different from the control when the 1st (orange), 2nd, and 3rd legs were removed. Removal of the 4th limb (purple) had no impact on locomotor performance for any of these variables. Asterisks denote significance at $p < 0.001$. 
Table 2.1: Average velocity, stride frequency, and stride length for four limb loss treatments and the control treatment in 85 ghost crabs (n=17).

<table>
<thead>
<tr>
<th>Variable</th>
<th>Control</th>
<th>Leg 1</th>
<th>Leg 2</th>
<th>Leg 3</th>
<th>Leg 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Running Speed (m/s)</td>
<td>2.043 ± 0.035</td>
<td>1.974 ± 0.065</td>
<td><strong>1.682</strong> ± <strong>0.060</strong></td>
<td><strong>1.825</strong> ± <strong>0.049</strong></td>
<td>2.052 ± 0.058</td>
</tr>
<tr>
<td>Stride Frequency (Hz)</td>
<td>11.109 ± 0.151</td>
<td><strong>12.215</strong> ± <strong>0.186</strong></td>
<td>11.854 ± 0.210</td>
<td><strong>12.126</strong> ± <strong>0.172</strong></td>
<td>11.554 ± 0.168</td>
</tr>
<tr>
<td>Stride Length (cm)</td>
<td>18.755 ± 0.189</td>
<td><strong>16.435</strong> ± <strong>0.237</strong></td>
<td><strong>14.307</strong> ± <strong>0.159</strong></td>
<td><strong>15.250</strong> ± <strong>0.198</strong></td>
<td>17.971 ± 0.196</td>
</tr>
</tbody>
</table>

Values represent averages ± s.e.m. Significantly different from control marked with an asterisk (*) and in bold (for ANCOVA results, see Table 2).

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

Stride length and frequency were not significantly different between individual limbs, whether leading or trailing (Table 2.2), and were therefore pooled per crab to test for effects of limb loss. Duty factor however was significantly different between individual limbs, specifically between trailing and leading limbs, and the data were therefore not pooled (Table 2.2). A Tukey HSD post-hoc test revealed higher duty factors in trailing legs when compared to the leading legs for all treatments. Stride length, frequency, and duty factor were recorded for all limbs except for the 4th pair of limbs, which were infrequently used during locomotion (Figure 2.3).
Figure 2.3: Graph showing the percentage of trials which used the 4th leg during stance phase for 78 Atlantic ghost crabs. Crabs with their 4th pair of limbs removed were excluded from this graph (N = 17).

Limb loss significantly impacted stride length, frequency, and duty factor (Table 2.2). Loss of the 1st, 2nd, or 3rd limbs were associated with shorter strides (Fig. 2.2B, Tables 2.1 and 2.2) and greater stride frequency (Fig. 2.2C, Tables 2.1 and 2.2) and duty factor (Fig. 2.4, Table 2.2) when compared to the control treatments. Removal of the 4th pair of limbs had no effect on stride length, frequency, or duty factor ($p > 0.05$).
Table 2.2: Results of ANCOVAs examining impacts of limb loss treatments on locomotor performance and differences among individual legs in ghost crabs.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Treatment</th>
<th></th>
<th></th>
<th>Individual leg</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>P</td>
<td>F</td>
<td>d.f.</td>
<td>P</td>
<td>F</td>
</tr>
<tr>
<td>Stride frequency</td>
<td>&lt;0.001*</td>
<td>8.637</td>
<td>4, 80</td>
<td>0.952</td>
<td>0.333</td>
<td>5, 313</td>
</tr>
<tr>
<td>Stride length</td>
<td>&lt;0.001*</td>
<td>10.229</td>
<td>4, 80</td>
<td>0.972</td>
<td>0.175</td>
<td>5, 313</td>
</tr>
<tr>
<td>Duty factor</td>
<td>&lt;0.001*</td>
<td>18.285</td>
<td>4, 80</td>
<td>&lt;0.001*</td>
<td>64.592</td>
<td>5, 313</td>
</tr>
</tbody>
</table>

Significant P-values corrected for False Discovery Rate are in **bold** and denoted with an asterisk, *.
Figure 2.4: Average 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. Grey bars indicate legs missing due to limb autotomy. Duty factors ± 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 $1^\text{st}$, $2^\text{nd}$, and $3^\text{rd}$ legs resulted in increased duty factors ($p < 0.001$). The duty factor for the $4^\text{th}$ legs were not quantified due to infrequent use during runs.
2.4.2 Novel limb couplings as result of autotomy of the median limbs

At high running speeds, intact crabs (control) raised their 4th pair of legs and ran on six of their eight legs using an alternating tripod gait (Fig. 2.5A). These limb couplings remained the same when the 1st (Fig. 2.5B) or 4th limbs (Fig. 2.5E) were removed. However, all ghost crabs that lost the 1st limbs ran quadrupedally on their 2nd and 3rd pairs of limbs—instead of hexapedally with all six remaining limbs—using a diagonal gait, continuing to raise the 4th pair of limbs as when intact (Fig. 2.5B).

Figure 2.5: A comparison of limb couplings (red) during running for intact (A) and autotomized treatments (B-E). (A) During high speed runs, crabs often lift their fourth limb pair and run with an alternating tripod gait on only six limbs, as highlighted by the red limbs. The 4th limb associated with the traditional tetrapod is highlighted in yellow, but was infrequently used during high-speed locomotion (see text). The black circles in (B-E) correspond to the intact limb coupling pattern, to highlight changes in gait with limb loss (gray shading). Loss of the (B) 1st and (E) 4th pair 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) 2nd and (D) 3rd pair of limbs result in a novel limb coupling. Kinematic data were collected for 85 crabs (n = 17 per group).
Figure 2.6: Box plot comparing between and within limb loss treatment differences of % stride for different number of limbs in stance. Each color represents a different number of limbs in contact with the ground during a stride (One leg = orange, two legs = blue, three legs = yellow, and four legs = green). The median % stride for each limb configuration in stance is shown for each limb loss treatment, with 25% and 75% quartiles extending from the median (thick bars), as well as minimum and maximum (thin bars). A colored asterisk indicates a significant difference from control at $p < 0.05$. Differences between limb loss treatments are represented with different letters at $p < 0.05$. Control and crabs without their 4th pair of limbs mainly relied on three legs on the ground during locomotion, whereas loss of the 1st, 2nd, and 3rd pair of limbs resulted in changes from the pattern observed in the control group. Following loss of the 1st pair of limbs, ghost crabs ran primarily with two limbs on the ground.
New limb couplings were formed by all ghost crabs when the 2\textsuperscript{nd} or 3\textsuperscript{rd} pair of limbs were removed, to enable the use of an alternating tripod gait. For instance, in control runs the 1\textsuperscript{st} and 3\textsuperscript{rd} limb on one side of the animal would be part of one tripod (Fig. 2.5A), whereas if the 2\textsuperscript{nd} pair of limbs is removed, the 1\textsuperscript{st} and 3\textsuperscript{rd} limbs would each be part of different tripods (Fig. 2.5C). The 4\textsuperscript{th} pair of limbs were never used in the control, or during the 1\textsuperscript{st} and 4\textsuperscript{th} limb autotomy treatments. Infrequent use of the 4\textsuperscript{th} pair of legs as a part of a tripod gait occurred when the 2\textsuperscript{nd} or 3\textsuperscript{rd} pair of limbs were removed; otherwise, crabs ran with a quadrupedal diagonal gait.

2.4.3  \textit{Limb loss induces static instability due to a shift in patterns of limb contact during strides}

Ghost crabs altered the amount time different number of limbs are in contact with the ground when the 1\textsuperscript{st}, 2\textsuperscript{nd}, and 3\textsuperscript{rd} pair of limbs were removed. Loss of the 4\textsuperscript{th} pair of limbs was indistinct from the control runs and in both cases ghost crabs used three limbs on the ground significantly more than two or one limbs on the ground (Fig. 2.6, Table 2.3). Loss of the 2\textsuperscript{nd} and 3\textsuperscript{rd} pair of limbs resulted in significant increases in single limb use compared to control runs (Fig. 2.6, Table 2.3). In these limb loss treatments crabs very rarely placed four limbs simultaneously on the ground. Loss of the 1\textsuperscript{st} pair of limbs resulted in a pattern of limb usage distinct from other limb loss treatments. In this treatment ghost crabs infrequently used three limbs on the ground, which is a stable configuration. Instead, they relied on statically unstable configurations by increasing use of two limbs and a single limb on the ground (Fig. 2.6, Table 2.3).
Figure 2.7: Effects of limb loss on average Static Stability Margins (SSM) for all limbs and different numbers of limbs on the ground. Each color represents a different number of limbs in contact with the ground during a stride (One leg = orange, two legs = blue, three legs = yellow, and four legs = green). Black dots indicates the average for all limbs combined. The average SSM ± S.E.M is shown for each limb loss treatment. There was a significant decrease in overall SSM with the loss of the 1\textsuperscript{st} and 2\textsuperscript{nd} pairs of limbs. There were no significant changes in SSM due to limb loss for one and two limbs on the ground. Loss of the 1\textsuperscript{st} pair of limbs resulted in significantly lower SSM compared to the other treatments and the control group. Since contact of four limbs on the ground only occurred with the loss of the 2\textsuperscript{nd} and 3\textsuperscript{rd} pairs of limbs, no statistical analysis was conducted on this category. The asterisk denotes significance at $p < 0.01$. 
On average, the SSM for all treatments were negative (Table 2.4), indicating that crabs were statically unstable and relying on dynamic stability mechanisms for locomotion. Positive SSM’s were calculated only when four legs were on the ground for ghost crabs with their 3rd pair of limbs removed, or when three legs were on the ground for intact crabs or with their 2nd, 3rd and 4th pair of limbs removed (Fig. 2.7, Table 2.4). All remaining SSM’s were negative. Ghost crabs with their 1st and 4th limbs removed as well as the control group did not make contact with four limbs during any of the recorded trials.

Effects of limb loss on SSM’s showed no significant differences between limb loss treatments for one and two legs in contact with the ground (Fig. 2.7, Table 2.4). Ghost crabs with the 1st pair of limbs removed exhibited significantly lower SSM’s with three legs on the ground compared to crabs from all other treatments, as well as the control group (Fig. 2.7, Table 2.4). SSM differences among treatments with four legs on the ground were not analyzed because this was limited to a few observations (N = 12) when the 2nd or 3rd pair of limbs were removed.
Table 2.3: Average 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.

<table>
<thead>
<tr>
<th>Contact period (% Stride)</th>
<th>Treatment</th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1st</td>
<td>2nd</td>
<td>3rd</td>
</tr>
<tr>
<td>Single limb contact</td>
<td>13.1 ± 1.5</td>
<td>24.6 ± 1.4</td>
<td>28.4 ± 2.5</td>
<td>20.4 ± 2.1</td>
</tr>
<tr>
<td>Two limb contact</td>
<td>17.6 ± 1.6</td>
<td>48.6 ± 3.6</td>
<td>29.9 ± 2.9</td>
<td>27.3 ± 2.1</td>
</tr>
<tr>
<td>Three limb contact</td>
<td>23.5 ± 2.2</td>
<td>0.5 ± 0.3</td>
<td>19.8 ± 2.9</td>
<td>26.3 ± 2.6</td>
</tr>
<tr>
<td>Four limb contact</td>
<td>N/A</td>
<td>N/A</td>
<td>1.5 ± 0.7</td>
<td>1.0 ± 0.6</td>
</tr>
</tbody>
</table>

Within Treatments

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>d.f.</th>
<th>P</th>
<th></th>
<th></th>
</tr>
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<tbody>
<tr>
<td></td>
<td>40.6774</td>
<td>3, 48</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>132.571</td>
<td>3, 48</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>22.5104</td>
<td>3, 48</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>33.7662</td>
<td>3, 48</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>53.2105</td>
<td>3, 48</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
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</table>

Between Treatments

<table>
<thead>
<tr>
<th></th>
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<th>d.f.</th>
<th>P</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>13.184</td>
<td>4, 79</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>31.147</td>
<td>4, 79</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>24.439</td>
<td>4, 79</td>
<td>&lt;0.001*</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values represent averages ± s.e.m.

Significant P-values corrected for False Discovery Rate are in **bold** denoted with an asterisk, *.

Results of Tukey HSD visible in Fig. 5
**Table 2.4:** Average Static stability margins for different numbers of limbs on the ground during stance as well as overall SSM including all limbs and results of ANCOVAs determining differences in Static stability margins between Treatments.

<table>
<thead>
<tr>
<th>SSM</th>
<th>Treatment</th>
<th>mixed-model ANCOVA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
<td>1&lt;sup&gt;st&lt;/sup&gt;</td>
</tr>
<tr>
<td>Single limb contact</td>
<td>-45.105 ± 2.473</td>
<td>-47.453 ± 2.054</td>
</tr>
<tr>
<td>Three limb contact</td>
<td>6.517 ± 1.45</td>
<td>-13.943 ± 7.417</td>
</tr>
<tr>
<td>Four limb contact</td>
<td>N/A</td>
<td>N/A</td>
</tr>
</tbody>
</table>

Values represent averages ± s.e.m. Significant P-values corrected for False Discovery Rate are in **bold** denoted with an asterisk, *.

### 2.5 DISCUSSION

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 allowing animals to run in their natural habitat and on their natural substrate, we were able to measure some of the fastest running speeds for this genus.
have been recorded in quantitative studies, except an observation of *O. ceratophalma* running at 4 m/s on the deck of a ship (Burrows and Hoyle, 1973). 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 consistent, fast running speeds observed in this study.

Although any limb loss had a destabilizing effect, ghost crabs modified their limb couplings and stride kinematics to maintain fast running speeds (> 1.5 m/s). Locomotor performance was affected only by the loss of the 2\textsuperscript{nd} and 3\textsuperscript{rd} limbs, which are also the largest limbs and the only autotomy treatments that required the animal to reorganize limb couplings.

### 2.5.1 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 (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 and sped up their strides, while increasing relative ground contact time after limb loss (Fig. 2.4 and 2.5). Similar locomotor adjustments have been observed in other animals as well. After limb loss, spiders increase the stance period of the remaining legs (Foelix, 2011) and increase their stride frequency (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 hind limbs (Hsieh, 2016). Even bipedal animals alter kinematics as a response to destabilizing events. Humans who encounter slippery conditions decreased stride lengths, which places the center of mass closer to the base of support, in order to avoid falling (Myung and Smith, 1997; You et al., 2001). We believe that ghost crabs, similar to the aforementioned examples, reduced stride lengths and increased duty factors to facilitate a stable gait while increasing stride frequency to maintain high running speeds similar to the intact condition.

As ghost crabs compensated for limb loss, they also spent significantly more time in statically unstable states during stance periods (Fig. 2.6 and 2.7). In fact, our results indicate that animals without their 1st pair of limbs had no statically stable periods in their stance periods at all. Animals moving at increasingly higher velocities experienced reductions in static stability, relying on dynamic stability to bridge these moments of static instability (Ting et al., 1994). The lack of static stability only further supports that ghost crabs rely on dynamic stability, corroborating many studies in other animals (Daley et al., 2007; Full et al., 2002; Sponberg and Full, 2008; Ting et al., 1994), as well as the ghost crab (Blickhan et al., 1993; Full et al., 2002).

2.5.2 Some limbs are costly to autotomize due to differential limb functions

Despite adjusting locomotor kinematics after limb loss, ghost crabs in this study were not able to fully compensate for the loss of the 2nd and 3rd pair of limbs. This
suggests that there are differential functions in the walking limbs of ghost crabs, with these two limbs in particular having a more important locomotor function than the other limbs. It is well-known that multi-legged animals, such as crustaceans and spiders, use their limbs differently during locomotion (Blickhan et al., 1993; Clemente and Federle, 2008; Goldman et al., 2006). For instance, the main propulsive forces in spiders are provided by the posterior limbs (Ehlers, 1939; Foelix, 2011) and 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 mode of locomotion (Pond, 1975). Cellar spiders use their anterior pair of legs in a sensory capacity, feeling for disturbances ahead as well as stable footholds, while using the remaining limbs for propulsion (Pinto-da-Rocha et al., 2007). Quadrupedal animals such as horses (Heglund et al., 1982), dogs (Lee et al., 1999), and lizards (Chen et al., 2006; Foster and Higham, 2012) also use their limbs differently during locomotion: the hindlimbs generate propulsive forces, whereas the forelimbs are used to slow the animal down and for maneuvering (Chen et al., 2006; Demes et al., 1994; Foster and Higham, 2012; Lee et al., 1999; Sullivan and Armstrong, 1978).

The spare leg hypothesis—originally inspired by studies in harvestman arachnids—proposed a redundancy in limb function such that other limbs can perform the role and function of a lost limb, offsetting potential negative impacts of autotomy (Brautigam and Persons, 2003; Guffey, 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). Pincers are usually not used for walking, except at very slow speeds (Herreid and Full, 1986) or to compensate for limb
loss (Barnes, 1975). One reason why the remaining limbs were not able to fill the role of either the 2\textsuperscript{nd} or 3\textsuperscript{rd} pair of limbs may lie in the size of these limbs. The 2\textsuperscript{nd} and 3\textsuperscript{rd} pair of limbs are the longest limbs in the ghost crab and have a merus that is volumetrically bigger. The merus houses the main locomotor muscles (flexor and extensor)(Perry et al., 2009), and volumetrically larger limb segment likely also house larger muscles (Biewener and Corning, 2005). Therefore, it would be reasonable to expect that these two limbs may produce the most propulsive forces. After loss of the 2\textsuperscript{nd} or 3\textsuperscript{rd} pair of limbs, the remaining limbs can take over the locomotor function of the limb, but may not be able to reproduce the same forces. In contrast, after loss of the 1\textsuperscript{st} pair of limbs, crabs ran exclusively on the 2\textsuperscript{nd} and 3\textsuperscript{rd} pair of limbs and were able to maintain intact running speeds (Fig. 2.2A).

Loss of the 4\textsuperscript{th} pair of limbs had no measurable effect on any aspect of locomotor performance. Not only are these limbs only used at slower speeds and then raised at faster speeds (Blickhan and Full, 1987; Burrows and Hoyle, 1973), their shorter length would require a great deal of potentially destabilizing body pitch to enable these limbs to contact the ground during high speed running. In garden and crab spiders that climb up a silk thread, the shortest limb pair is moved at twice the stride frequency compared to the other limbs (Ferdinand, 1981; Jacobi-Kleemann, 1953). While the 4\textsuperscript{th} pairs of limbs participate in locomotion at slow speeds in ghost crabs, it is possible that they originally evolved to serve another purpose. *Hemigrapsus nudus*, an intertidal crab of the family Grapsidae, has smaller 4\textsuperscript{th} pairs of limbs, similar to the ghost crab, which are used when climbing (Maginnis et al., 2014). The 4\textsuperscript{th} pair of limbs alternatively have evolved into specialized swimming limbs in crabs of the family Portunidae (Hartnoll, 1971). Among garden
spiders, differentially-sized limbs have been noted to result in slower flat running speeds when compared to spiders that have limbs of similar lengths; yet the short legs give the spider better climbing abilities (Foelix, 2011; Jacobi-Kleemann, 1953). It is likely that the ghost crab shares a similar functional distinction in their limbs as *H. nudus* or garden spiders.

2.5.3 *Realignment of limb couplings correlates with decreased performance*

Another source for the performance decrements observed with the loss of the 2\textsuperscript{nd} and 3\textsuperscript{rd} leg may lie in the realignment of limb couplings. In this study, ghost crabs used an alternating tripod gait for all limb autotomy treatments except following the loss of the 1\textsuperscript{st} pair of limbs, which resulted in a quadrupedal, diagonal gait. The limbs which formed the tripods were different depending on the treatment, requiring novel limb couplings following autotomy of the 2\textsuperscript{nd} and 3\textsuperscript{rd} limb pairs (Fig. 2.2). Similar rearrangements of limb couplings have been observed in other six and eight-legged animals when middle legs are autotomized (Delcomyn, 1991a; Delcomyn, 1991b; 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 which 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). In another study, tarantulas which lost limbs rearranged their limbs similarly like the ghost crabs in our study (Wilson, 1967).
Realignment of limb couplings is thought to occur due to the lack of proprioceptive feedback from missing or damaged limbs (Delcomyn, 1991a; Delcomyn, 1991b; Evoy and Fourtner, 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; Delcomyn, 1991b). 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.

2.5.4 Conclusion

Our findings failed to support our hypothesis that based on field observations of limb loss patterns, the removal of the 3\textsuperscript{rd} and 4\textsuperscript{th} pair of limbs would have the least effect on locomotor performance. Ghost crabs are remarkably robust to limb loss, suffering a maximum locomotor decrement of only \(~25\%\). Faced with the loss of a pair walking limbs, ghost crabs compensated for limb loss by adjusting their stepping and limb cycling patterns. The 2\textsuperscript{nd} and 3\textsuperscript{rd} pair of limbs, however, appear to be the most critical 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.

Two, mutually non-exclusive possibilities as to why the 2nd and 3rd pair of limbs are important to locomotor performance include: (1) these two limbs may serve locomotor roles which can’t 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; and (2) the removal of the 2nd and 3rd pair of limbs results in novel limb couplings, which likely requires sophisticated 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.
CHAPTER 3

FEED-FORWARD CONTROL STRATEGIES ENABLE SIDEWAYS-RUNNING ANIMALS TO OVERCOME LOCOMOTOR PERTURBATIONS

3.1 Abstract

The faster an animal runs, the less time it has to adjust to and recover from sudden perturbations using a combination of neural feedback, feed-forward control, preflexes, and distributed mechanical feedback. Feed-forward control and preflexes provide rapid, immediate responses to a perturbation and therefore play a key role in initial recovery mechanisms, and have been shown to be remarkably effective when responding to sudden changes in surface properties and large, destabilizing perturbations. Slip perturbations are of interest in particular because they are one of the leading causes of accident-related fatalities among humans. While slip recovery mechanisms have been well studied in bipedal organisms, we know very little about how a multi-legged animal responds to and recovers from slip perturbations. Additionally, what we do know is limited to forward-running animals; yet, sideways running provides a unique opportunity to explore how body orientation can influence neuromechanical mechanisms of recovery. The Atlantic ghost crab (*Ocypode quadrata*) is a high-speed, sideways runner. The goal of this study was to determine how these crabs respond to an unexpected slip perturbation and to characterize their recovery mechanisms. We hypothesized that these crabs would use feed-forward control to maintain stable locomotion, similar to that observed in cockroaches, to recover from a slip perturbation. Results indicate that although ghost crabs suffered severe slips averaging 40.23 ± 3.15 % carapace width, they kept their limb
timing kinematics constant and there were no measurable running speed decrements. This suggests that ghost crabs use feed-forward control and momentum to overcome the slip-induced destabilization. Ghost crabs did decrease their stride length in the stride following the initial perturbation, however, suggesting that neurological feedback control mechanisms do play a role later in the perturbation response. Our results show that fast running speeds and rapid response due to feed-forward control mechanisms play key roles in helping ghost crabs bridge periods of extreme instability.

3.2 Introduction

Locomotor stability is constantly challenged by the dynamically changing and demanding natural environment. Failure to counteract environmental perturbations can result in instability and accidents with potentially fatal consequences. Common perturbations include uneven terrain (Daley et al., 2006; Hsieh, 2016; Sponberg and Full, 2008), changes in substrate compliance (Hsieh and Lauder, 2001; Li et al., 2012; Moritz and Farley, 2004; Spence et al., 2010), inclines and declines (Birn-Jeffery and Higham, 2014; Gabaldón et al., 2004; Goldman et al., 2006; Higham and Jayne, 2004b; Lee, 2011), obstacles (Olberding et al., 2012; Tucker and McBrayer, 2012), and changes in surface friction (Clark and Higham, 2011; Myung and Smith, 1997; You et al., 2001). For instance, animals encountering changes in substrate compliance stiffen (Daley et al., 2006; Moritz and Farley, 2004) or soften their leg spring (Wilshin et al., 2017); lizards are hypothesized to use bipedalism to negotiate obstacles (Olberding et al., 2012; Parker and McBrayer, 2016); and animals moving up an incline prolong stance periods and
decrease stride length (Foster and Higham, 2012; Goldman et al., 2006; Higham and Jayne, 2004a), or decrease stride and stance periods (Jayne and Irschick, 1999), and alter limb functions (Lee, 2011).

Stable locomotor dynamics depend on neuromechanical control mechanisms, which coordinate limb movements and allow animals to respond to external stimuli (Büschges and Gruhn, 2007; Daley et al., 2006; Full and Koditschek, 1999; Höhne et al., 2011; Nishikawa et al., 2007; Noah et al., 2004; Pearson, 1995; Pearson, 2004; Sponberg and Full, 2008). Decades of research into locomotor control mechanisms have spanned multiple disciplines, resulting in definitions and roles of feed-forward and feedback control mechanisms which differ slightly in context. In kinesiology, feed-forward control often refers to anticipated postural adjustments which are based on prior experiences (Bouisset and Zattara, 1987; Santos et al., 2010). For instance, movement of limbs causes changes in the position of the center of mass, which in turn requires postural adjustments based on learned behavior to maintain stability (Bouisset and Zattara, 1987; Hinder and Milner, 2005; Höhne et al., 2011; Lam et al., 2006; Santos et al., 2010). Anticipated adjustments are also modulated via sensory feedback, such as visual, vestibular, and somatosensory cues (Alexandrov et al., 2005; Gordon et al., 2010; Lam et al., 2006; Seidler et al., 2004). For example, cats and humans use visual feedback of an obstacle to modulate feed-forward foot placement (Drew et al., 1996; Patla and Vickers, 1997; Patla and Vickers, 2003). The neurobiological perspective of feed-forward control is similar to that used in kinesiology, and considered to be an anticipatory adjustment of motor outputs mediated by the cerebellum. Specifically, the cerebellum adjusts and optimizes motor outputs, and predicts future sensory states to minimize errors, based on prior
experiences (Bastian, 2006; Pisotta and Molinari, 2014). In contrast, comparative biomechanics refers to feed-forward control as a repeated, rhythmic signal emanating from the central pattern generator which coordinates limb movements. The timing and phasing of this signal is then adjusted by the nervous system based on sensory feedback (Cruse, 1990; Cruse et al., 1995; Full and Koditschek, 1999; Nishikawa et al., 2007; Proctor and Holmes, 2010).

For this study, “feed-forward” and “feedback” control will strictly refer to the biomechanical locomotor control system definition, unless otherwise noted. We define feed-forward to be the signals sent out by the CPG that result in rhythmic limb movement. With “feedback” we refer to afferent sensory signals and subsequent efferent signals which alter limb movements by adjusting CPG, feed-forward timing characteristics and other properties of the neuromechanical system via the nervous system (Full and Koditschek, 1999; Nishikawa et al., 2007; Proctor and Holmes, 2010).

In addition to feed-forward and feedback mechanisms, animals also rely on other control mechanisms to maintain stability, such as preflexive mechanisms (mechanical feedback) resulting from the viscoelastic properties of the musculoskeletal system (Brown and Loeb, 2000; Full and Koditschek, 1999; Jindrich and Full, 2002; Nishikawa et al., 2007; Sponberg and Full, 2008). Feed-forward control and mechanical feedback can provide immediate stabilization during perturbations, which is especially important in fast running animals, as increases in running speed also decrease the time to respond to these perturbations, requiring these mechanisms to provide stability until neurologically mediated responses can take over (Brown and Loeb, 2000; Jindrich and Full, 2002;
Sponberg and Full, 2008). For example, cockroaches which were perturbed laterally showed signs of recovery within a step due to a combination of feed-forward control, and preflexive mechanisms (Jindrich and Full, 2002).

Slipping, in particular, is a perturbation of interest in human studies. Worldwide, falls due to tripping or slipping are one of the leading causes of accidental deaths, accounting for 40% of all injury-related deaths (Rubenstein, 2006; World Health Organization, 2007). Several studies in humans have investigated how falls occur, how to recover from slipping, and how to avoid slipping in the first place (Brady et al., 2000; Cham and Redfern, 2002a; Cham and Redfer, 2002b; Marigold and Patla, 2002; Marigold et al., 2003; Myung and Smith, 1997; Pai and Patton, 1997; You et al., 2001). In humans slips usually result in backward instability, which results in corrective responses to regain balance such as swinging the arms forward or trying to retract the slipping limb (Cham and Redfern, 2002a; Marigold et al., 2003). Initiating double support by bringing the swing leg down as soon as possible is perhaps the most crucial aspect of regaining balance, as this action improves stability by widening the base of support (BOS) (Marigold and Patla, 2002; You et al., 2001).

Studies on slipping in humans are plentiful; however, far fewer studies have investigated slipping and recovery in animals (except see: Clark and Higham, 2011; Phillips and Morris, 2001; Ross, 2015; Thorup et al., 2008). Clark and Higham (2011) investigated how guinea fowl, a bipedal bird, respond to slip perturbations and found similarities in recovery strategy to those in humans, such as moving the center of mass anteriorly to regain stability. While numerous studies have documented responses and
recovery strategies in bipedal animals, we know little about how animals with more limbs (i.e., multi-legged animals) respond to slip perturbations. Multi-legged animals likely experience slip perturbations differently than bipedal animals. It has been hypothesized that animals with four or more legs in a sprawled configuration are more stable on a horizontal plane (Full and Koditschek, 1999; Kubow and Full, 1999). The sprawled posture increases the base of support of the animal, which confers greater static stability compared to bipeds and quadrupeds which have adopted upright postures.

As most animals move anteriorly through their environment, they initially sense changes in substrate properties—such as compliance, elevation, and coefficient of friction—with their anterior-most contralateral limbs or antennae. In contrast, sideways moving animals, such as crabs, perceive these substrate changes with ipsilateral limbs on the leading side of the body. This difference in which limbs perceive perturbations is interesting as the neurological coupling relationships among limbs are the same in both forward and sideways moving arthropods (Fig. 3.1; Clarac and Chasserat, 1979; Evoy and Fourtner, 1973; Vidal-Gadea and Belanger, 2013; Wilson, 1967), with tighter neurological coupling of ipsilateral limbs than contralateral limbs (Cruse and Müller, 1986; Delcomyn, 1971). Most studies investigating the effects of perturbations on multi-legged animals have focused on anteriorly moving animals (Delcomyn, 1991a; Full and Koditschek, 1999; Jindrich and Full, 2002; Revzen et al., 2013; Sponberg and Full, 2008). Whether laterally moving animals respond similarly to perturbations as anteriorly moving animals is not known.
Figure 3.1: Relationship of pattern generators in eight-legged locomotion. A diagram representing the ipsilateral (green) and contralateral (blue) relationships between limb-specific pattern generators in a sideways moving crab (A) and forward moving spider (B).

We investigated the effects of slip perturbations on the locomotor performance of a semi-terrestrial, laterally-running decapod, the Atlantic ghost crab, *Ocypode quadrata* (Fabricius, 1787). The ghost crab is one of the fastest terrestrial invertebrates (Blickhan and Full, 1987; Blickhan et al., 1993; Burrows and Hoyle, 1973; Perry et al., 2009) and can maintain locomotor speeds above 1.5 m/s even after losing two limbs (Pfeiffenberger and Hsieh, in review).

The goal of our study was to determine how sideways moving animals, such as the ghost crab respond to and recover from slip perturbations. Because of their fast running speeds, we hypothesized that ghost crabs rely on feed-forward control to
overcome slip perturbations. We predicted that timing characteristics and limb cycling patterns would remain the same between strides if crabs used feed-forward control.

3.3 MATERIALS AND METHODS

3.3.1 Animal Collection and Experimental Setup

We collected 13 ghost crabs (carapace width: 30 – 40 mm), at the Two Mile Beach unit of the Cape May National Wildlife Refuge in New Jersey, USA (National Wildlife Refuge Special Use Permit # 1406, New Jersey Division of Fish and Wildlife Permit # 1658). Only those animals with all limbs intact and no visible injuries were retained for the study. All crabs were housed individually in plastic containers until their release after data collection, within 18 hours of capture. All data were collected in July and August 2014 while temperatures on the trackway ranged between 23 – 28 °C.

We ran crabs on a 350 x 60 cm enclosed trackway set up on the beach and filmed each trial with a high-speed camera (Photron SA-3, Photron USA Inc., San Diego, CA, USA) at 500 frames per second and a 1/2000 s shutter (Fig. 3.2A). The camera was positioned above the trackway to acquire a dorsal view. Two lateral views where obtained through two mirrors placed along the trackway at a 55° angle to the ground. A polished ceramic tile (20 cm x 15 cm) was placed in the middle of the field of view to elicit slipping. To facilitate motion tracking, we glued 1mm black glass beads using cyanoacrylate glue to the carapace (Fig. 3.2B). One to three runs were collected for each crab. Only runs in which the animal ran from one end of the trackway to the other, did not touch the sides of the trackway, and during which all limbs of the animal made
contact with the tile were retained. In order to characterize slip recovery during maximum performance, only the fastest run for each animal was included in the analysis. Crabs were released upon completion of data collection.

3.3.2 Data Analysis

All videos were digitized using Digitizing Tools (Hedrick, 2008) in MATLAB (version R2014a, The Mathworks Inc., Natick, MA, USA), which reconstructed tracked points into three-dimensional coordinates using direct linear transformation. We quantified five kinematic variables: running speed, stride length, stride frequency, duty factor, and the phase relationship of each limb in reference to the 3rd leading leg. Position data were filtered using a 4th order, low pass Butterworth filter (cutoff frequency 40 Hz). Running speed was calculated by numerically evaluating the first derivative of splined, filtered position data. Stride lengths were measured by digitizing the footfall positions of the individual limbs. Mean stride frequencies were determined by dividing the number of strides by time. Duty factor was calculated as the fraction of a stride during which each limb was in contact with the ground. These variables were measured for three strides: 1) the stride before the crab made contact with the tile ("control"), which served as our control reference in this study, 2) the stride in which the crab made initial contact with the tile ("initial slip"), and 3) the stride after initial contact ("secondary slip"). Whereas the control stride represented unperturbed running, both the initial and secondary slip strides were perturbed strides. We also quantified the slipping distance of each limb while in contact with the ceramic tile.
Figure 3.2: Experimental trackway (A) and illustration of Atlantic ghost crab (B). (A) An illustration of the experimental trackway with a slippery tile (20 cm x 15 cm) placed in the middle of the trackway. A high-speed camera captures a dorsal view and two lateral views through two mirrors positioned at 55° to ground. (B) A diagram of an Atlantic ghost crab, *Ocypode quadrata* (Fabricius, 1787), showing the alternating tetrapods (blue and orange) used in locomotion and the leg numbering scheme used in this study.

At the high running speed we investigated here, crabs raised their 4<sup>th</sup> legs such that they ran with an alternating tripod—rather than tetrapod—gait (Fig. 3.2B). As a result, depending on limb placement, either one or two limbs would contact the tile at initial slip. To facilitate analyses, we separated all runs into single or double contact trials based on the number of limbs contacting the tile at initial slip. In single contact trials, the leading 2<sup>nd</sup> limb made contact with the tile, whereas in double contact trials, the 1<sup>st</sup> and 3<sup>rd</sup> leading limbs made contact with the tile.
3.3.3 Statistical Analysis

We compared running speed of each animal between strides using a mixed-model ANCOVA, with carapace width as covariate, stride as factor, and individual as a random blocking factor. Stride frequency, stride length, duty factor, limb slip distances, and limb phasing were tested with mixed-model ANCOVAs with average running speed as covariate, stride and individual legs as factors, and individual as a random factor. All data were pooled if there were no differences between single and double contact trials.

All statistical analyses were performed using JMP Pro version 13 (SAS Institute Inc., Cary, NC, USA). P-values were adjusted for multiple testing using the False Discovery Rate (Benjamini and Hochberg, 1995).

3.4 RESULTS

3.4.1 Running speed remained constant despite large slip distances

The average running speed of ghost crabs during the initial slip stride and the secondary slip stride did not significantly differ from control ($F_{2,11} = 1.660, P = 0.211$; Table 3.1, Fig. 3.3). We also observed no falls or sudden stops as a result of the slip perturbation.

Upon encountering the perturbation, limbs within single contact runs slipp ed an average of $44.64 \pm 6.21\%$ carapace width, and limbs within double contact runs slipped $38.19 \pm 3.61\%$ carapace width. We found no significant differences in limb slip distances for limbs between the two initial limb contact conditions (single vs. double; $F_{5}$,
52 = 3.422, \( P = 0.0095 \), Tukey HSD post-hoc inconclusive), and within each tripod of single or double initial limb contact conditions (Fig. 3.4; single: \( F_{5,38} = 1.2739, P = 0.296 \); double: \( F_{5,14} = 3.197, P = 0.0393 \)).

**Table 3.1:** Results of ANCOVA analyses on average running speed, stride frequency, stride length, and duty factor for three strides in 13 Atlantic ghost crabs.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Initial</th>
<th>Secondary</th>
<th>d.f.</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Speed (m/s)</td>
<td>2.03±0.05</td>
<td>2.03±0.05</td>
<td>1.99±0.06</td>
<td>2, 24</td>
<td>1.66</td>
<td>0.2112</td>
</tr>
<tr>
<td>Stride frequency</td>
<td>12.58±0.27</td>
<td>12.60±0.32</td>
<td>13.90±0.36</td>
<td>2, 97</td>
<td>1.314</td>
<td>0.2733</td>
</tr>
<tr>
<td>Stride length</td>
<td>165.76±2.91</td>
<td>164.06±2.85</td>
<td>152.73±3.23</td>
<td>2, 97</td>
<td>16.197</td>
<td><strong>0.0001</strong></td>
</tr>
<tr>
<td>Duty factor</td>
<td>0.26±0.01</td>
<td>0.29±0.02</td>
<td>0.26±0.01</td>
<td>2, 97</td>
<td>0.215</td>
<td>0.2154</td>
</tr>
</tbody>
</table>
Figure 3.3: Average running speed of Atlantic ghost crabs for three strides. The average running speed (solid line) ± S.E.M. (colored area) over the % time frame of three strides, Control (green), Initial slip (blue), and Secondary slip (yellow). Atlantic ghost crabs did not experience any changes in running speed due to the slip perturbation.
Figure 3.4: Average individual limb slip distance for each initial limb condition.

Ghost crabs experienced considerable slipping, ranging from 2% to 127% carapace width. Overall, limb slippage averaged at 39.74 ± 3.15 % carapace width. All limbs (in red), regardless of the initial contact limb, experienced slipping. The 4th limbs (in grey) were not used while running along the trackway (arrow indicates running direction).

3.4.2 Perturbation had no effect on kinematic timing variables

There was no difference in stride frequency or duty factor among the three strides as ghost crabs traversed the slip surface (Table 3.1, Fig. 3.5A, C), however they did
reduce their stride lengths by 7% during the secondary slip stride—the stride immediately following initial contact with the slippery surface (Table 3.1, Fig. 3.5B).

We found that trailing limbs were more tightly coupled compared to the leading limbs. Leading leg 1 made contact with the ground significantly later than leading leg 3 during all three analyzed strides (Table 3.2, 3.3, and Fig. 3.6). In contrast, trailing limbs exhibited tight coupling, as the average limb phases for trailing legs 1 and 3 were not statistically different from each other, making ground contact nearly simultaneously (Tables 3.2, 3.3, and Fig. 3.6).

Although we detected a shorted stride length for the secondary slip stride, limb phasing patterns were not significantly different from control (single contact: $F_{1, 19} = 0.608, P = 0.445$; double contact: $F_{1, 43} = 0.243, P = 0.625$; Fig. 3.6A, B).

**Table 3.2:** Average limb phases ± s.e.m. for three strides in 13 Atlantic ghost crabs, using the third leading limb as the reference.

<table>
<thead>
<tr>
<th>Initial single</th>
<th>1st Leading</th>
<th>2nd Leading</th>
<th>1st Trailing</th>
<th>2nd Trailing</th>
<th>3rd Trailing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.13±0.02</td>
<td>N/A</td>
<td>N/A</td>
<td>0.03±0.03</td>
<td>N/A</td>
</tr>
<tr>
<td>Initial</td>
<td>N/A</td>
<td>0.48±0.03</td>
<td>0.52±0.03</td>
<td>N/A</td>
<td>0.54±0.04</td>
</tr>
<tr>
<td>Secondary</td>
<td>0.12±0.03</td>
<td>N/A</td>
<td>N/A</td>
<td>-0.03±0.02</td>
<td>N/A</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Initial double</th>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>N/A</td>
<td>0.52±0.01</td>
<td>0.49±0.02</td>
<td>N/A</td>
<td>0.46±0.02</td>
</tr>
<tr>
<td>Initial</td>
<td>0.13±0.02</td>
<td>N/A</td>
<td>N/A</td>
<td>0.01±0.02</td>
<td>N/A</td>
</tr>
<tr>
<td>Secondary</td>
<td>N/A</td>
<td>0.57±0.01</td>
<td>0.45±0.02</td>
<td>N/A</td>
<td>0.43±0.02</td>
</tr>
</tbody>
</table>
Table 3.3: Results of mixed-model ANCOVA’s comparing limb phasing within and between strides of single and double contact runs for 13 Atlantic ghost crabs.

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>d.f.</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Within stride</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single contact</td>
<td>185.487</td>
<td>5, 27</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Double contact</td>
<td>258.7498</td>
<td>5, 66</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td><strong>Between strides</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single contact</td>
<td>0.608</td>
<td>1, 19</td>
<td>0.445</td>
</tr>
<tr>
<td>Double contact</td>
<td>0.243</td>
<td>1, 43</td>
<td>0.625</td>
</tr>
</tbody>
</table>
**Figure 3.5:** Average stride frequency, stride length, and duty factor of 13 ghost crabs. Atlantic ghost crabs. Stride frequency and duty factor remained statistically similar throughout the three recorded strides, whereas stride lengths were reduced by 7% after encountering the slip perturbation.
**Figure 3.6:** Polarplots with the average limb phases ± s.e.m. for three strides in the Atlantic ghost crab indicating differences in limb coupling of leading and trialing limbs. Limb phases were calculated based on a reference leg (3rd leading leg in red) which was set as zero. Warmer colors (red, orange, and yellow) and cooler colors (blue, teal, and purple) represent the different tripods. Strides are represented such that the timing of strides is ordered from the outside-in. Runs were divided according to initial limb contact with either the 2nd limb (A, N = 4) or the 1st and 3rd limbs (B, N = 9). The 1st leading limb was significantly different from the other limbs in its tripod, regardless of initial limb condition. For initial limb condition with the 1st and 3rd limbs leading 2nd limb was significantly different form the other limbs in its tripod.
3.5 DISCUSSION

Ghost crabs experienced substantial limb slipping due to the perturbation, yet none fell or stopped running. Instead, ghost crabs maintained the same running speed regardless of the presence or absence of a perturbation. Locomotor kinematics were unchanged except for stride length, which decreased in the secondary stride. Our findings show that ghost crabs use momentum and constant limb cycling to navigate over the slip perturbation without suffering any quantifiable locomotor performance decrements.

3.5.1 Constant cycling of limbs for immediate stabilization

Feed-forward control and mechanical feedback are used to initially counteract perturbations (Brown and Loeb, 2000; Full and Koditschek, 1999; Kubow and Full, 1999; Spence et al., 2010; Sponberg and Full, 2008). This is especially important at fast running speeds when the available time for the nervous system to respond to perturbations decreases (Brown and Loeb, 2000; Full and Koditschek, 1999; Kubow and Full, 1999; More et al., 2010; Spence et al., 2010; Sponberg and Full, 2008). Whether the nervous system of animals can respond in a timely matter to perturbations depends on the muscle and neural physiology of the animal, as neural pathways and muscle response times are different between species (More et al., 2010). The scaling of neural responses with body size show that larger animals have greater latency periods compared to smaller animals, making it more difficult for larger animals to respond quickly to perturbations, likely resulting in slower movements that are more carefully planned out (More et al., 2010). For instance, the femoral extensor muscle in cockroaches that generates most of
the limb extension movement, takes between 16 and 20 ms to respond to stimuli (Ahn et al., 2006). Kinematic analyses and electromyographic recordings of muscle activity during obstacle negotiation showed cockroaches with stance times of ~40 ms—about twice as long as their muscle response time—used feed-forward control to overcome (Sponberg and Full, 2008). Response times of ghost crab muscles are not known; however, because ghost crabs are larger than cockroaches, we can reasonably assume that they have similar, or longer, sensory latency periods than cockroaches (More et al., 2010). Ghost crabs in this study had average step durations of 22 ± 3 ms, suggesting that they likely used feed-forward control to overcome the slip perturbation. To verify this finding, quantifying latency periods with electromyography (EMG) would be necessary, but is beyond the scope of this study.

The maintenance of constant limb cycling, when faced with perturbations to locomotor systems, have been used as kinematic indicators of feed-forward control (Full and Koditschek, 1999; Proctor and Holmes, 2010; Spence et al., 2010), as exemplified by cockroaches moving over an elastic surface while maintaining constant forward velocity and stride frequency at pre-perturbation levels (Spence et al., 2010). Likewise, in another study, rapidly laterally perturbed cockroaches did not alter their gait kinematics, or limb phasing, and did not require step transitions to stabilize, suggesting mechanical and feed-forward mechanisms were sufficient for recovery (Jindrich and Full, 2002). In contrast, cockroaches experiencing a sustained lateral perturbation decreased their stride frequency 110 ms after experiencing the perturbation, with mechanical feedback providing stability until sensory feedback ultimately decreased stride frequency (Revzen et al., 2013). Ghost crabs in our study maintained constant stride frequency, limb phasing, and duty factors
(Fig. 3.5, 3.6), which we conclude to be indicators of feed-forward control, especially when taking into account the short stance phases (22ms). However, taken alone, kinematic data do guarantee that constant limb cycling truly is caused by feed-forward control, as the animal may be altering muscle activity patterns to adjust limbs to maintain constant limb cycling in anticipation of the perturbation. Anticipation can be mediated using visual feedback well before contacting the actual perturbation (Drew et al., 1996; Patla and Vickers, 1997). In another example, guinea fowl experiencing sudden drops maintain constant limb cycling due to a proximo-distal gradient of control: proximal joints are feed-forward controlled and distal joints exhibit increased load sensitivity and high gain proprioceptive feedback (Daley et al., 2007).

Decreasing stride length is a common strategy used to maintain a stable gait. For instance, humans will anticipate slippery surfaces and shorten stride lengths and stance durations while in contact with the slippery surface in order to prevent falling (Bhatt et al., 2005; Bhatt et al., 2006; Cham and Redfern, 2002b; Marigold and Patla, 2002). Other studies investigating animal locomotion found that tail loss in lizards (Hsieh, 2016; Martin and Avery, 1998) or limb loss in crabs (Pfeifferberger and Hsieh, in review) can result in decreased stride length in order to maintain a stable gait. The observed decrease in stride length in the secondary slip stride in our study could have been due to several reasons. First, the slippery surface makes it difficult for the crab to generate propulsive forces or braking forces. As a result, ghost crabs could conceivably take more, shorter steps as their feet slipped. However, our data show that stride frequency did not change during a slip. Second, the slipping may have caused upwards pitching of the leading limbs causing decreased stride lengths either from the trailing limbs contacting the
ground sooner or the leading limbs being unable to extend as far in front of the body with each step. Unfortunately, we were unable to accurately quantify body pitch, roll, and yaw due to body rotation being subtle enough to be obscured by digitizing error. Lastly, the observed decreases in stride length may have been a neurological response to the slippery surface in order to maintain stable locomotor dynamics. While it is possible that some or none of these scenarios played a role in our observed changes in stride length, we believe that stride lengths were decreased as a neurological response (sensory feedback) to the slip perturbation. Between the initial slip and secondary slip stride, ghost crabs had between 71 – 83 ms to respond to the slip perturbation, which should be enough time for sensory feedback, even if their latency periods are twice as long as what has been measured in cockroaches (Ahn et al., 2006). EMG would be necessary to definitively support or reject this conjecture.

3.5.2 Momentum enables ghost crabs to overcome slip perturbations

Our study demonstrates the ability of ghost crabs to overcome slip perturbations using momentum, as has been reported elsewhere (Full and Tu, 1991; Kram et al., 1997; Pfeifferberger and Hsieh, in review; Sponberg and Full, 2008; Ting et al., 1994). Ross (2015), however, reports significant decreases in running speeds among ghost crabs following a slip perturbation. Notably, ghost crabs in that study ran at slower speeds (25 carapace widths/s) along a shorter trackway (50 cm long) compared our study (60 carapace widths/s, 350cm trackway length). This further supports our finding that momentum is used to overcome slip perturbations because higher running speeds are
associated with higher momentum. These findings also suggest that ghost crabs are less susceptible to slip perturbations at higher running speeds, similar to guinea fowl and humans. Guinea fowl that ran over slippery surfaces at speeds above 3 m/s avoided falls and exhibited higher tolerance to limb parameters which caused falls when moving at slower speeds (Clark and Higham, 2011). Likewise, humans exhibit greater tolerance against slip-induced backward instabilities at higher walking speeds compared to when moving at slower walking speeds (Bhatt et al., 2005). Theoretical models, such as the spring-mass model (Seyfarth et al., 2002; Seyfarth et al., 2003), and the lateral leg spring model (Schmitt and Holmes, 2000) also support these empirical findings, and predict animals which move at high running speeds should exhibit an increased tolerance to perturbations (Holmes et al., 2006; Schmitt and Holmes, 2000; Seyfarth et al., 2002).

3.5.3 Limb phasing less coordinated in leading limbs

While analyzing limb phases, we observed differing limb coupling relationships between the leading and trailing limbs. Phasing relationships indicating tighter coupling among ipsilateral than contralateral limbs have been well documented in several species of arthropods, including stick insects (Bässler and Büschges, 1998; Cruse et al., 1995; Rosenbaum et al., 2010), cockroaches (Delcomyn, 1971; Hughes, 1957), spiders (Spagna and Peattie, 2012; Wilson, 1967), and crabs (Barnes, 1975; Burrows and Hoyle, 1973; Clarac et al., 1987; Evoy and Fourtner, 1973). The pattern we observed showed the expected tight limb coupling of ipsilateral limbs only among the trailing limbs of the crabs, whereas leading limbs did not exhibit this coupling relationship (Fig. 3.6). In the
leading limbs, leading leg 1 made contact with the ground significantly later than leading leg 3, its ipsilateral counterpart. This pattern was observed for all perturbed and unperturbed strides, indicating that this is not caused by the slip perturbation but rather represents the normal limb coupling relationship in ghost crabs. Burrows and Hoyle (1973) mentioned that phase relationships between limbs became inconsistent at high speeds for the horned ghost crab, *Ocypode ceratophalma*, and Barnes (1975) noted that the Atlantic marsh fiddler crab, *Uca pugnax*, had different dominating stepping sequences between trailing and leading limbs: the leading limbs showed the typical alternating tetrapod sequence, whereas trailing limbs diverged from the normal pattern. While differences in the phase relationships between leading and trailing limbs have been previously observed, the underlying reason is not known. We offer potential explanations for our observations below.

One explanation may lie in the primary locomotor functions of the limbs. The main function of the leading limbs is to decelerate the animal during runs (Blickhan and Full, 1987; Burrows and Hoyle, 1973). Trailing limbs provide the propulsive forces ghost crabs use to attain fast running speeds, exhibiting significantly higher muscle activity compared to the leading limbs (Burrows and Hoyle, 1973). Anterior-posteriorly moving animals also use their trailing limbs (i.e., hindlimbs), to generate propulsive forces (Demes et al., 1994; Lee et al., 1999). It is possible that ghost crabs prioritize coordination of their trailing limbs, as pushing off with two limbs could result in instability if not timed properly. In contrast, a slight coordination mismatch in leading limbs would not affect propulsive force generation and likely minimally affect locomotor stability.
Another explanation could be that the leading 1st limb is not functionally as important in locomotion as the other leading limbs. We base this assumption on a prior study by the authors, which identified the 1st and 4th pair of limbs to play a lesser role in locomotion compared to the 2nd and 3rd pair of limbs (Pfeiffenberger and Hsieh, in review). It is possible that coordination of a functionally less important limb is not essential to the locomotor performance of ghost crabs.

3.5.4 Conclusion

Our study shows that ghost crabs use momentum and constant limb cycling to navigate over the slip perturbation without suffering any quantifiable locomotor performance decrements. Whether feed-forward control is used to overcome the slip perturbation cannot be established with certainty in this study, which would require observations of muscular or neural activity. However, the data strongly indicate that ghost crabs use feed-forward control, as they exhibited very short stance periods of 22 ms, which likely was not enough time for neural responses to the perturbation to become effective. Based on these findings, we confirm our hypothesis which stated that ghost crabs use feed-forward control to overcome slip perturbations. Future directions exploring the muscle activity of ghost crab limbs or describing the neural pathways of their locomotor muscles could give definite answers as to whether these animals employ feed-forward control to overcome slip perturbations.
CHAPTER 4

COMPARISON OF PELVIC FIN MORPHOLOGY ACROSS WALKING AND NON-WALKING ANTARCTIC NOTOTHENIOIDS

4.1 Abstract

Many aquatic animals interact directly with the substrate for locomotion. Among them are several species of fish that have evolved distinct features in their pelvic and pectoral fins to move on the bottom of the ocean. One of them is the Antarctic plunderfish, Histiodraco velifer, the only known member of its suborder Notothenioidei that bottom-walks. H. velifer uses its pelvic fins to punt along the substrate, however what features allow it do this behavior and why other notothenioids cannot is unknown. The goals of this chapter were to explore the morphological features of the pelvic girdle in this H. velifer, by comparing the morphology and material properties of H. velifer to those of four other notothenioids. Using micro CT scans and material testing, we determined the flexural stiffness of the fin rays as well as the bone mineral density of the pelvis and the fin rays.

We found that H. velifer had high flexural stiffness in its fin rays, comparable to what has been observed in bottom-walking rays and skates. H. velifer however did not have the highest material stiffness of the observed pelvic fin rays, indicating they achieve high flexural stiffness by the morphological features of their fin rays. Shape analysis of their pelvises exhibited differences in bone mineral densities, revealing radiating mineralization patterns, which may serve as struts to support pelvic musculature.
4.2 Introduction

Terrestrial legged locomotion evolved 385 – 359 million years ago, when fish first began leaving water to invade land (Pierce et al., 2013; Shubin et al., 2006). To be able to move on land, early tetrapods had to be able to support their body in a gravity dominated environment (Pierce et al., 2013; Shubin et al., 2006). Changes in their appendicular skeleton, including robust limbs, digits, and a broad pelvis, provided support to the body outside of water. These limbs had to interact with the ground, pushing against it to propel the body forward, while also supporting the body. Regardless of the number of limbs an animal possesses, all terrestrial animals use the same principles to generate propulsive and braking forces, forces generated by the limb musculature.

Aquatic animals such as fishes, reptiles, whales, pinnipeds, and sirens rely on water’s high viscosity in order to generate propulsive forces with their appendage. These forces are generated by oscillating their appendages, undulating their body, or both (Drucker and Lauder, 2002; Tytell and Lauder, 2004). Yet, many aquatic animals interact directly with the substrate for locomotion. Many turtles, such as snapping turtles (Willey and Blob, 2004), and red-eared sliders (Mazouchova and Hsieh, unpublished) have been described to use bottom-walking in addition to regular swimming. Other vertebrates, such as salamanders (Azizi and Horton, 2004), newts (Ashley-Ross et al., 2009), pinnipeds (Fish et al., 1988), and hippopotamuses (Coughlin and Fish, 2009) have also been described to walk on the bottom of lakes, rivers, and seas. Invertebrates, such as octopuses and crabs (Martinez et al., 1998), also use their appendages to bottom-walk. Elasmobranchs, such as benthic batoids (stingrays and skates), also bottom-walk on the
substrate. By moving their pelvic fins synchronously, an act called “punting”, batoids are able to generate propulsive forces by pushing off the substrate and then gliding with their enlarged pectoral fins (Macesic and Kajiura, 2010; Macesic and Summers, 2012). If the fins were to move asynchronously, i.e. in an alternating pattern, then this movement could be called “walking” (Macesic and Kajiura, 2010; Martinez et al., 1998). Epaulette sharks use their pectoral and pelvic fins to move on the sea bottom (Goto et al., 1999; Pridmore, 1994). They alternate their limbs to walk, similar to how terrestrial tetrapods would alter their limb movements (Goto et al., 1999; Pridmore, 1994). Several species of teleost fish, spanning different orders, also use their pectoral and pelvic fins to move along the bottom, with some species being capable to move onto land and even climb up structures (Kawano and Blob, 2013; Pace and Gibb, 2009; Wicaksono et al., 2017), such as mudskippers and the walking cavefish (Flammang et al., 2016; Wicaksono et al., 2017).

Fish that walk on substrate, either on land or underwater, are of particular interest to the field of functional morphology due to the relationship (“deep homology”) between fish fins and terrestrial tetrapod limbs (Flammang et al., 2016; Shubin et al., 2009; Standen et al., 2016). The components that interact with the substrate in bottom walking fish are the pectoral and pelvic fins. These fins are comprised of soft and spinous fin rays, called lepidotrichia. Each lepidotrichia has a dorsal and ventral component, called hemitrichia. The two hemitrichia are held together by connective tissues and elastic fibers, allowing the fin to deform when loaded, and then return to its original shape when unloaded (Alben et al., 2007; Taft et al., 2008). Proximal regions of the lepidotrichia are unsegmented and usually thicker, the attachment area for muscles and ligaments which
control the fin ray (Alben et al., 2007; Flammang et al., 2013; Lauder et al., 2011; Taft and Taft, 2012). Distal regions of lepidotrichia are usually segmented and fan out. The fins of walking fishes possess morphological differences which aid them in pushing against the substrate. For instance, frogfish (Antennariidae), use their modified, robust pectoral fins, which resemble jointed arms and hands (Arnold and Pietsch, 2012), and pelvic fins to walk along the seafloor in a tetrapod-like manner (Pietsch and Grobecker, 1987). Searobins (Triglidae), use the first three fin rays of their pectoral fins to move along the bottom of the seafloor, simultaneously using the many sensory neurons in their fin rays to detect prey (Jamon et al., 2007; Renous et al., 2000). They move their pectoral walking rays in a metachronal pattern, similar to slow moving crabs or cockroaches (Jamon et al., 2007; Martinez et al., 1998; Renous et al., 2000). The only teleost fishes we know of that use pelvic fins exclusively to bottom-walk are flying gurnards (Dactylopteridae), (Nelson, 1994; Poss and Eschmeyer, 1998), and the Seamoths (Pegasidae) which are heavily armored fish who use their long and slender pelvic fins to walk along the sea floor (Vincent, 1997). Mudskippers (Gobiidae) are amphibious fish and have evolved to traverse in and out of water. They use their pectoral fins, which have a “shoulder” and “intra–fin” joint, to vault their body forward, while using their pelvic fin as a support structure on land (Kawano and Blob, 2013; Pace and Gibb, 2009).

Changes in pectoral and pelvic fins may be the most evident external changes in morphology; however, the underlying, internal support structures are important for locomotion as well (Shubin et al., 2006; Shubin et al., 2009; Standen et al., 2014; Standen et al., 2016). For instance, movements of the pelvic fin rays are generally actuated by six muscles in teleosts, which originate on the pelvic plate (Winterbottom, 1973; Yamanoue
et al., 2010). To move the pelvic fins, the pelvic plate must be able to support contractions of the muscles that actuate the fin rays. The morphology of the pelvic girdle has been shown to be an excellent predictor of what type of locomotion terrestrial vertebrates use (Ekstrom and Kajiura, 2014; Lovejoy et al., 2009). Aquatic animals do not experience the same stresses in their skeleton due to the simulated microgravity of water compared to terrestrial animals, making it unlikely for teleosts to display this relationship (Ekstrom and Kajiura, 2014). Pelvic girdle shapes were found to correlate with locomotor mode in batoids, in some ways analogous to terrestrial vertebrate girdles, such as a broader, anterior-posterior expansion of the pelvis, and elongated lateral processes (Ekstrom and Kajiura, 2014). Whether bottom-walking teleosts have specific pelvic morphologies due to their interaction with the substrate is not known.

Material properties are an essential factor dictating the function of skeletal elements (Currey, 2002; Ennos, 2012; Vogel, 2003). As the fish moves along the bottom, it exerts forces onto the substrate, resulting in ground reaction forces that result in forward movement. Similar to terrestrial animals, the skeleton of the fish must be able to withstand these forces. In the case of bottom-walking fish, the fin rays must withstand bending forces, and they must do so every time the fin cycles and contacts with the ground (Ennos, 2012; Vogel, 2003). This requires the material also to be resilient to cyclical loading. Failure to withstand these forces can cause a catastrophic failure in the structure, i.e. breaking, or deforming the structure to a point where it does not return to its original shape (Biewener and Corning, 2005; Currey, 2002; Ennos, 2012; Vogel, 2003). Physical variables of interest that measure and describe the material properties of bending skeletal structures are flexural stiffness, the Young’s modulus, and the second moment of
area. Flexural stiffness is a measure of rigidity and describes how resistant a structure/material is to bending. The Young’s modulus, or elastic modulus, describes the material stiffness and is based on the relationship of stress and strain in solid materials. The second moment of area measures a shape’s resistance to deformations. These three variables share a close relationship, as flexural stiffness is the product of the Young’s modulus and the second moment of area (Ennos, 2012; Vogel, 2003). Due to this relationship, biological structures can alter their flexural stiffness by altering either the Young’s modulus, the second moment of area, or both.

In this study, I investigated seafloor locomotion in the bottom-walking Antarctic plunderfish, Histiodraco velifer, which uses its pelvic fins to punt against the substrate (Fig. 4.1). It is the only known member of its family (Artedidraconidae) and suborder (Notothenioidei) that walks on the bottom of the ocean (Janssen et al., 1993). Notothenioids live in Antarctic waters and are predominantly associated with the benthos, as they lack swimbladders and are negatively buoyant (Eastman et al., 2014; Lombarte et al., 2003; Near and Cheng, 2008). They also have greatly reduced skeletal density (about 3% of body weight), and feature persistent cartilage (Eastman et al., 2014), both of which may be adaptations to increase their buoyancy (Eastman and DeVries, 1982; Eastman et al., 2014). The pectoral girdle in some species, such as Chionodraco aureus, have been found to have large amounts of cartilage, being covered by a thin layer of bone (15 μm) accounting for 3% of the entire pectoral girdle (Eastman et al., 2014), yet little is known about the composition of the pelvic girdle in these fish.
Acquisition of live animals for study is near impossible, and even procuring preserved specimens can be a challenge due to the logistics of sampling in their geographic isolation. Using micro-source computed tomography (μCT) scanning, material testing, and the principles of beam theory, we determined the morphological and biomechanical characteristics of the pelvic girdle in *Histiodraco velifer* and how they relate to bottom-walking. We compared the pelvic plates and fin rays of *H. velifer* to four other benthic Antarctic notothenioids, which do not bottom-walk. We hypothesized that *H. velifer* will have higher bone density in the pelvis and fin rays, as well as fin rays with higher Young’s modulus and flexural stiffness, relative to four other species of notothenioids.
3.6 Materials and methods

3.6.1 Specimens

For this study, five species from the suborder Notothenioidei (Histiodraco velifer, Akarotaxis nudiceps, Gymnodraco acuticeps, Chaenodraco wilsoni, and Trematomus bernacchii) were procured from the personal collection of Dr.’s Christina Cheng and Art DeVriess from the University of Illinois, Urbana-Champaign (Fig. 4.2). All specimens were stored in 75% ethanol at 4 °C and were never fixed in formalin. Specimens were collected during several field seasons in Antarctica between the years 2008 and 2015.

3.6.2 Video recording and analysis

One Histiodraco velifer was captured by Dr. Paul Cziko in Antarctica in the winter of 2015/16 and placed in a flow-through tank at McMurdo Station. One video (30 frames per second, ventral view, video recording device unknown) was captured of the animal walking on the bottom of the tank on top of a 1cm by 1cm grid. We analyzed the video for basic locomotor kinematic variables (speed, stride frequency, distance, sweeping angle of fin, and duty factor) using Digitizing Tools (Hedrick, 2008) in MATLAB (version R2014a, The Mathworks Inc., Natick, MA, USA).
Figure 4.2: Phylogeny of the Suborder Notothenioidei, down to the family level. The shaded area indicates fish families belonging to the Antarctic clade of Nototothenioidei. The asterisk indicates the fish family with the only known species of bottom walking Nototothenioidei. The colored boxes indicate families of fish which are represented in this study: Histiodraco velifer (Artedidraconidae, red), Akarotaxis nudiceps (Bathydraconidae, blue), Gymnodraco acuticeps (Bathydraconidae, blue), Trematomus bernacchii (Notothenidae, green), and Chaenodraco wilsoni (Channichthyidae, yellow).

3.6.3 CT imaging and reconstruction

The pelvises of each specimen were dissected and extracted, then stored in 75% ethanol prior to scanning. For scanning, the pelvises of each fish were wrapped with 70% ethanol soaked gauze, then securely positioned inside a 5 x 5 cm cylinder. The pelvises
were micro-CT scanned at the Temple University micro-CT Core facility using a Bruker Skyscan 1172 micro CT scanner (Bruker, Kontich, Belgium). The pelvises were scanned at a resolution of 26.7 microns, at 89 kV and 112 uA, with an exposure of 350 ms and a 0.5 mm Aluminum filter.

All micro CT scans were reconstructed in NRecon ver. 1.7.1.0 (Bruker, Kontich, Belgium) with corrections for ring-artifacts, post-alignment, and beam-hardening.

3.6.4 Bone mineral density conversion

The output of CT scans is given in grey values ranging from 0 (black, lowest density) to 255 (white, highest density). While these values show differences of density within a scan, they are unreliable for comparisons across multiple scans. We placed two objects of known volume and bone mineral density (BMD) (0.25 g/cm$^3$ and 0.75 g/cm$^3$) into the scanning container such that they would not obstruct scans of the pelvises. We determined the relationship between BMD and grey values for our CT scan using a linear regression, with grey values as the dependent variable and BMD as the independent variable. Using this relationship, we could convert the grey values measured for the pelvis and fin rays into BMD.

3.6.5 Segmentation of CT scans

Using 3D Slicer ver. 4.6 (http://www.slicer.org; Fedorov et al., 2012), CT scans were segmented slice by slice to label the pelvis and individual fin rays. From these
labels, we created 3D models, and determined volumes and densities for each label using the “Label Statistics” function in 3D Slicer.

3.6.6 Fin ray material properties

The right pelvic fin from each specimen were carefully cleaned of excess soft tissue and each fin ray was carefully disarticulated, such that the dorsal and ventral hemitrichia of each lepidotrichia were separated. The first pelvic fin ray was not separated to dorsal and ventral components since it was a spinous fin ray in all five specimens. Fin rays were stored in notothenioid ringer’s solution (following recipe by O’Grady et al., 1982) at 4°C for at least 48 hours before material testing.

A three-point bending rig was set up in an Instron Mini 55 tensile testing unit (Instron, Norwood, MA, USA). The bottom span, sitting on a 500N force transducer, was 9 mm wide. The indenter was mounted atop. Each fin ray, dorsal and ventral part, were tested individually. They were placed such that the ventral side faced down, such that the load was applied along the dorso-ventral axis. A minimum amount of force (max 0.01N) was applied during the mounting to ensure no movement during the test. Fin rays were placed such that the indenter would contact the fin ray 6 – 9 mm from the base of the ray (proximal part). Each fin ray was loaded once at a speed of 0.1mm/s to a deflection of 0.5 mm. The loading rate was chosen such that viscoelastic responses of the bone are minimized. After the test, the indentation spot was measured and marked with a felt marker.
3.6.7 Second moment of area and density calculations of fin rays

The first and second fin ray were isolated from the other structures in the CT scans using FIJI (https://imagej.net/Fiji; Schindelin et al., 2012). We used an analytical package for FIJI developed to study bone morphology, called BoneJ (Doube et al., 2010), and aligned each fin ray along its long axis. Slice by slice, the bone mineral density, cross sectional area, and second moment of area were determined for each slice along the fin ray using the “Slice Geometry” function in BoneJ.

3.6.8 Calculations of flexural stiffness and apparent material stiffness

Using the formula below, which is derived from beam theory (1; (Ennos, 2012; Vogel, 2003), we calculated the flexural stiffness, which is the product the Young’s modulus or material stiffness $E$ and the second moment of are $I$, for each fin ray:

$$EI = \frac{Fl^3}{48Y_{max}}, \quad (1)$$

where $Y_{max}$ is the deflection of the fin ray, caused by the force $F$, and $l$ is the length of the span of the 3 point-bending rig. This equation assumes that the deflections are caused by bending and not shear. Deflections caused by shear in a three-point bending setup usually occur if the aspect ratio of height to length are below 15 (Spatz et al., 1996). In our study, aspect ratios ranged from 18 to 36, satisfying the assumption. In addition, we were able to determine the Young’s modulus for each fin ray by dividing flexural stiffness with the second moment of the area.
3.6.9 3D geometric morphometric analysis of pelvis shape

To analyze shape changes in the pelvic morphology of our five Notothenioid species, we placed fifteen landmarks on 3D models of each pelvis using IDAV Landmark editor (Wiley et al., 2005). Landmarks were chosen based on their repeatability in all pelvises and also placed such that the entire pelvis was encompassed by landmarks (Fig. 4.3).

Landmark files for each 3D model were exported and loaded into R (R Development. Using the geomorph package (Adams and Otárola-Castillo, 2013), landmark coordinates were Procrustes superimposed, which rotates and superimposes the landmark coordinates of all imported pelvises based on the centroid of each pelvis. The resulting Procrustes coordinates were then analyzed using a Principal Component Analysis (PCA).
Figure 4.3: Reconstructed model of the right pelvic plate of *Histiodraco velifer* with 15 landmark used in 3D geometric morphometric analysis.

3.7 Results

3.7.1 Video analysis

Analysis of this video and resulting kinematics represent the behavior of one fish and do not are not meant to depict the breadth and variety of kinematics of bottom-walking in *H. velifer*. The video depicted *H. velifer* taking four complete strides, which we defined as starting with the initiation of the stance phase of the fins, through the movement of the fish, retraction of the fins, and ending with the forward placement of the
fins (Fig. 4.4A). On average, *H. velifer* moved forward by 4.15 cm per stride and a stride frequency of 0.25 Hz. As *H. velifer* initiated the stroke phase, it accelerated to a peak speed of ~ 2 m/s before coming to a near stop at the end of the stance phase (Fig. 4.4B). On average, *H. velifer* had a speed of 1.09 ± 0.91 cm/s during the stroke. The stance period lasted an average of 2.9 ± 0.52 s, whereas the swing phase lasted an average of 1.1 ± 0.04 s. The average sweep angle for the pelvic fin was 83.9 ± 14.2°.

### 3.7.2 Pelvic volumes and densities

We determined volumes and densities of the right pelvic plate from the CT scans (Table 4.1). All scanned pelvises exhibited areas of low ossifications, resulting in incomplete pelvic structures (Fig. 4.5). *H. velifer* had the largest pelvic plate volume at 22.83 mm$^3$ and the highest density at 0.71 g/cm$^3$ (Fig. 4.5). *A. nudiceps* had the second highest density at 0.67 g/cm$^3$ with a pelvic plate volume of 4.26 mm$^3$. The pelvic plate of *T. bernacchii* had a density of 0.36 g/cm$^3$ and a volume of 4.59 mm$^3$. *G. acuticeps* had a pelvic plate density of 0.28 g/cm$^3$. The pelvis with the lowest density was that of the icefish *C. wilsoni*, which was 0.14 g/cm$^3$ with a volume of 4.41 mm$^3$. Pelvic plate reconstruction depended on the ossification or density of the pelvis. The pelvises of *H. velifer* and *A. nudiceps* (Fig. 4.6A and B) were the most complete, with a few structures missing, whereas *C. wilsoni* had the most incomplete pelvis (Fig. 4.6C). The patterns of ossification radiated from the pelvic fin joints medially and anteriorly (Fig. 4.6). The posterior region, along with lateral and ventral wings, are denser than the medial plate.
(see Fig. 4.6). In all scans, the posterior process of the pelvis is not showing as it is likely composed of cartilage.

**Figure 4.4:** *Histiodraco velifer* bottom-walking video sequence and velocity diagram. (A) Sequence of one full stride divided into six frames, beginning with initiating stance phase (0 sec), stroke (0 – 2.93 sec), end stance phase (2.93 sec), return stroke (3.16 – 3.83 sec), ending with initiating stance phase (3.83 sec). (B) Speed (BL/s) plotted against time (s) over 4 strides.
Table 4.1: Volumes (mm$^3$) and bone mineral densities (g/cm$^3$) for the right pelvic plate and the right pelvic fins of five Notothenioid species.

<table>
<thead>
<tr>
<th></th>
<th>Histiodraco velifer</th>
<th>Akarotaxis nudiceps</th>
<th>Gymnodraco acuticeps</th>
<th>Chaenodraco wilsoni</th>
<th>Trematomus bernacchii</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pelvic plate</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Volume</td>
<td>22.826</td>
<td>4.259</td>
<td>4.592</td>
<td>4.406</td>
<td>4.593</td>
</tr>
<tr>
<td>Density</td>
<td>0.707</td>
<td>0.666</td>
<td>0.280</td>
<td>0.137</td>
<td>0.359</td>
</tr>
<tr>
<td>Pelvic fins</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Density</td>
<td>1.269</td>
<td>1.387</td>
<td>0.756</td>
<td>0.488</td>
<td>0.950</td>
</tr>
</tbody>
</table>
Figure 4.5: Reconstructed right pelvic plates for (A) *Histiodraco velifer*, (B) *Akarotaxis nudiceps*, (C) *Gymnodraco acuticeps*, (D) *Trematomus bernacchii*, and (E) *Chaenodraco wilsoni*. For each species presented are a (1) ventral view, (2) dorsal view, and (3) a medial view.
3.7.3 Pelvic shape differences

A principal component analysis of the 3D geometric morphometric data shows separation in shape along PC axis 1 (57.07 % variation explained, Fig. 4.6) and PC axis 2 (24.77 % variation explained, Fig. 4.7). For PC 1, pelvises which loaded strongly exhibited broadening of the pelvic plate medio-laterally, shortening anterior-posteriorly, anterior elongation of the ventral process, and an increase in height (dorso-ventrally) of
the anterior process. Pelvises loading on the negative end of PC 1 exhibited narrowing of the pelvic plate medio-laterally, widening anterior-posteriorly, and the ventral process moved posteriorly. Positive loading on PC 2 was not very different from the reference landmarks, as pelvises that loaded heavily on PC 2 did so by up to 0.1 and exhibited slight elongation of the pelvis anterior-posteriorly. Negative loading on PC 2 exhibited elongation anterior-posteriorly on the proximal side of the pelvic plate, shortening anterior-posteriorly on the distal portion of the pelvic plate on the medio-lateral plane.

**Figure 4.7:** Principal component analysis depicting shape differences in the right pelvis of five Notothenioid species. Principal component axis 1 and 2 account for 81.84% of variability observed in this analysis. At the minima and maxima of each axis, two warp grids depicting the shape changes experience at each end. The left grid shows a dorsal view, whereas the right grid shows a lateral view.
C. wilsoni loaded heavily on both PC 1 and PC 2, being far removed from the remaining pelvises. A. nudiceps loaded negatively on PC 2, whereas G. acuticeps loaded positively on PC 2. Both loaded neutrally on PC 1. T. bernacchii loaded negatively on PC 1 and neutrally on PC 2. H. velifer loaded negatively on PC 1 and positively on PC 2.

3.7.4 Densities and volumes of the pelvic fins

All fin rays were segmented and their volumes and densities determined. These values reflect the average for all pelvic fin rays on the right side of each animal. H. velifer had the highest volume in fin rays at 50.78 mm$^3$ at a density of 1.27 g/cm$^3$. A. nudiceps had the highest observed BMD density in their fin rays at 1.39 g/cm$^3$ and the smallest volume at 7.59 mm$^3$. G. acuticeps had a fin ray volume of 10.94 mm$^3$ and a density of 0.76 g/cm$^3$. The second highest fin ray volume was found in T. bernacchii (14.87 mm$^3$) and had a density of 0.95 g/cm$^3$. The lowest density was found in the fin rays of C. wilsoni at 0.49 g/cm$^3$ with fin ray volume of 19.09 mm$^3$ (Table 4.1).

3.7.5 Proximo-distal reduction of CSA, BMD, and I_{NA} in individual fin rays

All fin rays experienced a decrease in cross-sectional area (CSA), bone mineral density (BMD), and second moment of area (I_{NA}), from the proximal base towards the distal tips (see Figures 4.8 – 4.12). The 1st fin ray, which was a spinous ray for all species, had the highest CSA, BMD, and I_{NA} (Figures 4.8 – 4.12B), when compared to the 2nd dorsal (Figures 4.8 – 4.12C) and ventral fin ray (Figures 4.8 – 4.12D) in all
species (Table 4.2). The fin rays of *H. velifer* had the highest CSA, BMD, and *I*<sub>NA</sub> compared to the other species.

**Figure 4.8:** Pelvic fin rays volumes and material properties for *Histiodraco velifer*. (A) Right pelvic fin, with dorsal view on the top and ventral view on the bottom. The second moment of area (*I*<sub>NA</sub>), Bone mineral density (BMD), and cross-sectional area (CSA) for the 1<sup>st</sup> fin ray (B), 2<sup>nd</sup> dorsal ray (C), 2<sup>nd</sup> ventral ray (D), depicting changes from the base (left) to the tip (right). (E) Color legend for *I*<sub>NA</sub>, BMD, and CSA.
Figure 4.9: Pelvic fin rays volumes and material properties for *Akarotaxis nudiceps*. (A) Right pelvic fin, with dorsal view on the top and ventral view on the bottom. The second moment of area ($I_{NA}$), Bone mineral density (BMD), and cross-sectional area (CSA) for the 1$^{st}$ fin ray (B), 2$^{nd}$ dorsal ray (C), 2$^{nd}$ ventral ray (D), depicting changes from the base (left) to the tip (right). (E) Color legend for $I_{NA}$, BMD, and CSA.
Figure 4.10: Pelvic fin rays volumes and material properties for *Gymnodraco acuticeps*.

(A) Right pelvic fin, with dorsal view on the top and ventral view on the bottom. The second moment of area ($I_{NA}$), Bone mineral density (BMD), and cross-sectional area (CSA) for the 1<sup>st</sup> fin ray (B), 2<sup>nd</sup> dorsal ray (C), 2<sup>nd</sup> ventral ray (D), depicting changes from the base (left) to the tip (right). (E) Color legend for $I_{NA}$, BMD, and CSA.
Figure 4.11: Pelvic fin rays volumes and material properties for *Trematomus bernacchii*. (A) Right pelvic fin, with dorsal view on the top and ventral view on the bottom. The second moment of area ($I_{NA}$), Bone mineral density (BMD), and cross-sectional area (CSA) for the 1<sup>st</sup> fin ray (B), 2<sup>nd</sup> dorsal ray (C), 2<sup>nd</sup> ventral ray (D), depicting changes from the base (left) to the tip (right). (E) Color legend for $I_{NA}$, BMD, and CSA.
Figure 4.12: Pelvic fin rays volumes and material properties for *Chaenodraco wilsoni*. (A) Right pelvic fin, with dorsal view on the top and ventral view on the bottom. The second moment of area (I<sub>NA</sub>), Bone mineral density (BMD), and cross-sectional area (CSA) for the 1<sup>st</sup> fin ray (B), 2<sup>nd</sup> dorsal ray (C), 2<sup>nd</sup> ventral ray (D), depicting changes from the base (left) to the tip (right). (E) Color legend for I<sub>NA</sub>, BMD, and CSA.

3.7.6 Fin ray MTS and flexural stiffness

The most force required for deflection was measured in the first fin rays of each species. First fin rays were spinous rays, and not soft rays like the other fin rays, and therefore were tested as whole. Forces ranged between 0.024 and 0.660 N to deflect to 0.5 mm (Table 4.2).

Forces and deflection distances were used to calculate the flexural stiffness, *EI* (Fig. 4.13A). Flexural stiffness ranged between 1.20 – 34.42 Nmm<sup>2</sup>, with *H. velifer* consistently having the highest flexural stiffness for all fin rays. Young’s modulus ranged
between 1151.36 and 17253.68, with *A. nudiceps* having the highest Young’s modulus for all fin rays (see Fig.4.13B).

As a statement of caution, we must acknowledge that our material stiffness values may be inflated. While the effects of bone storage in ethanol are mixed, some indicate that storage in ethanol can increase the elastic modulus due to dehydration and change the material properties. Rehydrating bones may not restore the bone to prior material properties (Vesper et al., 2017).
Table 4.2: Averages ± standard deviation for cross-sectional area (CSA), bone mineral
density (BMD), and second moment of area ($I_{NA}$), and the force required to reach 0.5 mm
deflection for each fin ray among five species of Notothenioids.

<table>
<thead>
<tr>
<th>Species</th>
<th>Force</th>
<th>CSA Average</th>
<th>BMD Average</th>
<th>$I_{NA}$ Average</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>H. velifer</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>0.660</td>
<td>0.232 ± 0.202</td>
<td>1.438 ± 0.005</td>
<td>0.004 ± 0.045</td>
</tr>
<tr>
<td>2nd D</td>
<td>0.327</td>
<td>0.195 ± 0.261</td>
<td>1.153 ± 0.003</td>
<td>0.003 ± 0.011</td>
</tr>
<tr>
<td>2nd V</td>
<td>0.327</td>
<td>0.158 ± 0.082</td>
<td>1.112 ± 0.311</td>
<td>0.002 ± 0.002</td>
</tr>
<tr>
<td><em>A. nudiceps</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>0.238</td>
<td>0.153 ± 0.235</td>
<td>1.436 ± 0.007</td>
<td>0.003 ± 0.035</td>
</tr>
<tr>
<td>2nd D</td>
<td>0.072</td>
<td>0.074 ± 0.136</td>
<td>0.929 ± 0.0001</td>
<td>0.0003 ± 0.001</td>
</tr>
<tr>
<td>2nd V</td>
<td>0.077</td>
<td>0.073 ± 0.154</td>
<td>0.945 ± 0.0001</td>
<td>0.0009</td>
</tr>
<tr>
<td><em>G. acuticeps</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>0.043</td>
<td>0.120 ± 0.260</td>
<td>1.128 ± 0.0007</td>
<td>0.001 ± 0.001</td>
</tr>
<tr>
<td>2nd D</td>
<td>0.085</td>
<td>0.067 ± 0.183</td>
<td>0.850 ± 0.0003</td>
<td>0.0004 ± 0.001</td>
</tr>
<tr>
<td>2nd V</td>
<td>0.058</td>
<td>0.075 ± 0.165</td>
<td>0.856 ± 0.001</td>
<td>0.0004 ± 0.001</td>
</tr>
<tr>
<td><em>T. bernacchii</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>0.085</td>
<td>0.138 ± 0.172</td>
<td>1.200 ± 0.002</td>
<td>0.001 ± 0.033</td>
</tr>
<tr>
<td>2nd D</td>
<td>0.075</td>
<td>0.119 ± 0.169</td>
<td>0.846 ± 0.0007</td>
<td>0.001 ± 0.005</td>
</tr>
<tr>
<td>2nd V</td>
<td>0.065</td>
<td>0.103 ± 0.186</td>
<td>0.816 ± 0.0006</td>
<td>0.008 ± 0.004</td>
</tr>
<tr>
<td><em>C. wilsoni</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1st</td>
<td>0.132</td>
<td>0.102 ± 0.081</td>
<td>0.771 ± 0.003</td>
<td>0.002 ± 0.005</td>
</tr>
<tr>
<td>2nd D</td>
<td>0.024</td>
<td>0.053 ± 0.072</td>
<td>0.652 ± 0.0003</td>
<td>0.001 ± 0.0008</td>
</tr>
<tr>
<td>2nd V</td>
<td>0.040</td>
<td>0.071 ± 0.078</td>
<td>0.683 ± 0.0004</td>
<td>0.0006 ± 0.001</td>
</tr>
</tbody>
</table>
**Figure 4.13:** Graph showing the (A) Flexural stiffness and (B) Young’s modulus for the 1st fin ray (1), 2nd dorsal (2D), and 3rd ventral (2V) among five Notothenioid species. Each species is depicted as a different symbol, see legend upper right corner.

### 3.8 Discussion

3.8.1 Locomotor kinematics resemble those of other bottom-walkers

Pelvic fins are rarely used exclusively in bottom-walking fish, as most fish that bottom-walk use their pectoral fins (i.e. searobins) or a combination of both pectoral and
pelvic fins (i.e. frogfishes). Fish that use pelvic fins exclusively to bottom-walk among teleost fishes are the seamoths (Pegasidae) and flying gurnards (Dactylopteridae) (Nelson, 1994; Poss and Eschmeyer, 1998; Vincent, 1997). Seamoths and flying gurnards punt along the seafloor similarly to *H. velifer*; however, no kinematic or morphological data regarding their bottom-walking behavior exists. In our study, the Antarctic plunderfish *Histiodraco velifer*, punted along the seafloor using their pelvic fins at average velocities of 1.09 ± 0.91 cm/s (0.07 ± 0.06 BL/s; Fig. 4.3). Another bottom-walker, the streaked gurnard, *Trigloporus lastoviza* (Triglidae), moved at an average speed of 1.83 cm/s (0.09 BL/s), generating the propulsive forces with their specially modified fins (Jamon et al., 2007; Renous et al., 2000). In another study, frogfishes moved slowly along the sea-bottom (0.01 BL/s, (Pietsch and Grobecker, 1987; Yamanoue et al., 2010)) using their pectoral and pelvic fins. Frogfish primarily generate propulsion with their highly modified pectoral fins, which they sweep through an angle of 50°, whereas the pelvic fins provided little propulsive forces (Pietsch and Grobecker, 1987). In our study, *H. velifer* swept their pelvic fins through an arc of 83.9°, generating almost all of the displacement with these fins.

Few studies exist on bottom-walking teleost fishes that exclusively use their pelvic fins. Instead, we compare our findings to the African lungfish (*Protopterus annectens*), a sarcopterygian fish, and several species of batoids, which use their pelvic fins to bottom-walk on aquatic substrates (King et al., 2011; Macesic and Kajiura, 2010). African lungfish either walk or punt, using cycling their pelvic fins with a stride frequency of 0.5 Hz and a duty factor of 0.46. Pectoral fins are sometimes used for support, but main propulsion is generated by the pelvic fins (King et al., 2011). Macesic
and Kajiura (2010) compared the punting kinematics of four species of batoids. They generated speeds ranging between 0.20 and 0.41 body-lengths per second, which propelled them to distances ranging between 0.32 and 0.48 body-lengths. Whereas *H. velifer* propelled itself forward by sweeping motion of the pelvic fins, batoids use their pelvic fins to push off the substrate and glide. The duty factor of *H. velifer* in bottom walking was 0.72, whereas batoids had duty factors ranging from 0.29 to 0.44 (Macesic and Kajiura, 2010). It is possible that the duty factor could indicate the presence of glide phases in bottom-walking animals.

### 3.8.2 Fin flexural stiffness is highest in *Histiodraco velifer*

This is the first study comparing flexural stiffness of limb elements in bottom-walking teleosts. Flexural stiffness is an important component of material properties as it expresses the resistance of a body to bending, which is an essential functional component of fin rays (Ennos, 2012; Flammang et al., 2013; Lauder et al., 2011; Taft, 2011; Vogel, 2003). In our study, comparison of the five notothenioid species showed that *H. velifer*, the only fish in our dataset that is known to bottom-walk, had the highest flexural stiffness among the fin rays which were tested (Fig. 4.13A). It is likely that flexural stiffness facilitates this behavior, which supports our hypothesis that bottom-walking fish have higher flexural stiffness than non-bottom-walking fish. Our hypothesis was inspired by research on punting in batoids (Macesic and Summers, 2012), which found that punting species had higher pelvic flexural stiffness than non-punting, more sedentary batoids. Unfortunately, only a few studies have investigated the material properties of
teleost bone. In comparison to the flexural stiffness of punting, benthic batoids (56.14 – 126.1 Nmm$^2$), *H. velifer* was found to have lower flexural stiffness in its fin rays compared to the propterygia of these batoids, yet *H. velifer* had considerably larger flexural stiffness in their fin rays compared to another teleost, the bluegill sunfish (0.565 Nmm$^2$) (Flammang et al., 2013).

While *H. velifer* had the highest flexural stiffness, it did not possess the highest Young’s modulus of the five species of notothenioids we compared (Fig. 4.13B). *Akarotaxis nudiceps* had the highest Young’s modulus, whereas *H. velifer* ranked in the middle of the groups and even had the lowest Young’s modulus for the 2$^\text{nd}$ ventral fin ray. As we mentioned in the introduction, flexural stiffness is a product of the Young’s modulus and the second moment of area, which means that deficits in one variable can be compensated for by the other in order to achieve high flexural stiffness. This suggests that *H. velifer* achieves high flexural stiffness by having a large second moments of area ($I$), which our study confirms (see Fig 4.8, and Table 4.3). For instance, rib bones in the great sculpin achieve high flexural stiffness due to their second moment of area rather than their Young’s modulus (Horton and Summers, 2009). The rostrum of billfishes increases in Young’s modulus from the proximal origin to the distal tip; however, flexural stiffness decreases along the same path due to changes in rostrum geometry, which have been hypothesized to provide support in lateral striking motions of the rostrum for stunning and hunting prey (Habegger et al., 2015). Similar strategies are again observed in batoids, where punting skates had high flexural stiffness but not particularly high Young’s modulus (Macesic and Summers, 2012). Finally, even in birds,
feather shafts also rely on their geometry to produce higher second moments of area rather than increasing their Young’s modulus (Bachmann et al., 2012).

The observed values in this study for Young’s modulus (1.15 – 17.25 GPa) fall within the reported values of other studies which evaluated teleost bone material properties, which range from 6.5 GPa in the ribs of *Myoxocephalus polyacanthocephalus* (Horton and Summers, 2009) to 15.5 GPa in intramuscular bone of *Clupea harengus* (Rho et al., 2001). For comparison, batoid pelvic propterygia used during punting had a Young’s modulus of 0.14 to 2.53 GPa (Macesic and Summers, 2012), overlapping with the material stiffness range in our study.

### 3.8.3 Pelvic shape increased density for support

In this study, pelvic plates had lower BMDs compared to their fin rays (Table 4.1). All notothenioids (except 4 – 5 species; (Eastman et al., 2014)) are negatively buoyant, and swim only occasionally, spending a lot of time on the substrate. Reduction in bone mineral density and the retention of cartilage in the skeleton are hypothesized to alleviate the lack of a swimbladder (Eastman and DeVries, 1982). Acellular bone, which comprises most teleost skeletons, can remodel bone according to the repeated loads it experiences (Cohen et al., 2012; Horton and Summers, 2009). Differences in the BMD of pelvic plates therefore may be an indicator of the animal’s reliance on their pelvic girdle. Therefore, fish that rely on their pelvic girdle, like a bottom walker, would be expected to have increased mineralization which support the musculature that interacts with the fin.
rays. Mineralization was highest in the pelvises of *H. velifer* and *A. nudiceps*, of which the former is a known bottom-walker (Fig. 4.6).

The distribution of mineralization in all pelvises concentrated on the pelvic fin joints and the surrounding area. We also noticed a striated pattern emanating from the pelvic fin joints alone the pelvic plates. Since the muscles attach along the pelvic plate, it is likely that the distribution of mineralization acts as increased support for the pelvic musculature while minimizing the bone mineral density of the pelvis. Distribution patterns of mineralization therefore also may be indicative of bottom walking. In contrast, *C. wilsoni* had the lowest mineralization observed in this study (Table 4.1, Fig. 4.6). This finding is in agreement with prior studies that have assessed the skeletal morphology in other icefish. They have particularly large amounts of cartilage in their pectoral girdles (Eastman et al., 2014; Iwami, 1985), which makes it unsurprising to see a similar pattern in the pelvic girdle.

A comparison of notothenioid pelvic shapes in this study showed differences across species with changes in pelvic dimensions, however the basic morphology remained the same across the species (Fig. 4.7). There was no loss or addition of structures, such as processes or wings. The most drastic characteristic which was observed was in the ventral wing, which was highly enlarged and anteriorly expanded in *C. wilsoni* compared to the other four species (see Fig. 4.5 and 4.7). In their study on bottom-walking batoids, Ekstrom and Kajiura (2014) correlated pelvis shape and locomotor mode. Clear morphological differences (i.e. elongation of lateral processes, broadening and anterior elongation of the pelvis, etc) were observed in the pelvises of
bottom-walking and swimming batoids (Ekstrom and Kajiura, 2014). It is possible that teleosts do not exhibit this relationship (Ekstrom and Kajiura, 2014).

3.8.4 Proximo-distal changes in fin rays suggest regionalized functions

Notothenioid fin rays in this study had larger cross-sectional areas proximally, which decreased gradually towards the distal end (Fig. 4.8 – 4.12). In a similar trend, there was a decrease in second moment of area proximally, and bone mineral density (Fig. 4.8 – 4.12). Bluegill sunfish decreased the second moment of area from 0.0009 mm$^4$ on the proximal end to 0.0003 mm$^4$ distally in the 2nd pectoral fin ray (Lauder et al., 2011), which are in range of values we observed in our study. Bluegill sunfish also decreased the Young’s modulus from 1.5 GPa proximal to 0.67 GPa on the distal end. A mechanical explanation may lie in the need to counteract increasing moment arms on the distal end by increasing the cross-section proximally. This arrangement would level out the stress experienced across the structure (Currey, 1984). Another explanation, may lie in the function of the fins and how they are used. Muscles and tendons attach on the proximal side of the fin rays, which take up space and require firm structural support (Alben et al., 2007; Lauder et al., 2011; Taft, 2011; Taft and Taft, 2012). The fin ray arrangement, where two hemitrichia are held together by collagen and elastic fibers, enables the fins to properly control the stiffness and form of the fin by muscle contractions (Alben et al., 2007; Lauder et al., 2011). For instance, by contracting a set of muscles on one hemitrich but not the other, the actuated hemitrich is pulled towards the body, whereas the other stays still, causing curvature in the fin (Flammang et al., 2013;
Lauder et al., 2011; Taft and Taft, 2012). Curling of the distal ends of fin rays is unlikely if the material would resist bending. For this matter, distal ends of fin rays are segmented, which gives them more flexibility as well as stability from perturbation (Flammang et al., 2013; Taft, 2011).

Notothenioid fin rays (excluding spinous ray), appear to have minimal segmentation and branching. Segmentation, if it occurs, is found in the distal half of the fin rays (Fig. 4.8 – 4.12). Taft (2011), while assessing differences between benthic and pelagic pectoral fin ray morphology, found differences in segmentation of fin rays, with benthic fin rays having segmentation on the distal half of the ray, whereas pelagic fin rays had segmentation along most of the fin. It was hypothesized that the benthic fin rays are regionalized by function, providing strength proximally to withstand forces while allowing flexibility distally. Similar concepts are used in the insect wings of Manduca sexta, where proximal areas were higher in flexural stiffness, providing rigidity, and distal regions and wing edges were more flexible, facilitating aerodynamic force production (Combes and Daniel, 2003).

3.8.5 Conclusion

*H. velifer* is a benthic, bottom-walking fish which uses pelvic-punting, reminiscent of batoids, to propel itself along the seafloor. This is the first study comparing flexural stiffness of limb elements in bottom-walking teleosts. While *H. velifer* had the highest flexural stiffness, it did not possess the highest Young’s modulus of the five species of notothenioids we compared. This study demonstrates the
importance of the pelvic skeleton in bottom-walking fish. *H. velifer* appears to depend more on skeletal geometry (i.e. second moment of area) than material stiffness to provide a functional pelvis for bottom-walking. Our knowledge on material properties and morphology of bottom-walking teleosts is very limited. Future studies comparing the pelvic and pectoral girdles of other bottom-walking fish could reveal evolutionary patterns in bottom-walking morphology and material properties.
CHAPTER 5

CONCLUSION

In this Dissertation, I investigated how animals which move in challenging situations depend on control mechanisms, biomechanics, and morphology. Particularly, I looked at control mechanisms and biomechanics in an eight-legged, terrestrial decapod, the Atlantic ghost crab, and the morphology and biomechanics of the Antarctic plunderfish, a nototheniod fish from Antarctica, which can walk on the bottom of the ocean.

In the second Chapter, I filmed Atlantic ghost crabs, which had been subjected to paired limb removal treatments, running in an enclosed trackway in the field using high-speed video. This was the first study that investigated the effects of limb loss on locomotion in ghost crabs. By running animals in their natural setting, we elicited some of the fastest recorded running speeds for this genus. The findings failed to support my hypothesis that based on field observations of limb loss patterns, the removal of the 3rd and 4th pair of limbs would have the least effect on locomotor performance. Ghost crabs are remarkably robust to limb loss, suffering a maximum locomotor decrement of only ~25%. Faced with the loss of a pair of walking limbs, ghost crabs compensated for limb loss by adjusting their stepping and limb cycling patterns. The 2nd and 3rd pair of limbs, however, appear to be the most critical for maintaining overall running performance. We observed dramatic changes in limb timing characteristics and decrements in running speed with the loss of either one of these limbs; whereas the impacts were far more modest following the loss of any of the other limbs.
In Chapter 3, ghost crabs subjected to a slippery tile experienced severe slipping with their limbs, however none of the animals fell or stopped because the slipping. Instead, the momentum of the ghost crabs carried them over the slippery surface, while simultaneously maintained constant locomotor kinematics. The constant cycling of limbs, coupled with an average stance time of 22ms, which is likely to short for a neural response, indicate that ghost crabs used feed-forward control to maintain stability. The findings of this study corroborate prior findings as well as locomotor models, which predict that fast running speeds make animals less susceptible to perturbations. Lastly, odd limb coupling patterns were observed in this study that don’t agree with prior findings. I hypothesized that the observed changes may be due to differential limb functions, and that limbs important to locomotion are prioritized in limb coordination.

In Chapter 4, I investigated the shape and material properties of the pelvic girdle of the bottom-walking Antarctic plunderfish, *Histiodraco velifer*. The volume, bone mineral density, and material properties were compared to four other notothenioids which do not bottom-walk. Comparative data on the material properties of fin rays in bottom-walking teleosts are rare, making this the first study to determine the material properties of bottom-walking pelvic fin rays in teleosts. As hypothesized, *H. velifer* exhibited the highest flexural stiffness among the tested notothenioids. However, the Young’s modulus was not the highest in *H. velifer*. This further suggests that *H. velifer* achieves high flexural stiffness through fin morphology, i.e. increasing its second moment of area. The pelvic plates showed low mineralization, with *H. velifer* having the highest bone mineral density in the pelvic plate. There were no major shape changes in the pelvic plates of the five notothenioids studied in this Chapter. Using volume renderings to display bone
mineral densities, patterns of mineralization were found on most pelvises. These radiating striations of mineralization likely align with muscle fiber of the fin musculature to provide support for muscle contractions. These findings suggest that *H. velifer* can attain a stable pelvis to support bottom-walking by geometric arrangement of mineralization.
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