



How to waddle with a paddle: a study of duck hindlimb anatomy, kinematics, and muscle function across behaviors and species

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HARVARD UNIVERSITY
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How to waddle with a paddle: a study of duck hindlimb anatomy,
kinematics, and muscle function across behaviors and species

A DISSERTATION PRESENTED
BY
KARI REBECCA TAYLOR-BURT
TO
THE DEPARTMENT OF ORGANISMIC AND EVOLUTIONARY BIOLOGY

IN PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY
IN THE SUBJECT OF
ORGANISMIC AND EVOLUTIONARY BIOLOGY

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ABSTRACT

The most impressive animal movements often arise from animals that have specialized their anatomy, muscle function, and kinematics (the way they move) for a specific behavior or environment. However, specialization may come at a cost, as most animals require their structures and muscles to perform multiple behaviors. Despite the specialization of their hindlimb (pelvic limb) for swimming, ducks are able to use their hindlimbs to move on land and to takeoff, in addition to swimming at the surface and diving. How are they able to use the same structures (the hindlimb, pelvis, and foot) and muscles that drive them to perform so many behaviors? What structures and traits are associated with their specialization for aquatic locomotion, and are there tradeoffs in locomotor ability that come with this specialization? In my dissertation, I begin to explore the answers to these questions by studying the kinematics (Chapter 2, 4), muscle function (Chapter 2, 3), and anatomy of ducks (Chapter 4) across behaviors and across the duck phylogeny.

In **Chapter 2**, I examined kinematics and function of a bi-articular hindlimb muscle, the lateral gastrocnemius (LG), during aquatic and terrestrial takeoffs in mallard ducks. Unlike many waterbirds, mallards are capable of vertical takeoffs from both land and water, regimes where force production and demands on the animal differ. Mallards change their kinematics and LG function with takeoff medium. Importantly, the knee moves in the opposite direction, extending during terrestrial takeoff to launch the body into the air but flexing during aquatic takeoff to contribute to caudal motion of the foot. The LG powers both ankle extension and knee flexion, so it undergoes

larger excursions and higher shortening velocities during aquatic takeoffs than terrestrial, making tuning of the muscle's force-length and force-velocity properties across takeoff media challenging.

The function of the mallard LG was explored in greater depth in **Chapter 3** by focusing on how it operates during cyclical behaviors like walking and swimming. Post-activation potentiation (PAP) is a less well-studied physiological property of muscle that represents an increase in force and rate of force development in muscle after recent activation. PAP could therefore impact LG function during behaviors that require repeated muscle activation. Using an *in situ* muscle preparation, I controlled mallard LG cycle frequency, length change, and activation parameters to mimic surface swimming. As hypothesized, PAP affected LG function, resulting in a gradual increase in muscle force, rate of force development, and work production over several cycles despite no change in activation. The degree of PAP depended on cycle frequency. This work was novel because our knowledge of mallard LG function during cyclical behaviors *in vivo* allowed me to make connections between these behaviors and PAP of the muscle observed *in situ*.

Ducks exhibit a wide range of swimming abilities, including highly terrestrial species, ducks that swim well but only at the surface, foot-propelled divers, and wing-and-foot-propelled divers. In **Chapter 4**, I explored how anatomy and movement relate to specialization for swimming in ducks. Some hindlimb structures differed among behavioral groups, including the proportional length of the femur and tarsometatarsus, the shape of the femur and tibiotarsus, and the lateral cnemial crest size. However, only the increased size of the cnemial crest is consistent with the anatomical features seen in other avian swimming specialists and is thought to contribute to increased knee stabilization (by increasing the moment arm of knee extensors) and to powering distal limb movement (by increasing attachment surface for the digital flexors). I examined how kinematics varied among surface swimmers (mallards), foot-propelled divers (scaup), and wing-propelled divers (eiders) during walking, surface swimming, and diving. All three species increased speed by increasing stride

length for all behaviors. Cycle frequency was increased with speed only during walking, remaining constant during surface swimming and diving. Eiders increased stride length with diving speed more rapidly than the others, perhaps enabled by their simultaneous use of wings and feet. During walking, eiders used higher cycle frequencies and both eiders and scaup exhibited higher body angles, perhaps an indication of lower terrestrial stability than the surface swimming mallards.

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Dedicated to my family, Brian, Timmy, Josie, and my parents

CHAPTER 1. Introduction

Watching a whale breach, a bee buzz among the flowers, or an impala deftly outmaneuver a pursuing cheetah highlights the amazing breadth and diversity of animal locomotor strategies, often with the most extreme cases of specialization resulting in the most impressive behaviors. However, most animals require their structures and muscles to perform multiple behaviors, so specialization for one behavior or environment may come at a performance cost in other environments or behaviors. The waddle of the duck is one example of this tradeoff—from terrestrial theropod and volant avian ancestors, these birds have become adept swimmers with a cost of more awkward movement on land. Moving through water places different demands on the body than moving terrestrially, so swimming in birds is related to anatomical adaptations and corresponding changes in kinematics and muscle function. In this dissertation, I present three chapters exploring the hindlimb kinematics and the function of a hindlimb muscle (the lateral gastrocnemius) across behaviors in mallard ducks (Chapters 2 and 3) as well as differences in anatomy and kinematics among ducks from across the duck phylogeny with different swimming strategies (Chapter 4).

1.1 Evolution of Swimming in Birds

Birds are derived from terrestrial ancestors who acquired flight while maintaining terrestrial ability. Bipedal locomotion and flight each require considerable anatomical specialization, so the ability of an aerial, bipedal animal to develop a third locomotor strategy is impressive. Non-avian theropods excelled at bipedal terrestrial locomotion, but the hindlimb and tail were paired with the large caudofemoralis acting as a limb retractor. The origin of flight released the tight link between the tail and hindlimb, with the tail and caudofemoralis muscle reduced and with smaller hip and larger knee excursions (Gatesy & Middleton, 1997; Benson & Choiniere, 2013). When considering the morphospace of the lengths of the three hindlimb elements in theropods, Gatesy & Middleton (1997) found that non-avian theropods only take up 24% of the occupied morphospace while avian theropods take up about 87%. The diversity in hindlimb morphology observed in extant birds was only possible by the dissociation of the tail and hindlimb during terrestrial locomotion (Benson & Choiniere, 2013), and this diversity makes birds an ideal group in which to explore the cost of specialization vs. the ability to be locomotor generalists.

Birds have become highly aquatic swimmers many times throughout their evolutionary history (Habib & Ruff, 2008), and the fossil record is rich with swimming birds (Nudds *et al.*, 2012; Zinoviev, 2015). Theropods are generally thought to have been capable of swimming, but very few were specialized swimmers prior to birds (Hutchinson & Allen, 2009). One notable exception is the *Spinosaurus*, which retains the enlarged tail observed in other basal theropods and is thought to have been a tail-propelled swimmer (Ibrahim *et al.*, 2020), a distinct strategy from the wing and foot-propelled swimming observed in birds. The first aquatic birds likely appeared in the early Cretaceous (Lim *et al.*, 2000; Nudds *et al.*, 2012). The Chinese Cretaceous bird *Gansus* retained some primitive features, including an unreduced fibula and the presence of a pubic symphysis (Nudds *et al.*, 2012),

but also exhibited derived traits typical of swimming birds, including a well-developed cnemial crest on the tibiotarsus, elongated toes (Nudds *et al.*, 2012), and webbed feet (You *et al.*, 2006). *Gansus* was originally suggested to resemble a duck in morphology and habit but analysis of hindlimb segment lengths suggests that it resembled grebes or comorants more than ducks (Nudds *et al.*, 2012).

Hesperornis is another swimming bird from the early Cretaceous that had very large, lobed toes, like grebes, that may have facilitated lift-based swimming (Benton, 2015). However, *Hesperornis* ankle morphology resembled that of loons and likely permitted tarsometatarsus rotation, unlike the ankle anatomy of grebes that restricts motion to flexion-extension (Zinoviev, 2015). An analysis of hindlimb anatomy across the avian tree placed *Hesperornis* among the foot-propelled swimmers (Hinić-Frlog & Motani, 2010). The early appearance of swimming birds in the fossil record suggests that all modern birds may have been derived from a similarly amphibious ancestor (You *et al.*, 2006), and this possibility underscores the importance of understanding the evolutionary history of hindlimb morphology and how adaptations to swimming may affect hindlimb function.

Extant swimming birds include foot-propelled and wing-propelled species. Birds that are extremely specialized for swimming experience a tradeoff in the specialized limb, with a reduced flying ability or loss of flight in wing-propelled swimmers (Habib, 2010) and a reduced terrestrial ability in foot-propelled swimmers (Raikow, 1973; Johnsgard, 1987). The avian order Anseriformes includes a wide range of swimming abilities (Figure 1.1), with (1) highly terrestrial species, including screamers and some sheldducks and sheldgeese (tribe Tadornini); (2) surface swimmers, including swans, geese, and the dabbling ducks (tribe Anatini); (3) foot-propelled swimmers, including the diving ducks (tribe Aythyini), some sea ducks (tribe Mergini), stiff-tailed ducks (tribe Oxyurini), and the torrent duck (*Merganetta armata*); and (4) wing-and-foot-propelled swimmers which are exclusively found among the sea ducks (Figure 1.1).

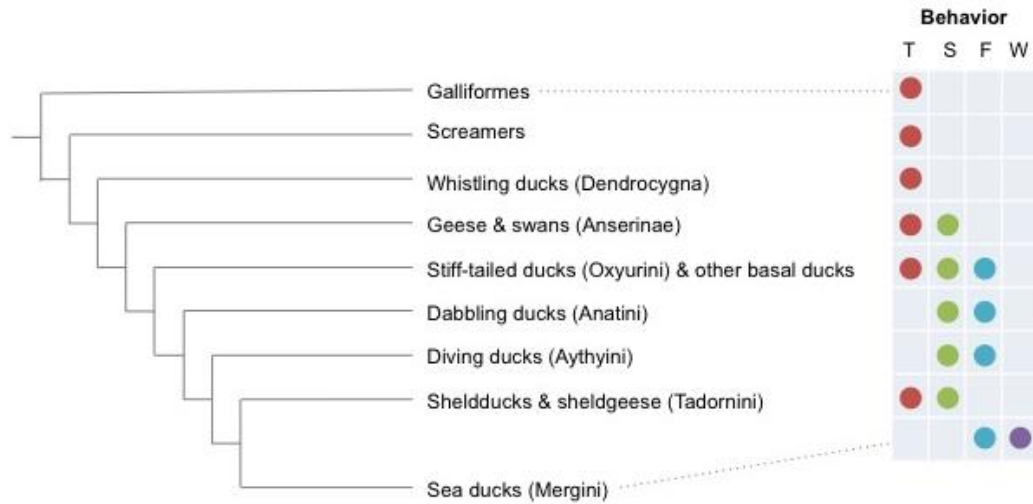


Figure 1.1 Relationships and swimming behaviors among ducks and other closely related groups. Common names are given, with scientific names in parentheses, as appropriate. Behavior categories are terrestrial (T, red), surface swimming (S, green), foot-propelled diving (F, blue), or wing-and-foot-propelled diving (W, purple). Assigned behaviors include both the indicated group and closely related species.

This diversity in swimming behaviors in closely related species is unusual and provides an exciting opportunity for comparing anatomy and kinematics in relation to locomotor strategies and degree of specialization for swimming. In Chapter 4 of this dissertation, I describe the variation in hindlimb and pelvic skeletal anatomy across the duck evolutionary tree. This anatomical survey includes species from all groups represented in Figure 1.1 and thus includes ducks with different swimming behaviors. I also describe hindlimb kinematics during walking, surface swimming, and diving in three species with different swimming abilities and representing three different tribes: Anatini, Aythyini, and Mergini.

1.2 Adaptations of the Skeletal Anatomy for Foot-Propelled Swimming

Foot-propelled swimming birds have convergently evolved a common suite of hindlimb characters. In swimming birds, the foot is enlarged to form a paddle or hydrofoil (Raikow, 1973; Johansson & Lindhe Norberg, 2000; You *et al.*, 2006). This enlargement is accomplished by lengthening the digits (Raikow, 1973; You *et al.*, 2006) and webbing connecting the toes (Raikow, 1973; Nudds *et al.*, 2012) or lobes around each toe (Johansson & Lindhe Norberg, 2000; Johansson & Lindhe Norberg, 2001). The cortex of the bones tends to take up a larger proportion of the cross-sectional area of long bones (Habib & Ruff, 2008) and the distal limb bones that move through the fluid occasionally become flattened perpendicular to the flow (Johnsgard, 1987; Lovvorn, 1990; Habib & Ruff, 2008). The increased cortical area may help to counteract buoyancy, the flattened bone may decrease drag on the propulsive appendage, and both adaptations may help resist stresses produced by moving the limbs through water (Habib & Ruff, 2008). The cnemial crests are enlarged relative to other birds to allow greater muscle attachment and longer moment arms (Johnsgard, 1987; You *et al.*, 2006; Clifton *et al.*, 2018). Highly derived foot-propelled diving birds have shorter femora and longer distal hindlimb segments (Johnsgard, 1987; Gatesy & Middleton, 1997; Johansson & Lindhe Norberg, 2001), an elongate pelvis with narrow width, and anteriorly position acetabula (Johnsgard, 1987; Hinić-Frlog & Motani, 2010; Ibáñez & Tambussi, 2012; Stoessel *et al.*, 2013; Anten-Houston *et al.*, 2017). Presumably these adaptations work together to keep the proximal leg closely tucked to the body and the distal leg positioned near the body midline (Hinić-Frlog & Motani, 2010). These characters guided my hypotheses about how duck hindlimb and pelvic anatomy would vary with swimming ability (Chapter 4).

Extreme adaptation of the hindlimb to swimming is often associated with a cost or tradeoff with other forms of locomotion. Enlargement of the foot is thought to cause decreased maneuverability on land (Raikow, 1973) and would certainly decrease terrestrial locomotor efficiency

by increasing the inertia of the limb. Birds that are highly specialized for swimming, such as diving ducks, loons, and grebes have reduced walking ability (Raikow, 1973; Johnsgard, 1987). The hindlimbs are often shifted posteriorly in these birds, presumably as an adaptation for more effective swimming propulsion; but the resulting posterior placement of the foot makes maintaining the foot directly under the center of mass while standing difficult (Raikow, 1973). This tradeoff is clear within ducks where more terrestrial species retain anteriorly positioned hindlimbs to balance weight support over the foot and facilitate walking, while species that are more specialized for swimming have posterior hindlimb placement (Raikow, 1973). When considering specialization for swimming and its costs, it is therefore critical to consider both anatomy and kinematics.

1.3 Avian Hindlimb Kinematics

1.3.1 Moving on land:

The avian hindlimb originally evolved for terrestrial locomotion and has since been adapted to other behaviors; however, most birds retain the ability to move on land by walking or hopping, the former of which was examined in ducks (Chapter 4) and will be the focus of the present discussion. Terrestrial strides are divided into two segments: swing, when the limb is not in contact with the ground, and stance, the support phase of the stride. Much of the limb movement during terrestrial locomotion results from joint excursions at the knee and ankle rather than the hip, at least at slow speeds (Gatesy & Biewener, 1991; Johnston & Beckoff, 1992; Biewener & Corning, 2001; Carr, 2008). Leg motion is often thought to be restricted primarily to planar flexion/extension, and adduction/abduction of the femur is mechanically prevented by the body (adduction) and the pelvic antitrochanter (abduction; Kambic *et al.*, 2014). However, birds are also capable of substantial long-axis rotation of the femur and tibiotarsus in order to turn, transversely displace the feet, or conduct other maneuvers (Kambic *et al.*, 2014). Increases in limb excursion are almost entirely in limb

retraction as the amount of limb protraction remains fairly constant; this is likely due to the need for foot placement under the cranially positioned center of mass (Gatesy & Biewener, 1991).

The degree of crouching also affects terrestrial locomotion. Larger birds tend to have more upright postures while smaller birds tend to be more crouched (Gatesy & Biewener, 1991). Smaller birds have relatively larger limb excursions, which can be attributed to this more crouched posture and to relatively larger feet (Gatesy & Biewener, 1991). As a result of this morphology, larger birds tend to increase speed by increasing cycle frequency while smaller birds increase speed by increasing limb excursion (Gatesy & Biewener, 1991; Abourachid, 2001). The abduction (or splay) of the duck femur is thought to be an adaptation for swimming (Abourachid & Höfling, 2012; Provini *et al.*, 2012A) and impacts femoral retraction during walking. Because of reduced femoral retraction, ducks increase stride length with a lateral waddle, which is costly both because of the energy lost to the lateral movements and the longer swing phase relative to other birds (Abourachid, 2001). The consequences of specialization for aquatic locomotion is even higher in other birds which no longer walk digitigrade and rather move in a plantigrade fashion or scoot along the ground on their abdomen (Johnsgard, 1987).

1.3.2 Taking off

The ‘ground-up’ and ‘wing-assisted incline running’ theories of the evolution of flight require significant contributions of the hindlimb (Earls, 2000; Dial, 2003; Heers & Dial, 2012; Heers *et al.*, 2014; Heers & Dial, 2014; Heers *et al.*, 2016; Heers *et al.*, 2018). The hindlimb continues to play a critical role in takeoff in modern birds. Birds jump prior to the onset of flapping during takeoff (Provini *et al.*, 2012B), with the hindlimbs contributing between 59% and 100% of the takeoff speed (Raikow, 1973; Earls, 2000; Tobalske *et al.*, 2004; Berg and Biewener, 2010; Abourachid & Höfling, 2012; Provini *et al.*, 2012B; Chin and Lentink, 2017). The hindlimbs dominate force production for the first phase of takeoff (Provini *et al.*, 2012B), regardless of whether the bird is terrestrial or more

specialized for perching (Earls, 2000).

Swimming birds that remain somewhat terrestrial retain the ability to perform vertical takeoffs (Williamson *et al.*, 2001); however most hindlimb-propelled swimmers, particularly those of larger size, require large aquatic runways in order to build speed prior to takeoff (Johnsgard, 1987; Lovvorn & Jones, 1994). This is generally attributed to reduced wing area for underwater efficiency or reduced selection pressure for slow-flight ability in uncluttered aquatic habitats (Lovvorn & Jones, 1994). However, considering the critical role that the hindlimb plays in takeoff in other birds, changes in the hindlimb for improved swimming performance may well affect flight takeoff performance. Because the hindlimb contributes to takeoff in both aquatic and terrestrial environments but propulsive force production differs between water and land, this behavior is an interesting system in which to explore the demands placed on the body by similar behaviors in different environments. A study examining aquatic and terrestrial takeoffs in mallard ducks is presented in Chapter 2.

1.3.3 Swimming

The proximal hindlimb joints are thought to be more stationary during swimming than during terrestrial locomotion. The hip and the knee undergo substantially smaller excursions during surface swimming in mallards than during walking; thus, ankle extension is the primary hindlimb motion during the propulsive stroke (Biewener & Corning, 2001; Carr, 2008). Although they are not aquatic birds, studies in swimming galliform chicks show that joint motion follows a similar pattern between walking and swimming, with the initiation of movement occurring at the knee, followed by the ankle (Johnston & Beckoff, 1992). Similar to ducks, the ankle showed the largest difference in timing and excursion between swimming and walking (Johnston & Beckoff, 1992), indicating that muscles acting at the ankle are important for both swimming and walking. However, the muscles at the hip and knee must remain activated and produce force in order to stabilize the limb and prevent

motion about these joints as the ankle extends to generate hydrodynamic force at the foot (Carr, 2008; Hinić-Frlog & Motani, 2010; Clifton *et al.*, 2018).

In dabbling ducks and most other avian swimmers, the hindlimbs move caudally and ventrally during swimming (Johansson & Lindhe Norberg, 2001), much like the limb excursion that occurs during walking. These motions are thought to be drag-based as the foot pushes water backwards to generate propulsive forces (Lovvorn & Liggins, 2002). However, birds that move their feet cranio-caudally ventral to the body may use both drag-based propulsion during the first part of the power stroke and lift-based propulsion at the end of the power stroke (Johansson & Norberg, 2003). Lateral limb movements that may be favorable for swimming, such as adduction/abduction of the femur, are typically restricted by morphology. The highly reduced femur in grebes and loons is laterally splayed (Johnsgard, 1987), permitting lateral motion of the foot, which is thought to be associated with lift production (Johansson & Lindhe Norberg, 2001; Clifton & Biewener, 2018). Although grebes tend to move their feet from a lateral to medial/dorsal position (Johansson & Lindhe Norberg, 2001), loons move their feet along a lateral arc posterior (rather than ventral) to the body (Clifton & Biewener, 2018).

1.4 Muscle Function in Terrestrial & Aquatic Environments

The properties of muscles tend to reflect a balance of the demands placed on them (Hill, 1956). The force-length and force-velocity properties of muscle are the foundation for our understanding of muscle function. The force-length property describes force production vs. sarcomere or muscle length, where maximum force production occurs over a limited, intermediate range of lengths (Gordon *et al.*, 1966; McMahon, 1984; Lieber, 1992; Biewener & Patek, 2018). Muscle force and velocity are inversely related such that maximal forces are only possible at low shortening velocities and vice versa (Hill, 1938; McMahon, 1984; Lieber, 1992; Biewener & Patek,

2018). Consequently, muscle power (force x velocity), with high values being especially important for explosive behaviors like takeoff, is maximal at intermediate velocities while maximum efficiency occurs at slightly lower velocities (Hill, 1956; McMahon, 1984; Lieber, 1992; Biewener & Patek, 2018). By limiting muscle shortening velocities and sarcomere lengths used *in vivo*, muscle function can be tuned or ‘optimized’ for certain contractile characteristics.

For birds, however, the hindlimbs perform many behaviors, requiring the ability to generalize the function of the muscles that drive them to power a broad range of kinematics. Propulsion differs between terrestrial and aquatic environments because of the physical properties and relevant forces in each medium. Previous studies examining muscle use across aquatic and terrestrial environments have observed differential muscle activation, force production, length changes, and work production depending on the environment (Kamel *et al.*, 1996; Biewener and Gillis, 1999; Gillis & Biewener, 2000; Biewener & Corning, 2001; Gillis and Blob, 2001; Carr, 2008; Foster *et al.*, 2018). The hindlimb of foot-propelled swimming birds must be able to power locomotion in both terrestrial and aquatic environments with an anatomy that may be specialized for one environment or the other; I explore this challenge by examining aquatic and terrestrial takeoffs in mallard ducks (Chapter 2). In addition, variation in length change, shortening velocity, and activation patterns between environments can affect muscle function given its force-length, force velocity, and contraction history-dependent properties. How muscle function changes with contraction history is especially important during cyclical behaviors, like walking and surface swimming, where hindlimb muscles undergo repeated, closely timed contractions. In Chapter 3, I examine how one contraction history-dependent property (post-activation potentiation) might affect force production during surface swimming and other cyclical behaviors. In assessing the function of muscle, it is important to consider the evolutionary history, anatomy, and kinematics of an animal as well as the properties of the muscle.

1.5 How Do Duck Hindlimb and Muscle Function Vary Across Behaviors and Species?

Birds, like any organism, are the product of their evolutionary history, and this context should be remembered when studying the remarkable ability of birds to inhabit and excel in a wide range of ecological niches and locomotor regimes. Birds that perform hindlimb-propelled swimming have modified the theropod hindlimb to specialize as swimmers, which permits them to swim more efficiently but comes with a penalty in terrestrial locomotion ability. When considering the tradeoffs that aquatic birds may face, we should consider the evolutionary history of their propulsive structures and how it has shaped their anatomy, the full range of kinematics and behaviors that these structures support (Chapter 4), and the demands these behaviors place on the locomotor muscles (Chapters 2 and 3).

In Chapter 2, I examined the movements of the hindlimb and function of a hindlimb muscle during aquatic and terrestrial takeoffs in mallard ducks. Unlike many other waterfowl, mallards are capable of taking off vertically from both land and water. Force production is very different in aquatic and terrestrial environments, and takeoff is a challenging behavior, so the ability of mallards to becoming airborne from both substrates is impressive. Because the hindlimb plays an important role in initiating flight, I focused on the hindlimb joint excursions (hip, knee, ankle, metatarsophalangeal) and on the lateral gastrocnemius (LG) muscle, a large ankle extensor and knee flexor.

In Chapter 3, I explored a phenomenon known as post-activation potentiation (PAP) and how it might impact LG function during cyclical behaviors like walking and swimming. PAP is contraction-history dependent change in muscle function where a muscle that has been recently activated produces higher forces and has a higher rate of force development in subsequent contractions. PAP could impact LG function during walking and swimming because cyclical

behaviors require repeated muscle activation. By using an *in situ* muscle preparation, I was able to control the LG cycle frequency, length change, and activation parameters to mimic surface swimming while measuring muscle force production and work (force x length).

In Chapter 4, I investigated how anatomy and movement relate to specialization for swimming in ducks. In the first part of Chapter 4 I examined hindlimb and pelvic anatomy across the duck evolutionary tree, predicting that more specialized swimmers (i.e., ducks that dive) would have anatomical adaptations resembling those found in other groups of specialized swimmers. I also expected to find a gradient of anatomical traits across swimming abilities, from terrestrial, to surface swimmers, to foot-propelled divers, to wing-and-foot propelled divers. In the second part of Chapter 4, I examined how body size normalized kinematics varied among surface swimmers (mallard), foot-propelled divers (scaup), and wing-propelled divers (eider) during walking, surface swimming, and diving.

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CHAPTER 2. Aquatic and terrestrial takeoffs require different hindlimb
kinematics and muscle function in mallard ducks

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2.0 Abstract

Mallard ducks are capable of performing a wide range of behaviors including nearly vertical takeoffs from both terrestrial and aquatic habitats. The hindlimb plays a key role during takeoffs from both media. However, because force generation differs in water versus on land, hindlimb kinematics and muscle function are likely modulated between these environments. Specifically, we hypothesize that hindlimb joint motion and muscle shortening are faster during aquatic takeoffs, but greater hindlimb muscle forces are generated during terrestrial takeoffs. In this study, we examined the hindlimb kinematics and *in vivo* contractile function of the lateral gastrocnemius (LG), a major ankle extensor and knee flexor, during takeoffs from water versus land in mallard ducks. In contrast to our hypothesis, we observed no change in ankle angular velocity between media. However, the hip and metatarsophalangeal joints underwent large excursions during terrestrial takeoffs but exhibited almost no motion during aquatic takeoffs. The knee extended during terrestrial takeoffs but flexed during aquatic takeoffs. Correspondingly, LG fascicle shortening strain, shortening velocity, and pennation angle change were greater during aquatic takeoffs than during terrestrial takeoffs because of the differences in knee motion. Nevertheless, we observed no significant differences in LG stress or work, but did see an increase in muscle power output during aquatic takeoffs. Because differences in the physical properties of aquatic and terrestrial media require differing hindlimb kinematics and muscle function, animals such as mallards may be challenged to tune their muscle properties for movement across differing environments.

2.1 Introduction

Navigating between terrestrial and aquatic environments presents a challenge for animals because differences in solid and fluid properties change the demands for generating propulsive forces for locomotion. As a result, movement across these media likely requires differing kinematics

and muscle function (Kamel *et al.*, 1996; Biewener and Gillis, 1999; Biewener and Corning, 2001; Gillis and Blob, 2001; Foster *et al.*, 2018). In particular, previous studies have reported differences in hindlimb kinematics and muscle function between walking and surface swimming in ducks (Biewener and Corning, 2001; Carr, 2008; Provini *et al.*, 2012A).

Mallards, *Anas platyrhynchos* (Linnaeus, 1758), are dabbling ducks that readily move between water and land, and their behavioral repertoire includes nearly vertical takeoffs in both environments (Raikow, 1973). Consequently, it is likely that mallards also face the challenge of modulating hindlimb movement and muscle function during takeoff depending on the takeoff medium, particularly because the hindlimb contributes to the first part of takeoff when the body is still in contact with the substrate. Previous work on terrestrial takeoff has examined takeoffs from solid surfaces by focusing on distal hindlimb kinematics and substrate reaction forces (Earls, 2000; Tobalske *et al.*, 2004; Berg and Biewener, 2010; Provini *et al.*, 2012B; Chin and Lentink, 2017; Crandell *et al.*, 2018) or wing musculature (Williamson *et al.*, 2001). Others have considered behaviors at the water's surface, including paddle-assisted flight (Norberg & Norberg, 1971; Gough *et al.*, 2015), mating displays (Clifton *et al.*, 2015), hydroplaning (Aigeldinger & Fish, 1995), and steaming (Gough *et al.*, 2015). However, no previous studies have examined how vertical takeoff varies between water and land, and none have reported hindlimb muscle function or the kinematics of proximal joints (hip and knee) during takeoff. Therefore, our goal here was to compare the hindlimb kinematics and *in vivo* function of the lateral gastrocnemius (LG), a major ankle extensor and knee flexor (Biewener and Corning, 2001; Clifton *et al.*, 2018), in response to differences in propulsive requirements for aquatic versus terrestrial takeoffs in mallard ducks.

Similar to other tetrapods that use their hindlimbs for jumping, in many avian species the hindlimbs play an important role in flight takeoff. When taking off from the ground or a perch, birds extend their legs as the wings unfold, with the first downstroke of the wings occurring late in

hindlimb extension or after the feet have left the ground or perch (Earls, 2000; Provini *et al.*, 2012B). Depending on the species, the hindlimbs are responsible for 59-100% of the body's velocity at the time of toe-off (Earls, 2000; Tobalske *et al.*, 2004; Berg and Biewener, 2010; Provini *et al.*, 2012B; Chin and Lentink, 2017), with the remainder made up by an active upstroke or rotations of the body (Provini *et al.*, 2012B). Because birds take off from a variety of substrates, takeoff performance may be compromised if behaviors are not adjusted to substrate properties (Crandell *et al.*, 2018). Substrate compliance can affect jumping kinematics, with more compliant substrates linked to lower takeoff velocities and larger joint excursions (Giatsis *et al.*, 2004; Astley *et al.*, 2015; Crandell *et al.*, 2018). Waterfowl face perhaps the most disparate tasks of taking off from both terrestrial and aquatic environments. Consequently, adjusting their kinematics to water and land may present challenges for hindlimb muscle tuning.

How locomotor forces are produced necessarily differs between aquatic and terrestrial environments, and these differences can lead to changes in limb kinematics and muscle shortening. On land, an animal's limbs and body are supported and accelerated by ground reaction forces, which are directly proportional to the force produced by skeletal muscles, whereas in water, hydrodynamic forces produced by propulsive appendages (in this case, the duck's feet) accelerate the body and depend on the foot's relative velocity squared (drag force) and on the acceleration of the added mass of fluid displaced by the foot (acceleration reaction force; Daniel, 1984; Biewener and Patek, 2018). Consequently, in order to increase hydrodynamic forces, the duck's feet must be moved faster (hypothesis 1 [H1]), requiring greater muscle shortening and contraction velocities (H2). This has particular importance for the function of ankle extensors, such as the LG, which power foot motion. Although active modulation could result in higher hindlimb joint velocities and muscle shortening velocities, the increased compliance of a fluid versus solid substrate can also result in larger limb

excursions under a given applied force (Hsieh, 2003; Giatsis *et al.*, 2004), potentially contributing to higher joint excursions and muscle shortening velocities.

How much propulsive force is required differs between terrestrial and aquatic habitats. On land, force must be generated both to support an animal's body weight and to accelerate the body. In contrast, while in water, body weight support is reduced or eliminated because of an animal's buoyancy. However, during aquatic takeoffs, the force required to launch the body is increased by the water's resistance to the bird exiting the water (drag on the body, surface tension) and by water entrained in a bird's plumage, increasing its effective body mass. Nevertheless, we expect that buoyancy will dominate relative to these other forces, resulting in higher forces being required for terrestrial takeoffs (H3). Consistent with this, many animals produce higher forces and increase muscle activation on land versus in water (Kamel *et al.*, 1996; Biewener and Gillis, 1999; Gillis & Biewener, 2000; Gillis and Blob, 2001; Foster *et al.*, 2018); although this pattern does not universally hold (Gillis and Blob, 2001). Of most relevance to this study, previous work has shown that the mallard LG produces higher stresses (i.e., force/fiber cross-sectional area) during walking than during surface swimming (Biewener and Corning, 2001).

Thus, we hypothesized that mallard hindlimb kinematics and LG force production would change with takeoff substrate, specifically by producing greater (H1) hindlimb joint extension velocities and (H2) muscle shortening velocities during aquatic takeoffs to generate the required hydrodynamic forces, while producing (H3) greater hindlimb muscle forces during terrestrial takeoffs to accelerate the body and support body weight. Because these differing demands for force versus velocity likely impact hindlimb muscle function, an animal's ability to balance these demands is of particular interest given that skeletal muscles are well known to exhibit a tradeoff between their force and velocity contractile properties (Hill, 1938; McMahon, 1984; Lieber, 1992; Biewener and Patek, 2018). In this study, differences between terrestrial and aquatic takeoffs in mallard ducks were

examined by measuring hindlimb kinematics of both proximal and distal joints and *in vivo* function of the LG, a major ankle extensor that generates foot motion during both aquatic and terrestrial locomotion.

2.2 Materials & methods

2.2.1 Animals & Training

Wild caught (n=8) and farm-raised (n=4) mallard ducks (Table S2.1) were fed *ad libitum* and housed in an indoor-outdoor enclosure with both terrestrial and aquatic areas. Experiments were performed during May-November. This period includes a full molt of the primary feathers in late summer that typically renders the ducks flightless for a few weeks. Animals were only studied during times when they had flight ability, either prior to molt or after the feathers had regrown. All animal procedures were approved by Harvard University's Institutional Animal Care and Use Committee under protocol number 20-09. Wild birds were collected under Massachusetts Division of Fisheries & Wildlife Permits 011.15SCB, 007.17SCB, and 093.18SCB and U.S. Fish & Wildlife Permit MB005348-0.

Birds were trained in the flight arena, a long hall with high ceilings. Training involved first acclimating the birds to two areas: the takeoff area—either a platform (0.6 m x 0.4 m) for terrestrial takeoffs or an acrylic water tank (1m x 1 m x 0.25 m depth) for aquatic takeoff—and the landing area at the other end of the hall. Birds were rewarded with treats (millet) and withdrawal of the researcher when they remained in either location. Researcher approach encouraged the birds to move from the takeoff area to the landing area. A small barrier placed at the front of the platform or tank encouraged vertical takeoffs (Figure S2.1A). The barrier height was gradually increased after successful practice trials at previous heights. During all experiments, animals were placed on or guided to the takeoff area. After a period of acclimation (30s-2 min), birds were encouraged to take

off by researcher approach, although some birds took off spontaneously. Following takeoff, birds rested in the landing area or were returned to the takeoff area for a rest period of 1-5 minutes.

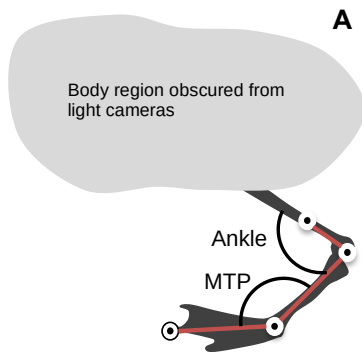
Animals trained in the flight arena readily translated their training to takeoffs in the X-ray setup, where they took off from the same platform for terrestrial takeoffs as for the light video and from a smaller tank (0.66 m x 0.4 m x 0.3 m depth) for aquatic takeoffs (Figure S2.1B). To avoid researcher exposure to the X-ray beam, motion of a glove attached to a long rod was used as a takeoff stimulus.

2.2.2 Kinematics

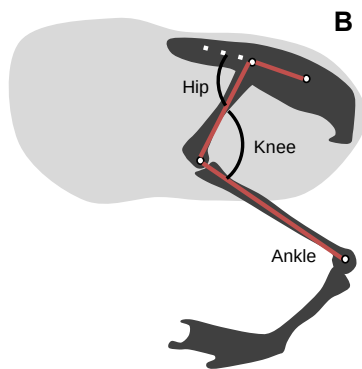
Timing

We used kinematic parameters to determine when the ‘power stroke’ of takeoff commenced and ended. All time-varying data, including *in vivo* LG recordings, are plotted relative to the kinematic power stroke, where time=0 is the beginning and time=1 is the end of takeoff power stroke. For terrestrial takeoffs, the first forward and/or upward motion of the body was considered the start of takeoff, with the end of takeoff being when the toe left the ground (i.e., toe-off). For aquatic takeoffs, we considered the first movement of the foot caudally and/or inferiorly as the start of takeoff. During aquatic takeoffs, the webbing of the foot collapses prior to the foot leaving the water, at which point in time the foot likely no longer contributes to powering takeoff as it is drawn forward in the water. We therefore considered collapse of the webbing to be the end of the aquatic takeoff power stroke. Although normalized time was used to compare relative timing of kinematics and measures of muscle function, real times (s) were used for calculating durations and velocities (joint motion duration, joint angular velocities, muscle shortening velocities, and muscle power).

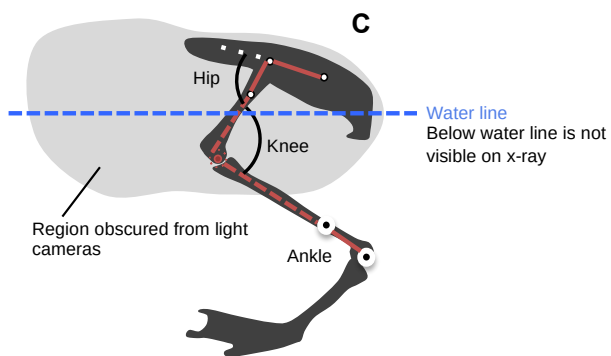
Figure 2.1. Methods for measuring joint angle changes (A-C) and LG function (D). (A-C) The key applies to panels A-C. The digitized points (indicated) were used to calculate joint angle change. (A) Markers on the tibiotarsus, ankle, MTP, and toe were visible in light video and were used to calculate ankle and MTP joint angles. (B) During terrestrial takeoff, anatomical landmarks on synsacrum, hip, femur, knee, and ankle were visible in X-ray video. These points were used to calculate knee and hip angles. (C) During aquatic takeoffs, the hip joint angle was calculated as in (B), but the water blocked the X-ray visualization of distal points. Instead, we combined light visualization of the ankle and tibiotarsus and X-ray visualization of the hip and femur. The projected directions of the tibiotarsus and femur were used to find the intersection of these bones (i.e., the knee) based on their measured lengths. Knee angle was calculated using the calculated knee position. (D) Schematic of a lateral view of the left hind leg with electrodes implanted to measure *in vivo* LG function. An outline of the hind leg is shown in light gray and the bones are shown in dark gray. The LG (pink) is a longitudinal cross-section, showing the placement of the sonomicrometry crystals (white circles) used to measure length of a fascicle segment ($L_{fas, seg}$) and pennation angle. The location of the EMG electrodes is shown (x) but electrodes were placed medially or laterally to the sonomicrometry plane, not in the same plane. Placement of the tendon buckle (black E shape) on the LG tendon to measure LG forces is also shown.



Ankle & MTP: Aquatic & Terrestrial



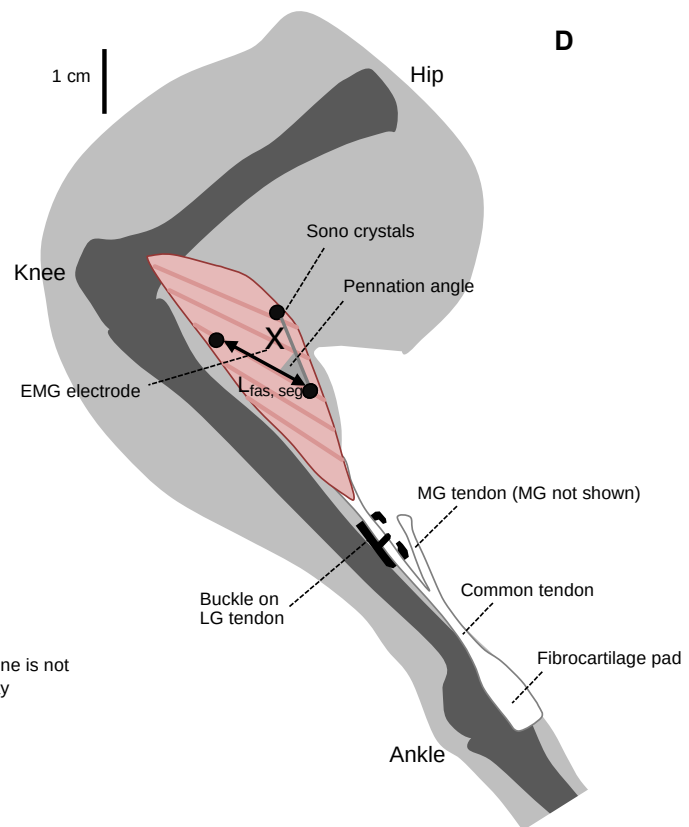
Hip & Knee: Terrestrial



Hip & Knee: Aquatic

Key for Digitizing Figures (left)

- Anatomical landmark visible on X-ray
- Marker visible for light camera
- Calculated
- Observable Segment
- - - Projected Segment



Ankle & Metatarsophalangeal Joint

Takeoffs were elicited in the flight arena, as described above. We used high-speed light video to measure movement of the externally visible portions of the leg (distal tibiotarsus, tarsometatarsus, and foot; Figure 2.1A & Movie 1; N=5 individuals, n=15 trials, three trials per individual for each takeoff substrate). Takeoffs were recorded at 250 fps (shutter 1-1.5 ms) using IDT NR5-S1 cameras (Integrated Design Tools, Inc., Pasadena, CA, USA) equipped with Nikon AF Nikkor 24-85 mm zoom lenses (Nikon Inc., Melville, NY, USA). We used a wand of known length to calibrate the 3D space using easyWand5 (Theriault *et al.*, 2014). The tip of the toes, metatarsophalangeal (MTP) joint, ankle, and the distal tibiotarsus (Figure 2.1A) were marked with white latex and black marker pen, and these points were digitized using DLTdv6 (Hedrick, 2008) to calculate MTP and ankle joint angles. Note that in the example movie (Movie 1), the camera view is oblique rather than perfectly perpendicular since two cameras with a lateral view were used to capture 3D kinematics (Figure S2.1A).

Hip & Knee

We used videoradiography to examine the proximal joints for each type of takeoff (N=3 individuals, n=6 trials, two trials per individual for each condition) because these joints are not visible or well-identified using light video. We recorded lateral view X-ray images from a C-arm (Model 9400, OEC-Diasonics Inc.; modified by Radiological Imaging Services) using a high-speed Photron Fastcam 1024 PCI camera (Photron USA, Inc., San Diego, CA, USA, 500 fps, shutter 1 ms). These images were de-distorted using a grid and XMALab (v. 1.4.0; Knörlein *et al.*, 2016).

For terrestrial takeoffs, we performed a 2D analysis using anatomical landmarks to identify the synsacrum, hip, knee, and ankle, so that hip and knee joint angles could be calculated (Figure 2.1B). However, the water tank used for aquatic takeoffs was sufficiently large that the X-ray beam

could not penetrate the water, blocking views of the knee and ankle joints. However, the synsacrum and proximal femur were above the water line and thus visible on X-ray video. We combined light video using the IDT cameras (125 fps) synchronized with the X-ray video to calculate the location and angle of the knee (Figure 2.1C). Using a metal calibration object in view of both the light and X-ray videos, the light video of the distal leg was aligned with the X-ray recordings of the proximal leg. 3D coordinates from the light video were then projected onto a plane parallel to the X-ray plane. The X-ray recordings yielded locations for the synsacrum and hip and the direction of the femur. The light video recordings yielded the location of the ankle and the direction of the tibiotarsus. The locations and angle of the knee were then calculated using these digitized points and measured lengths of the femur and tibiotarsus (Figure 2.1C).

Splay Correction for X-ray Analysis

Unlike the 3D analysis used to calculate the ankle and MTP joint angles, the planar X-ray video analysis used to calculate the hip and knee joint angles is susceptible to errors caused by out-of-plane motion (Kambic *et al.*, 2014). Because the mallard femur is splayed relative to the body midline, we measured splay angle (α) for each individual and used it to calculate a duck-specific correction factor. Specifically, we took the x, y coordinates for the synsacrum, hip, knee, and ankle derived from X-ray recordings as described above. We then calculated a z-coordinate (i.e. estimated distance out of plane due to splay) for each point based on splay angle (α) for each individual. The z-coordinate for the synsacrum and hip were set to 0 since they were defined as in-plane. The z-coordinate for the knee was $L_F \sin(\alpha)$, where L_F is the length of the femur. The z-coordinate for the ankle was represented by z_a . We used the x, y, z coordinates for each point to define the vectors along each bone: synsacrum (S), femur (F), and tibiotarsus (T).

$$S = \langle U_S, V_S, W_S \rangle = \langle x_h - x_s, y_h - y_s, 0-0 \rangle$$

$$F = \langle U_F, V_F, W_F \rangle = \langle x_k - x_h, y_k - y_h, L_F \sin(\alpha) - 0 \rangle$$

$$T = \langle U_T, V_T, W_T \rangle = \langle x_a - x_k, y_a - y_k, z_a - L_T \sin(\alpha) \rangle$$

where U, V, and W are the vector components and subscripts s, h, k, and a represent the synsacrum, hip, knee, and ankle, respectively. All of these values could be calculated from known values except for W_T , which was dependent on z_a , which we had not calculated. The magnitude of the T vector is the length of the tibiotarsus (L_T), which we also measured. Thus, we calculated W_T as follows:

$$L_T = \sqrt{U_T^2 + V_T^2 + W_T^2}$$

$$W_T = \sqrt{L_T^2 - U_T^2 - V_T^2}$$

With all vectors defined, we then calculated the joint angles as the angle between vectors.

$$\Theta_{\text{knee}} = 180 - \cos^{-1}[F \cdot T / (L_F \cdot L_T)]$$

$$\Theta_{\text{hip}} = \cos^{-1}[S \cdot F / (L_S \cdot L_F)]$$

The splay correction allowed our otherwise 2D analysis to more accurately determine the hip and knee angles. Although unaccounted for out-of-plane motion could produce errors in our measured knee and hip angles, these errors are likely small (i.e., a 10° change in the splay angle would result in $< 1^\circ$ error in the observed angular excursions of the hip and knee joints) and are unlikely to produce changes in kinematic patterns that we observed (see Results).

Filtering

To account for digitizing errors, raw kinematics traces were smoothed in Python (v. 3.7) by applying a 4th order low pass Butterworth filter with a cutoff frequency of 20 Hz for the hip, knee, and ankle joints and 50 Hz for the MTP joint. Kinematics timescales were normalized to the duration of the power stroke, and the data were interpolated in 100 time steps during the power stroke to average across trials and individuals.

2.2.3 *In vivo* Muscle Measurements:

Surgery

In vivo LG function was measured during aquatic and terrestrial takeoffs in N=10 individuals. We anesthetized (via mask induction) the animals using a 1-2% isoflurane: oxygen gas mixture. Three 2.0 mm sonomicrometry crystals (Sonometrics Corporation, London, Ontario, Canada) were implanted in the LG, one at each end of a mid-belly fascicle and one proximal to, but in-line with, the crystal at the superficial end of the fascicle (Figure 2.1D). The crystals and lead wires were secured with 5-0 silk suture (Ethicon, Cincinnati, OH, USA). This configuration of crystals allowed us to measure length changes along the fascicle and calculate pennation angle using the law of cosines (Figure 2.1D). We also implanted silver fine wire hook electrodes (0.1 mm, with 0.5 mm exposed tips and 2-3 mm spacing, California Fine Wire Company, Grover Beach, CA, USA) to obtain electromyography (EMG) recordings. An E-shaped tendon buckle was sutured in place on the LG portion of the gastrocnemius tendon, proximal to where the medial gastrocnemius (MG) tendon joins with the LG tendon to form the common tendon (Figure 2.1D). The tendon buckle design followed Biewener & Corning (2001) and was built by mounting a strain gauge (Type FLA-1-11, Tokyo Sokki Kenkyujo Co., Ltd., Tokyo, Japan) on the middle arm of an E-shaped piece of 1 mm thick stainless-steel. After soldering 36 gauge lead wires and insulating with oven-cured epoxy (AE-10, Micromasurements Group, Inc., Raleigh, NC, USA), the transducer was subsequently coated with polyurethane M-coat A (Micromasurements Group, Inc.) to minimize tissue reaction. All transducer lead wires were passed subcutaneously where they emerged to an external custom-designed 12 pin miniature connector that was secured to the animal's back with 2-0 Vicryl sutures (Ethicon, Cincinnati, OH, USA) between the wings. This connector location allowed us to disconnect the birds from the cables between recording bouts and ensured that the connectors and cables did not inhibit motion of the wings or feet.

Data Collection

Birds were permitted to recover from surgery overnight. Animals were administered flunixin (4 mg kg⁻¹, intramuscularly pectoralis) every 12 hours for the duration of the experiment following surgery (2-3 days). After recovery, we recorded animals performing terrestrial and aquatic takeoffs; takeoffs were elicited as described in the “Animals and Training” section. Signals were transmitted from the animal’s back connector via ~8 m shielded multi-lead cable to recording equipment located outside of the flight arena. The sonomicrometry signals were recorded using a Sonometrics Digital Ultrasonic Measurement System (TR-USB Series 8) and sonoLAB data acquisition software (Sonometrics Corporation, London, Ontario, Canada). The tendon buckle signal was passed through a bridge amplifier (Vishay Instruments, Raleigh, NC, USA), and the EMG signals were passed through Grass P-511 Series amplifiers (Grass Instruments, West Warwick, RI, USA) before being recorded at 5000 Hz by a BIOPAC MP150 data acquisition system (BIOPAC Systems, Inc., Goleta, CA, USA) using the accompanying AcqKnowledge software (v. 4.1.1). High-speed light videos of the takeoffs were also recorded as described above. A voltage pulse trigger that illuminated an LED light in the cameras’ fields of view was used to synchronize across software programs and video. This resulted in synchronized kinematics (MTP and ankle joints) and LG force, EMG, fascicle length, and pennation angle. Due to the challenging nature of the experiments, not all data were obtained for all individuals. A list of animals used and the data collected from each is provided in the supplemental information (Table S2.1).

Following data collection, animals were sacrificed, and the hindlimb was dissected. The tendon force buckle was calibrated by removing the common gastrocnemius tendon from the ankle, distal to the thick fibrocartilage pad. Kevlar thread (DuPont de Nemours, Inc.) was tied proximal to the fibrocartilage pad and attached to a calibrated piezoelectric force transducer (model 9203, Kistler

Instrument Corporation, Amherst, NY, USA) or an ergometer lever arm (Aurora Scientific Inc., Aurora, Ontario, Canada). By first freezing the aponeurosis with liquid nitrogen and then pulling on the instrumented tendon or by activating the muscle, we simultaneously recorded the tendon buckle voltage output with the force transducer or lever arm output to obtain dynamic calibrations of tendon force; least-squares linear regression fits had $r^2 > 0.94$ ($p < 0.05$). Following calibration, we dissected the hindlimb to confirm the alignment of the sonomicrometry crystals and to measure resting LG fascicle length, resting pennation angle, and muscle mass. The muscle was bisected along the muscle midline, revealing the locations of the implanted sonomicrometry crystals. Length measurements were made with calipers to a precision of 0.1 mm and angles were measured with a swing arm protractor. The distance between the crystals (inter-crystal distance) and the full length of the fascicle in which the crystals were implanted (resting fascicle length) were measured. In order to calculate a mean fascicle length and pennation angle, measurements were taken at 3-6 midline fascicles distributed along the proximo-distal axis of the muscle.

Data Processing

Sonomicrometry crystals were implanted mid-belly to record from as large a portion of the LG muscle's fascicle length as possible (Figure 2.1D). Measurements of fascicle strain were obtained from the fascicle segment recordings. Resting fascicle length and inter-crystal distance were measured post-mortem, and we multiplied the ratio of these distances (fascicle length/inter-crystal distance) to convert the sonomicrometry output to fascicle length. Fascicle strain was calculated as (instantaneous length – resting length)/ resting length. Fascicle shortening velocity was calculated from smoothed (4th order low-pass Butterworth filter with 30 Hz cutoff) fascicle length data over the duration of shortening and normalized by resting fascicle length to $L \cdot s^{-1}$.

Muscle-tendon force was filtered using a 4th order low-pass Butterworth filter with a cutoff frequency of 20 Hz. Muscle physiological cross-sectional area (PCSA in cm²) was calculated as $(\cos(\alpha) * m) / (\rho * l_{fas})$ where α is the pennation angle (Figure 2.1D), m is muscle mass (kg), ρ is density (assumed to be $1.06 * 10^{-3}$ kg cm⁻³), and l_{fas} is fascicle length (cm). Muscle stress (kPa) was calculated by dividing force by PCSA. Muscle work (J) was calculated as the area under the force vs. fascicle length curve for the portion of takeoff when both force was being produced and the fascicles were shortening. Work was converted to mass-specific work by dividing by muscle mass. Average mass-specific muscle power was calculated as work / (time * muscle mass).

2.2.4 Statistics

Statistical tests were performed in R (v. 3.6.0). All reported values are mean $\pm$ s.e.m., with significance based on $\alpha = 0.05$. We first obtained the average of all measurements for each individual and then plotted and performed statistical tests on the individual averages, which allowed us to account for the different number of trials per individual and/or permitted paired comparisons between aquatic and terrestrial takeoffs. Prior to performing t-tests, normality was verified using a Shapiro-Wilk test. If this assumption was not violated ($p > 0.05$), we compared the aquatic and terrestrial takeoffs using one-tailed t-tests. If the normality assumption was violated (Shapiro-Wilk $p < 0.05$), we instead used a one-tailed, Wilcoxon nonparametric test. Kinematics were compared using paired tests. LG measurements were compared using unpaired tests. For a subset of animals, we recorded from the LG during both aquatic and terrestrial takeoffs. For some measures with sufficient samples with paired data, we also tested for differences using paired, one-tailed t-tests or Wilcoxon tests, as appropriate, to take into consideration large differences between individuals. All figures display data from all individuals and report paired statistics for kinematics and unpaired

statistics for LG measures; any statistics that result from paired tests for LG measures are indicated in the text.

2.3 Results

2.3.1 Kinematics during terrestrial versus aquatic takeoffs

The kinematics of aquatic versus terrestrial takeoffs differed substantially (Movie 1). Qualitatively, during terrestrial takeoffs leg extension coincided with wing upstroke and there was very little overlap with wing downstroke. Many, but not all, terrestrial takeoffs were preceded by a counter-movement, where the wings remained folded, legs were very flexed, and the head was drawn near the body by flexing the neck. The neck began to extend as the wings unfolded and the body angle shifted from horizontal to vertical. Neck extension and shifts in body angle continued throughout the remainder of takeoff. As the legs began to extend, the wings were elevated dorsally and cranially in upstroke. At the point of toe-off, the wings changed direction and were depressed ventrally and slightly caudally for downstroke, the neck was fully extended, and the body angle was nearly vertical. In contrast, during aquatic takeoffs, leg extension coincided with ventral depression of the tail and wing downstroke. Prior to takeoff, the legs were drawn to a very flexed position such that the foot was parallel with and very near to the water's surface. The wings were drawn cranially. The wings were oared through the water with ventral and caudal motion, nearly synchronously with the extension of the leg and ventral depression of the tail. As in terrestrial takeoff, the neck extended and the body angle shifted from horizontal to nearly vertical throughout the takeoff. The wings, tail, and legs reached their final position quickly to propel the bird out of the water; once clear of the water, the wings were elevated dorsally and flapping commenced. The feet also paddled once clear of the water, occasionally striking the water at the surface or, if the water was cleared, cycling mid-air.

Power stroke duration of aquatic takeoffs (0.13 ± 0.018 s) did not differ significantly ($p = 0.087$, Table 2.1) from terrestrial takeoffs (0.16 ± 0.012 s). Because each limb joint only moves during a portion of the power stroke (Figure 2.2), we also compared the duration of motion for each joint, with motion onset based on when joint angular velocity exceeded zero (or fell below zero for the knee during aquatic takeoffs) and motion ending when velocity returned to zero. No significant differences were found for the duration of joint motion between aquatic and terrestrial takeoffs for any of the joints measured ($p > 0.05$, Figure 2.3A, Table 2.1).

Hindlimb kinematics of individual joints differed between terrestrial and aquatic takeoffs. Terrestrial takeoffs involved extension of all hindlimb joints (Figure 2.2). In contrast, during aquatic takeoffs hip (Figures 2.2A & 2.3B) and MTP (Figures 2.2D & 2.3B) joint angles changed very little; angular excursions at these joints were significantly lower than for terrestrial takeoffs (Table 2.1), resulting in significantly lower angular velocities at these joints during aquatic takeoffs (Figure 2.3C, Table 2.1). The direction of knee motion reversed, with the knee being extended during terrestrial takeoffs but flexed during aquatic takeoffs. This change in direction resulted in significantly different knee angular excursions (Figures 2.2B & 2.3B; Table 2.1) and velocities (Figure 2.3C; Table 2.1), although the magnitudes of the excursion ($p = 0.370$) and velocity ($p = 0.450$) were not different. The ankle underwent a larger angular excursion during aquatic than terrestrial takeoffs (Figures 2.2C, 2.3B; Table 2.1), but no significant difference was observed in ankle extension angular velocity (Figure 2.3C; Table 2.1).

Figure 2.2. Hindlimb joint kinematics differ for aquatic versus terrestrial takeoffs. (A) Hip, (B) knee, (C) ankle, and (D) MTP joint angles (mean (solid line) $\pm$ s.e.m. (shading)) during aquatic (blue) and terrestrial (black) takeoff plotted against the proportion of the takeoff power stroke, as determined by kinematic analysis (see methods for more detail). Hip and knee angles were calculated from two trials each for three individuals (n=6 trials) for aquatic and terrestrial takeoffs. Ankle and MTP averages were calculated for three trials for each condition from five individuals (n=15 trials).

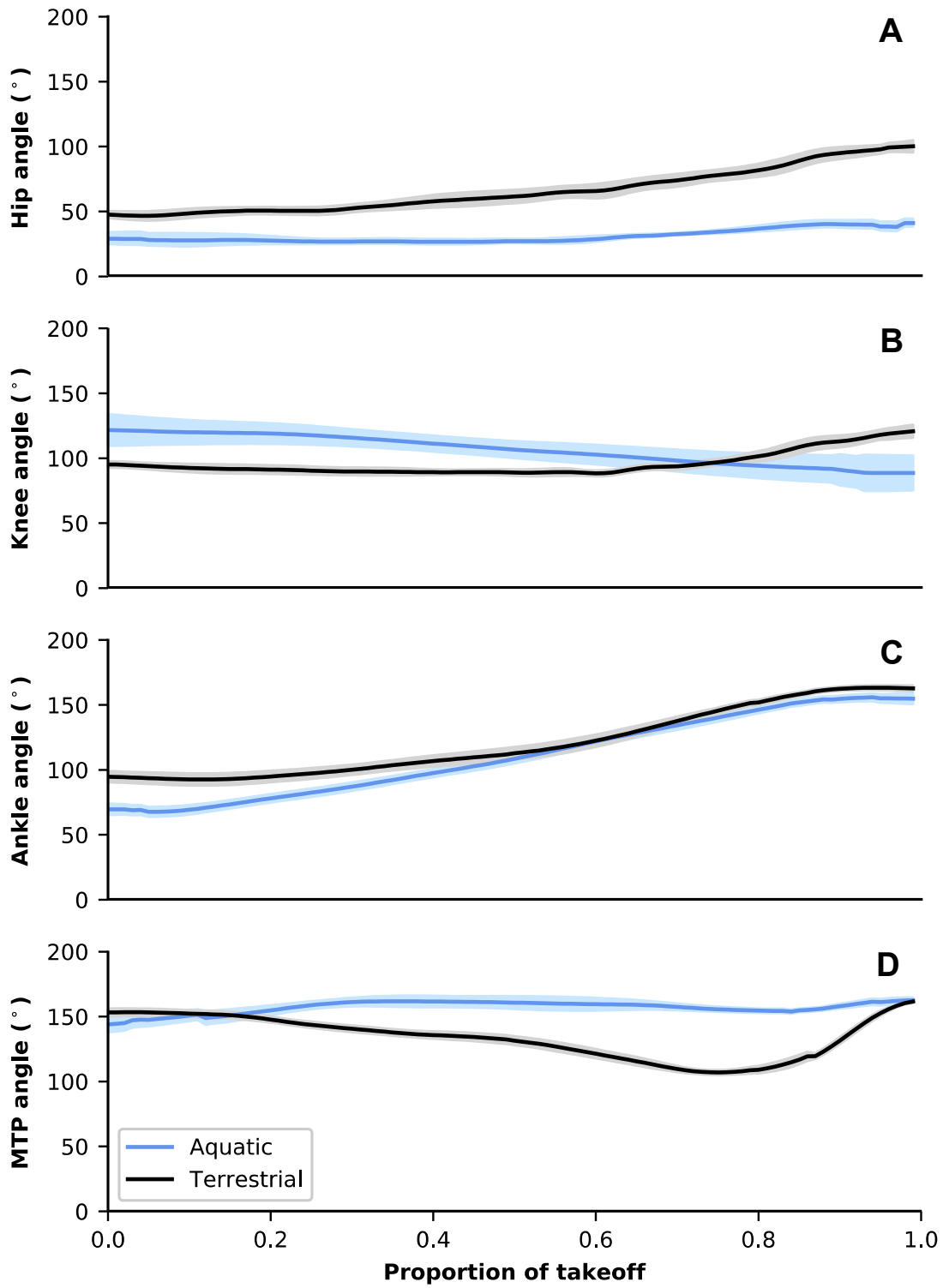


Figure 2.3. Kinematic parameters varied between aquatic and terrestrial takeoffs for some joints but not others. Comparisons by joint of (A) duration of movement, (B) angle changes, and (C) angular velocity during aquatic (blue) and terrestrial (white) takeoffs. Boxplots show the median (thick line), upper and lower quartiles, and highest and lowest values (vertical lines), excluding outliers. Hip and knee data were calculated from two trials each for three individuals (n=6 trials) for aquatic and terrestrial takeoffs. Ankle and MTP averages were calculated for three trials for each condition from five individuals (n=15 trials). Significant differences found using one-tailed t-tests or Wilcoxon tests are denoted (*, $p < 0.05$; see Table 2.1). n.s., not significant.

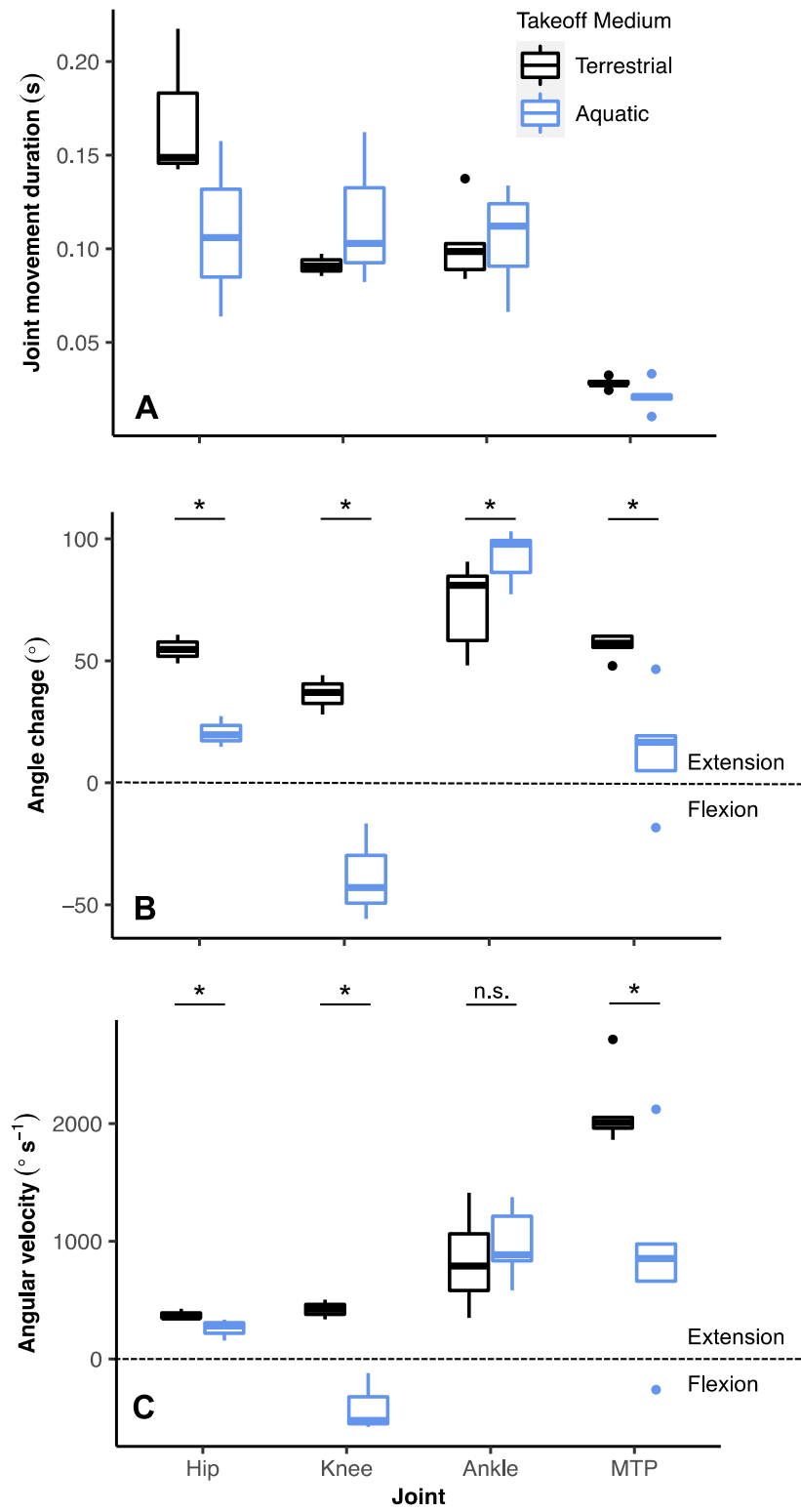


Table 2.1. Comparisons of total power stroke duration and hindlimb joint motion duration, angular excursion, and velocity by joint during terrestrial and aquatic takeoffs.

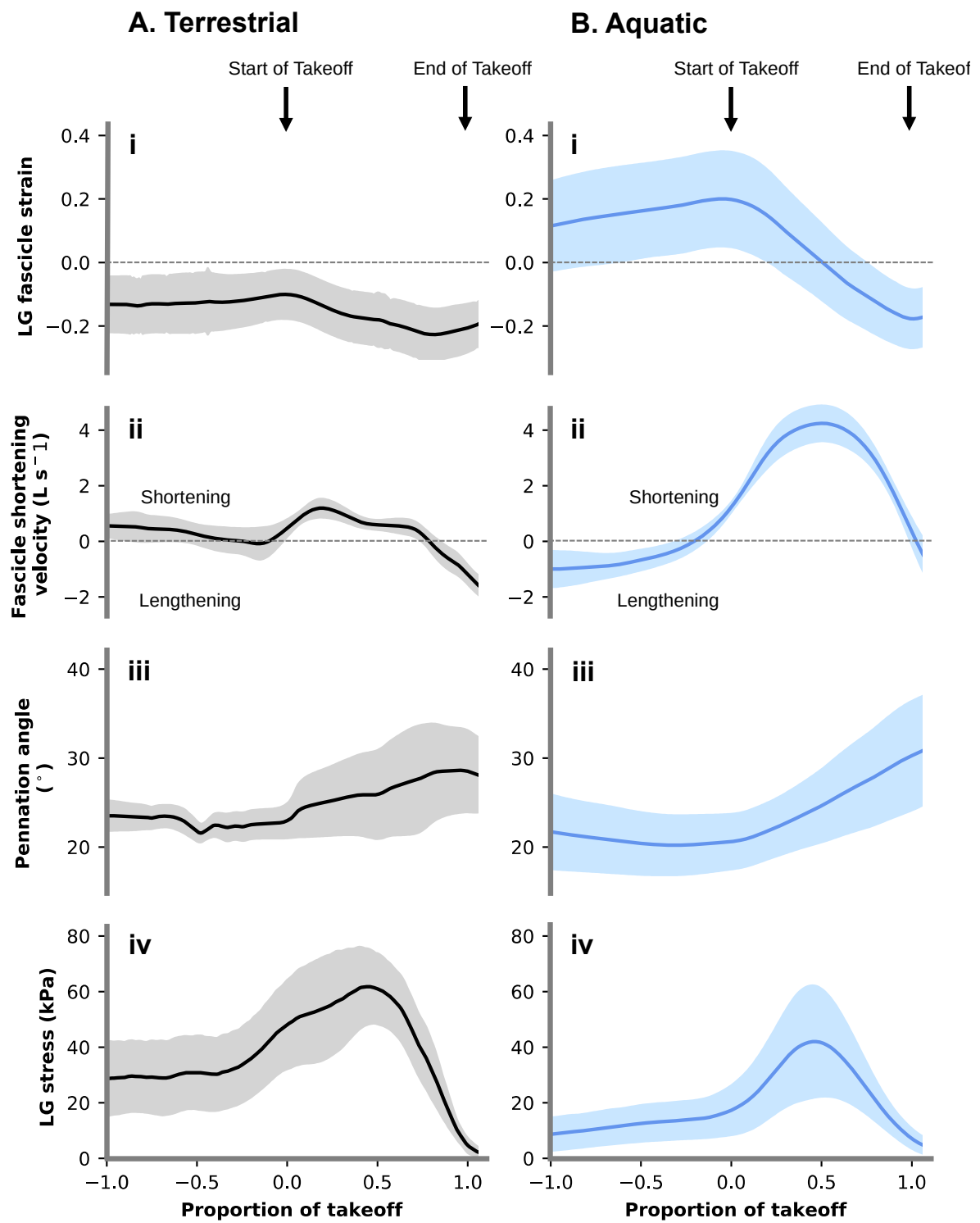
	Duration (s)	Angular Excursion (°)	Angular Velocity (° s ⁻¹)
Total Power Stroke			
Terrestrial	0.16 ± 0.012		
Aquatic	0.13 ± 0.018		
p-value	0.087		
Hip			
Terrestrial	0.170 ± 0.024	54.8 ± 9.3	375 ± 26
Aquatic	0.109 ± 0.069	20.6 ± 3.7	257 ± 53
p-value	0.234	0.016	0.038
Knee			
Terrestrial	0.091 ± 0.035	36.4 ± 4.7	421 ± 48
Aquatic	0.116 ± 0.036	-38.5 ± 11.5	-406 ± 143
p-value	0.462	0.022	0.024
Ankle			
Terrestrial	0.102 ± 0.021	72.6 ± 18.3	840 ± 414
Aquatic	0.105 ± 0.027	92.8 ± 10.7	979 ± 316
p-value	0.853	0.031	0.238
MTP			
Terrestrial	0.028 ± 0.003	56.2 ± 5.0	2120 ± 340
Aquatic	0.021 ± 0.008	13.8 ± 23.6	870 ± 851
p-value	0.149	0.011	0.022

Bolded p-values indicate that the paired, one-way t-test showed a significant difference between aquatic and terrestrial takeoffs.

2.3.2 LG function during terrestrial versus aquatic takeoffs

Average time-varying patterns of LG fascicle strain, fascicle shortening velocity, pennation angle, and stress for aquatic and terrestrial takeoff trials are shown in Figure 2.4. Representative traces from an individual with nearly complete data sets are shown in Fig. S2.2, including activation (EMG). A terrestrial trial from a second individual is also displayed, demonstrating the variation in the timing of force production among individuals (Figure S2.2). The rapid lengthening of the LG fascicle prior to takeoff (Figure S2.2A) corresponds to a countermovement (example in Movie 1). However, because not all animals used a large countermovement, this lengthening pattern was not observed in the LG strain pattern averaged across individuals (Figure 2.4A). Similarly, the shortening observed prior to force production for the example terrestrial takeoff (Figure S2.2A, black traces) was not observed across all individuals (Figure 2.4A, Figure S2.2A gray traces), and we suspect this shortening results from a shift in body orientation that flexes the knee in preparation for takeoff during this trial. Note that LG fascicles operate at greater strains during aquatic takeoffs due to the extremely flexed position of the ankle joint and, on average, LG stress is higher prior to terrestrial takeoffs due to weight support (Figure 2.4). Normalized timing of pennation angle change (terrestrial (T): $n=6$; aquatic (A): $n=5$), fascicle shortening (T: $n=7$; A: $n=6$), force production (T: $n=5$; A: $n=4$), and EMG activity (T: $n=6$, A: $n=2$) are shown in Figure 2.5A. When comparing the onset and ending of each of these measures, we found that LG fascicle shortening ($p=0.022$) and force production ($p=0.004$) began earlier and force production ended earlier ($p=0.043$) during terrestrial takeoffs. All other timing parameters, including the onset and ending time of LG pennation angle change and EMG activity, the ending time for fascicle shortening, and the duration of all four measures, did not differ significantly with takeoff medium ($p > 0.05$).

Figure 2.4. LG muscle function differs between aquatic and terrestrial takeoffs. Mean (solid lines) $\pm$ s.e.m. (shaded regions) time varying patterns of LG (i) fascicle strain (T: n=6, A: n=6), (ii) fascicle velocity (T: n=6, A: n=5), (iii) pennation angle (T: n=6, A: n=5), and (iv) stress during (T: n=4, A: n=4) (A) terrestrial and (B) aquatic takeoffs. Data are plotted against the proportion of takeoff, with the start and stop times of takeoff determined from kinematics (see methods for more detail). The gray box indicates the power stroke of takeoff. Negative velocities indicate fascicle lengthening while positive velocities indicate fascicle shortening.



The ranges of LG fascicle strains used during terrestrial versus aquatic takeoffs are shown in Figure 2.5B. When comparing data from all animals (T: n=7; A: n=6), the maximum strains (T: -0.05 ± 0.08 ; A: 0.15 ± 0.08) and minimum strains (T: -0.27 ± 0.07 ; A: -0.26 ± 0.06) did not significantly differ between aquatic and terrestrial takeoffs (maximum strain: $p = 0.059$, minimum strain: $p = 0.223$), but both maximum ($p = 0.016$) and minimum fascicle strain ($p = 0.006$) were significantly greater during aquatic takeoffs when based on paired t-tests ($n = 5$). In addition, the magnitude of LG shortening strain was significantly lower ($p = 0.011$) for terrestrial (0.22 ± 0.07) compared with aquatic (0.41 ± 0.06) takeoffs (Figure 2.6B). Given the similar shortening durations (Figure 2.5A), LG fascicle shortening velocity was also significantly greater ($p = 0.004$) during aquatic (4.12 ± 0.82 L s⁻¹, n=6) versus terrestrial (1.41 ± 0.17 L s⁻¹, n=6) takeoffs (Figure 2.6C). Pennation angle change (Figure 2.6D) was significantly larger during aquatic takeoffs than terrestrial takeoffs when compared using a paired t-test (difference: $4 \pm 1^\circ$, $p = 0.018$, n=4), although this difference was not observed when using an unpaired t-test and all animals ($p = 0.127$; T: $10 \pm 2^\circ$, n=7; A: $15 \pm 4^\circ$, n=4).

There was no significant difference in the LG stresses during terrestrial (79 ± 12 kPa) versus aquatic (53 ± 15 kPa) takeoffs ($p = 0.116$; T: n=4, A: n=4; Figure 2.6A). LG mass-specific work (T: 8 ± 1 J kg⁻¹; A: 15 ± 5 J kg⁻¹) did not differ significantly between media ($p = 0.1$); however, there was a significant difference ($p = 0.05$) in average muscle power output (T: 43 ± 3 W kg⁻¹; A: 172 ± 72 W kg⁻¹; sample sizes for work and power T: n=3, A: n=3; Figure 2.6E,F).

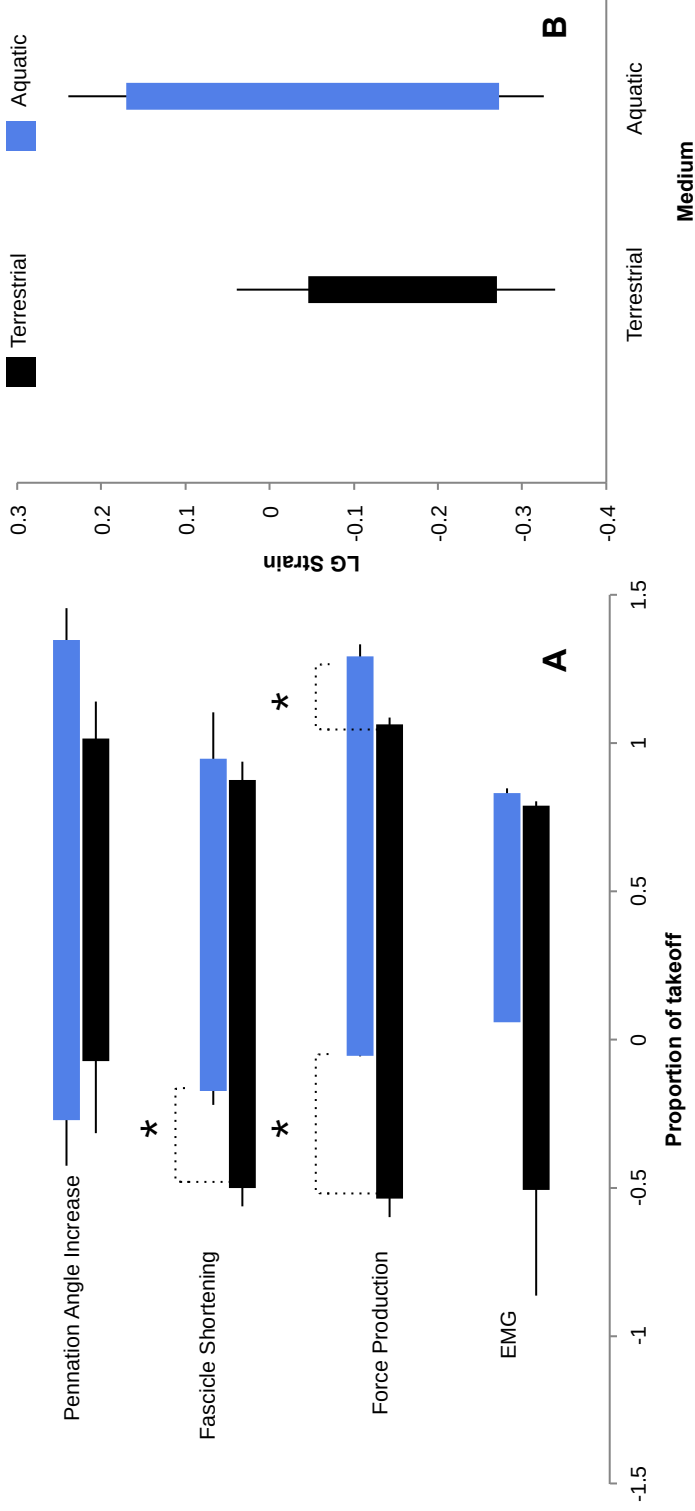


Figure 2.5. LG timing parameters and length changes vary between takeoff media. (A) Relative timing of changes in pennation angle (terrestrial (T): $n=6$, aquatic (A): $n=5$), fascicle shortening (T: $n=7$; A: $n=6$), force production (T: $n=5$; A: $n=4$), and EMG activity T: $n=6$, A: $n=2$). Times were compared using unpaired t-tests or Wilcoxon tests. Force production began ($p=0.004$) and ended ($p=0.043$) significantly earlier during terrestrial takeoffs. Shortening also began earlier during terrestrial takeoffs ($p=0.022$). All other start and end times and durations were not significantly different ($p>0.05$). (B) LG fascicle strains (i.e., normalized lengths) observed during aquatic versus terrestrial takeoffs. Maximum strain ($p=0.059$) and minimum strain ($p=0.223$) were not significantly different when considering all animals (T: $n=7$; A: $n=6$, data shown), but both the maximum strain ($p=0.016$) and minimum strain ($p=0.006$) were larger for aquatic takeoffs versus terrestrial takeoffs in animals for which we have paired data ($n=5$), using a paired t-test. Values are mean $\pm$

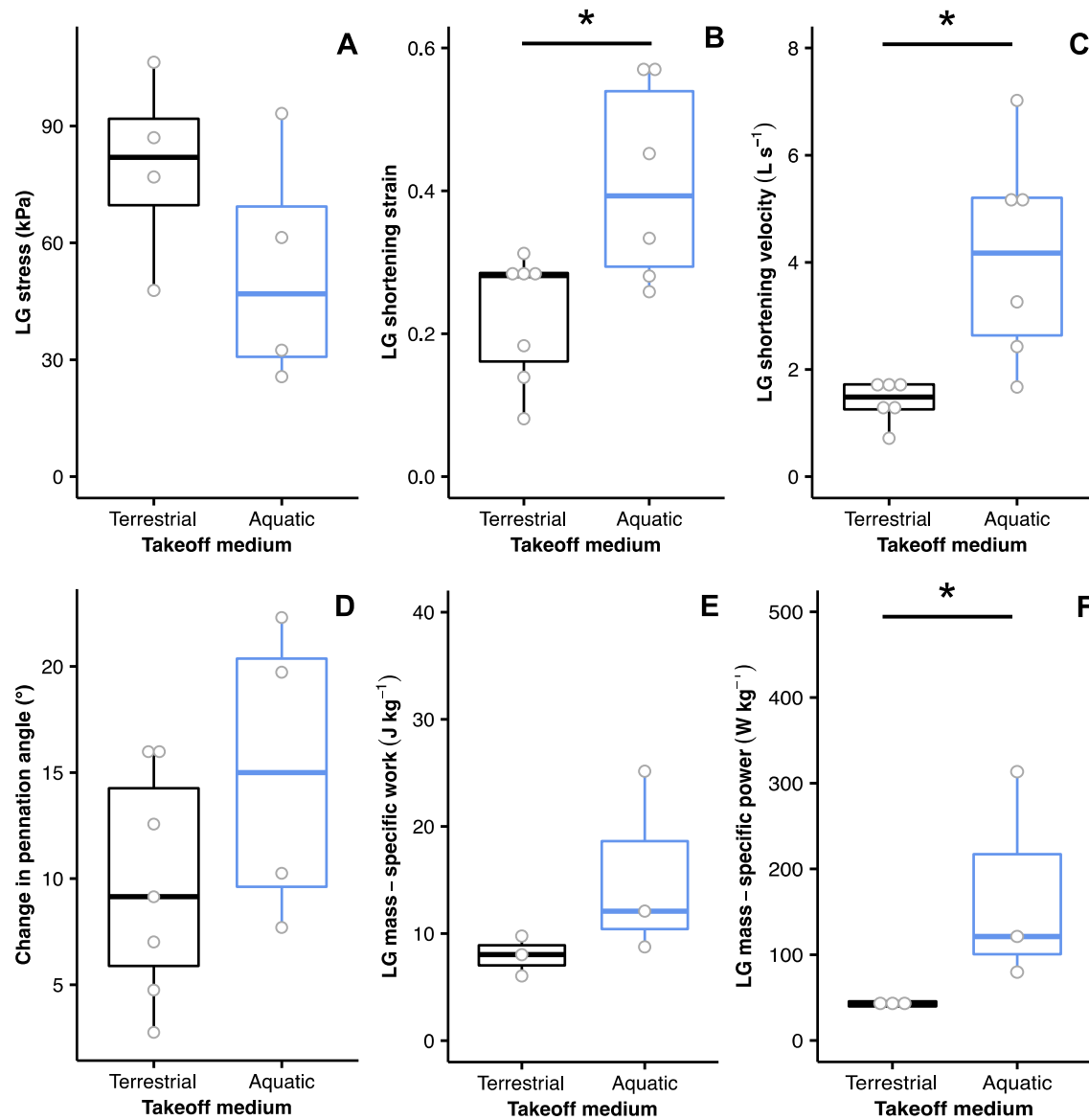


Figure 2.6. *In vivo* LG function differs for some key contractile parameters between aquatic and terrestrial takeoffs. Boxplots showing LG (A) stress (T: n=4 individuals; A: n=4), (B) shortening strain (T: n=7, A: n=6), (C) shortening velocity (T: n=6; A: n=6), (D) pennation angle change (T: n=7; A: n=4), and mass-specific (E) work and (F) power (T: n=3; A: n=3) for aquatic (blue) and terrestrial (black) takeoffs. Whereas muscle stress did not differ significantly ($p=0.116$), fascicle shortening strain ($p=0.011$) and velocity ($p=0.004$) were significantly larger for aquatic versus terrestrial takeoffs. Pennation angle change was not significantly different when compared using an unpaired t-test ($p=0.127$, plotted above) but pennation angle change was significantly larger during aquatic takeoffs than terrestrial takeoffs when compared using a paired t-test ($p=0.018$, $n=4$). Work was not significantly different between takeoff

Figure 2.6 (*Continued*)

media ($p=0.2$), but power was significantly higher during aquatic takeoffs ($p=0.05$). Boxplots show the median (thick line), upper and lower quartiles, and highest and lowest values (vertical lines), excluding outliers. Averages for individual ducks are shown as open circles. Significant differences between aquatic (blue) versus terrestrial (black) takeoffs found using 1-tailed t-tests or Wilcoxon tests are denoted (*, $p < 0.05$; see Table 2.1).

2.4 Discussion

Animals that regularly move between aquatic and terrestrial environments must be able to manage the different physical properties of fluids and solids. On land, ground reaction forces are used to support or accelerate an animal's body and are proportional to the forces produced by an animal's muscles. However, in water, propulsive forces are hydrodynamic and depend on the square of the velocity of the body part moving through the water. We, therefore, expected to see differences in both hindlimb kinematics and muscle function of mallards to accommodate differences in environment during takeoff. Specifically, we hypothesized that (H1) hindlimb joints would move at higher angular velocities during aquatic takeoffs than terrestrial takeoffs, that (H2) higher angular velocities at the ankle joint would be powered by higher LG shortening velocities, and that (H3) the LG would produce greater forces during terrestrial than aquatic takeoffs. We did not find support for the hypothesis that mallard hindlimb joints move at higher angular velocities in water (H1, Figure 2.3C); however, we did observe substantial differences between the kinematics of aquatic versus terrestrial takeoffs. Of particular interest, the knee reversed its motion from flexion during aquatic takeoffs to extension during terrestrial takeoffs with, unexpectedly, no change in ankle angular velocity (Figures 2.2 & 2.3). The change in knee kinematics reflects the underlying difference in LG muscle function, which shortens at higher velocities during aquatic takeoffs than terrestrial takeoffs (H2, Figure 2.6C). Finally, although two of the three animals for which we have paired data produced greater muscle stress during terrestrial compared with aquatic takeoffs, overall,

the difference in LG muscle stress was not significant (Figure 2.6A) and failed to support our hypothesis regarding LG force (H3).

2.4.1 Takeoff kinematic patterns & implications for muscle length change

One way to address the challenge of moving on or through media with differing properties is through different kinematics. Changes in kinematics represent a combination of active control and passive interactions with the environment (Gillis & Biewener, 2000).

Our study of takeoff performance, facilitated by X-ray video analysis, confirms changes in mallard hindlimb kinematics, particularly at the knee, between the two media. The knee joint reverses its direction of rotation, extending during terrestrial takeoffs but flexing during aquatic takeoffs (Figures 2.2, 2.3, 2.7). The overall excursion and angular velocity for each type of takeoff is the same but in opposite directions. Knee extension causes cranial motion of the foot while knee flexion and extension at the hip, ankle, and MTP joints cause caudal motion (Figure 2.7). Thus, knee flexion during aquatic takeoffs contributes to caudal motion of the foot in the water caused by ankle extension, rather than opposing it (Figure 2.7), allowing the knee and ankle joints to work together to enhance foot motion. Similarly, aquatic specialist frog species tend to reduce knee extension during swimming to prevent it from counteracting the propulsive foot motion induced by ankle extension (Richards, 2010). We expect that the change in knee joint rotation was the result of active control, acting to increase foot velocity and hydrodynamic force production. Knee extensors almost certainly contribute to terrestrial takeoff by helping to accelerate the body's center of mass off the ground. Knee flexors (including the LG) likely contribute to knee motion during aquatic takeoff but not terrestrial takeoff. Although the LG is active during both aquatic and terrestrial takeoffs, antagonist activity of knee extensors could prevent the LG from causing knee flexion during terrestrial takeoff.

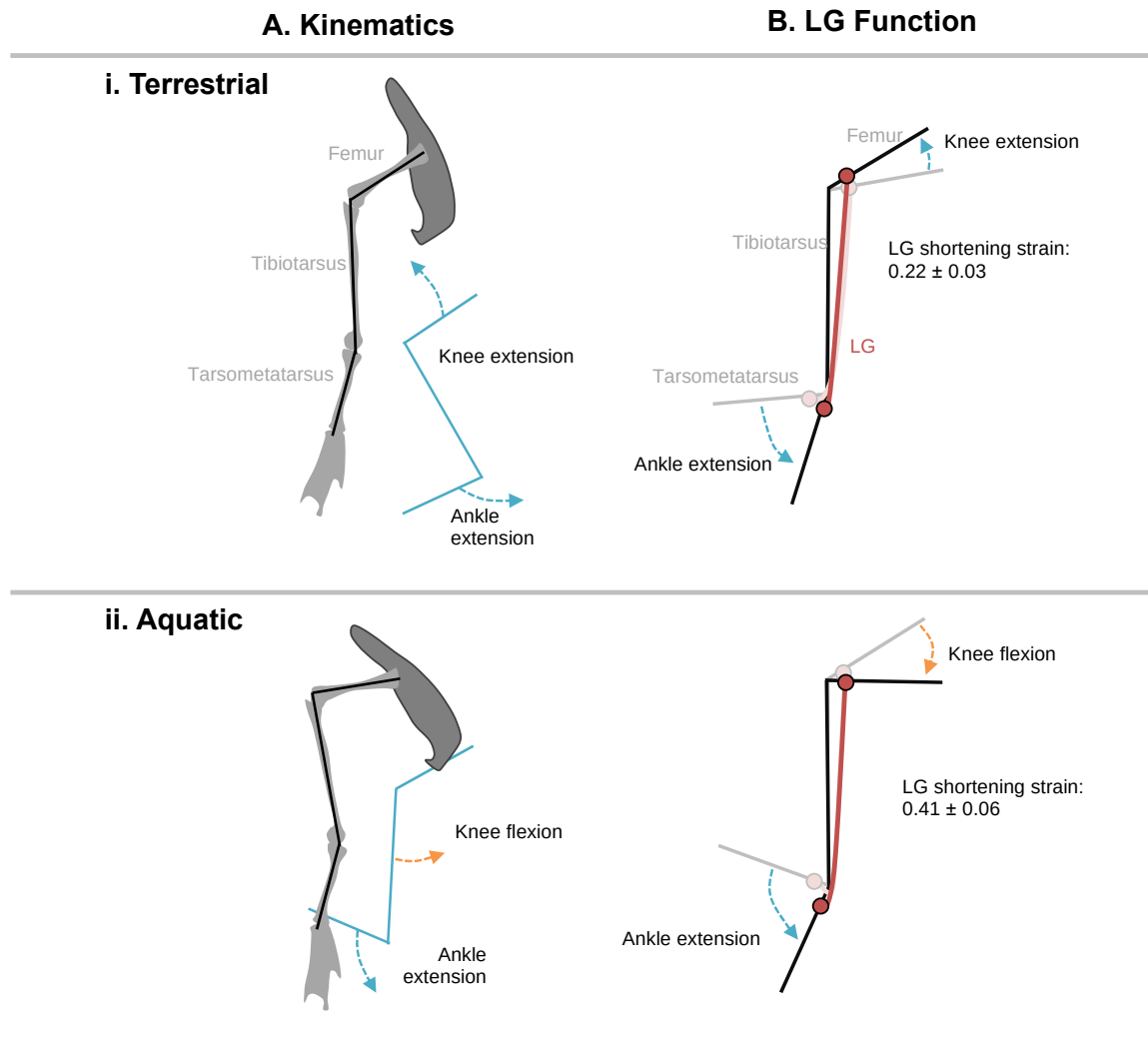


Figure 2.7. Knee and ankle kinematics have implications for LG function. (A) Joint angles at the beginning of takeoff are shown in light gray/blue for the beginning of takeoff and dark gray/black for the end of takeoff. (B) A simplified model of the femur, tibiotarsus, and tarsometatarsus is shown. The LG is represented by the red/pink bands, with the origin and insertion (circles) locations exaggerated for clarity. For ease of comparison, the tibiotarsus is held stationary. The hindlimb segment/muscle positions are light gray/pink for the beginning of takeoff and black/red for the end of takeoff. During terrestrial takeoff, knee extension and ankle extension both help launch the body (A). However, these joint actions oppose each other, resulting in lower LG shortening strain as the LG shortens to extend the ankle but lengthens as the knee extends, and decreasing shortening velocity (B). During aquatic takeoff, knee flexion and ankle extension are both contribute to backward motion of the foot (A). Both motions are powered by large shortening strains and high shortening velocity by the LG (B).

Unexpectedly, the ankle joint exhibited similar kinematics between aquatic and terrestrial takeoffs (Figures 2.2 & 2.3). Although angular excursion of the ankle differed significantly between the two media, the difference was much lower than we observed for the other joints, and ankle angular velocity did not differ significantly. Although this result might suggest that the LG and other muscles driving ankle extension would exhibit similar contractile patterns across both types of takeoff, because the LG crosses both the knee and ankle joints, the reversal in knee motion for aquatic versus terrestrial takeoffs profoundly impacts LG contractile function. During terrestrial takeoffs, the LG shortens to extend the ankle, but this is opposed by knee extension (Figure 2.7), resulting in more limited LG shortening and reduced velocity during terrestrial takeoffs (Figure 2.6). In contrast, because the knee flexes during aquatic takeoffs, LG shortening is greater and occurs with higher velocity to contribute to knee flexion as well as ankle extension (Figure 2.7).

Changes in the kinematics of the MTP and hip joints between terrestrial and aquatic takeoffs likely alter the roles played by the muscles acting at these joints. Both joints undergo large excursions during terrestrial takeoffs but experience almost no angle change during aquatic takeoffs (Figures 2.3, 2.7). The MTP first flexes, as the more proximal joints extend, and then extends rapidly at the end of takeoff from land (Figure 2.2). For aquatic takeoffs, MTP motion is negligible, as the joint is held close to 180° , allowing the foot and tarsometatarsus to act as a propulsive paddle. Being held rigid during a power stroke seems to be a characteristic pattern of MTP kinematics for aquatic locomotion, as it is also observed during surface swimming, when flexion of the MTP only occurs during the recovery stroke (Provini *et al.*, 2012A). The hip joint, which exhibits large extensions during terrestrial takeoff, also undergoes little motion during aquatic takeoffs. This pattern is also consistent with other types of avian locomotion, in which the hip undergoes large excursions during walking but is relatively stationary during surface swimming (Carr, 2008; Provini *et al.*, 2012A). These hip kinematic patterns are reflected in the length change patterns of hip extensor muscles, which

undergo more shortening during walking than during swimming (Carr, 2008). Thus, although we did not measure muscle function at these joints, we would predict that muscles acting at the hip and MTP joints also change roles between aquatic and terrestrial takeoff, with terrestrial takeoffs requiring greater muscle shortening compared with aquatic takeoffs, which would require nearly isometric contractions to stabilize these joints.

2.4.2 LG stress, work & power

Unexpectedly, we observed no significant differences in LG muscle stress or work but did see a significant difference in muscle power output in our *in vivo* recordings across takeoff media (Figure 2.6). The small sample size for these variables may have contributed to our inability to distinguish differences that, in fact, exist for muscle stress and work. That muscle work did not differ was perhaps less surprising, given that the increase in muscle shortening during aquatic takeoffs was balanced by a decrease in LG force for two out of the three animals for which paired results were obtained. However, the decrease in LG force observed for most animals was not enough to offset the increase in LG shortening velocity, resulting in significantly higher LG power during aquatic takeoffs.

It is interesting to consider how a demanding task like takeoff can be managed by a major leg extensor muscle, such as the LG, which must accommodate differing contractile lengths and velocities depending on the medium in, or on, which the animal moves. Both force-length and force-velocity effects (McMahon, 1984; Lieber, 1992; Biewener & Patek, 2018) would decrease the force generating ability of the LG muscle during aquatic takeoffs. Given that LG stress did not decrease significantly, it seems likely that increased neural recruitment compensated for force-length and force-velocity effects. However, our limited success in obtaining paired EMG recordings of the

LG in multiple individuals across both takeoff behaviors (Table S2.1) prevented us from confirming this.

2.4.3 LG pennation angle changes and muscle gearing

When pennate muscles contract, their fibers can rotate, changing their pennation angle. Fascicle shortening and rotation, therefore, can both contribute to whole muscle shortening (Brainerd and Azizi, 2005; Azizi *et al.*, 2008). The ratio of muscle velocity to fascicle velocity defines a muscle's gear ratio, which has been shown to change in relation to changes in muscle force requirements during shortening contractions (Azizi *et al.*, 2008). Muscle gear ratios are predicted to be higher under low force behaviors, allowing for higher whole muscle shortening velocities, and lower during high force behaviors, keeping the fascicles' force more aligned with the line of action of the muscle (Azizi *et al.*, 2008). Consequently, we hypothesized that terrestrial takeoffs would involve lower muscle gear ratios to favor greater force generation, whereas aquatic takeoffs would favor increased gear ratios to enhance whole muscle shortening velocity. Although we did not see a difference in normalized muscle force, or stress, we did observe larger changes in LG pennation angle, consistent with a higher gear ratio, during aquatic takeoffs than terrestrial takeoffs when compared using a paired t-test (Figure 2.6).

2.4.4. Implications of moving between water and land

Animals that regularly move between environments face the challenge of using the same propulsive structures and muscles in media having very different physical properties. As made obvious by their webbed feet and waddling gait, many waterfowl, such as mallards, exhibit specializations for swimming that are thought to have compromised their walking ability (Biewener

and Corning, 2001; Provini *et al.*, 2012A) and increased the energetic cost of terrestrial locomotion relative to other birds (Pinshow *et al.*, 1977; Griffin and Kram, 2000; Nudds *et al.*, 2010).

As previously discussed, force production varies between environments. On land, animals need to support body weight as well as generate ground reaction forces to accelerate the body. In water, animals produce hydrodynamic forces, which depend on the speed of the propulsive structure (drag) and the acceleration of the added mass of fluid displaced by the structure (Daniel, 1984). Thus, we predicted that muscle force production would be higher during terrestrial locomotion and joint angular and muscle-shortening velocities would be higher during aquatic locomotion. These hypotheses are consistent with previous comparisons of kinematically similar behaviors where higher muscle EMG activity (Kamel *et al.*, 1996; Biewener and Gillis, 1999; Gillis & Biewener, 2000; Gillis and Blob, 2001; Foster *et al.*, 2018) and higher impulses (Nauwelaerts & Aerts, 2003) are observed during terrestrial locomotion, while faster limb movements are observed during aquatic behaviors (Wilkinson, 2014; Clifton *et al.*, 2015).

That our results don't support these hypotheses is likely a result of their simplicity. For example, we expected the demand for hydrodynamic force would be lower because body weight is supported by buoyancy. However, when moving through water, the ducks also experience other sources of energy loss and demand for force. For example, when taking off from water, the ducks would need to produce force to compensate for drag experienced by the body or accelerating the extra mass of water entrained in the feathers. In addition, as the foot moves faster and hydrodynamic force increases, larger muscle force will be required to balance hydrodynamic force. Thus, velocity and force must simultaneously increase during aquatic locomotion (Richards, 2011), as evidenced by our observation of higher LG power (i.e., force x velocity) during aquatic versus terrestrial takeoffs (Figure 2.6).

Another important difference between moving on land versus water is the compliance of water relative to the rigid terrestrial takeoff platform used in our study. Differences in compliance can affect kinematics and performance. For example, takeoff velocity was lower on more compliant substrates for frogs (Astley *et al.*, 2015; Reynaga *et al.*, 2019), birds (Crandell *et al.*, 2018), and humans (Giatsis *et al.*, 2004). When jumping from the water column, frogs extended their legs faster than during terrestrial jumps (Wilkinson, 2014) and frogs that reduced slip of their feet during aquatic jumps had higher jump heights (Nauwelaerts *et al.*, 2004). More compliant, dissipative substrates, such as sand, also require more mechanical work and incur a higher energetic cost to walk and run over (Lejeune *et al.*, 1998). The higher compliance of a fluid substrate and the resulting ‘slip’ of the propulsive appendage relative to the fluid also results in a loss of mechanical energy (Hsieh, 2003; Li *et al.*, 2012; Astley *et al.*, 2015; Reynaga *et al.*, 2019), and compromises the ability of animals to store elastic energy (Li *et al.*, 2012; Reynaga *et al.*, 2019). Because slip (motion of the appendage relative to the fluid) represents energy loss, animals need to produce additional work (Hsieh, 2003; Li *et al.*, 2012) through larger joint excursions or higher forces.

In order to account for differences between environments, ducks are not limited to only changing hindlimb kinematics. Ducks and other waterfowl use their wings and tail to assist foot propulsion during aquatic behaviors, including during vertical takeoffs (Williamson *et al.*, 2001). Although characterizing the contributions of the wings and tail to aquatic takeoff is beyond the scope of the current study, they are clearly key to achieving vertical takeoff in mallards. In fact, the wings and feet working in unison is a common strategy for birds moving at the water’s surface and has been described for behaviors like taxiing prior to takeoff or paddle-assisted flight (Norberg & Norberg, 1971; Gough *et al.*, 2015), and hydroplaning and/or steaming (Aigeldinger & Fish, 1995; Gough *et al.*, 2015). An inverse dynamics study pairing ground reaction forces (GRF, measured using a force plate) and fluid dynamic forces (measured using PIV), although challenging to carry out,

could elucidate the relative contributions of the hindlimbs, wings, and tail during takeoff. Although not the focus of the current study, preliminary results demonstrated a strong but not significant relationship between GRF and LG stress ($r_2=0.88$, $p=0.06$, Figure S2.3), suggesting that LG function plays an important role in terrestrial and, we would argue, aquatic takeoffs.

Linked to the kinematic differences they produce, changes in muscle function also facilitate movement in different media. Based on the knee kinematics we observed for aquatic versus terrestrial takeoffs, it is clear that changes in motor coordination patterns must occur, altering the timing of when and which muscles are activated (i.e. knee flexors versus extensors). Differential recruitment of LG synergists could also play a role. Biewener and Corning (2001) observed that the mallard MG and LG have similar force contributions during walking but the MG generates much less force than the LG during surface swimming. This suggests that increased MG recruitment may account for the additional force needed to accelerate the body during terrestrial takeoffs, which could explain why we found no significant change in LG stress between terrestrial and aquatic takeoffs.

Because muscle force is maximized over a limited range of length changes and shortening velocities, changes in these parameters could limit force, work, and power production. The LG exhibited higher shortening velocities during aquatic takeoffs than terrestrial takeoffs, which likely compromises its force production for a given amount of muscle recruitment, requiring the recruitment of more fibers and more energy expenditure to produce similar forces. We also observed that the LG operated over longer fascicle lengths and generated force over a broader range of strains during aquatic takeoffs than for terrestrial takeoffs (Figure 2.5B), suggesting length-dependent shifts in its force-generating ability. Mapping the LG's *in vivo* muscle use patterns during aquatic and terrestrial takeoff to muscle force-length and force-velocity properties would, therefore,

allow us to determine whether these changes in strain and shortening velocity involve a tradeoff in muscle force production.

2.5 Conclusions

Locomotion on or through different environments places demands on the mechanisms by which animals generate propulsive forces that require changes in both motor behavior and muscle function. We found that hindlimb kinematics and the function of at least one key propulsive muscle (LG) change between terrestrial and aquatic takeoffs. Because aquatic takeoffs require larger and faster fascicle length changes, LG muscle function is likely altered by changes in neural activation as well as by its intrinsic force-length and force-velocity properties, which may be compensated by differential recruitment of other leg muscles or other parts of the body that assist in propulsion. This study highlights the challenges animals face when moving through or on different media and how muscle function may be tuned across behaviors.

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2.7 Competing Interests

No competing interests declared.

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2.9 List of Symbols and Abbreviations

A	Aquatic
EMG	Electromyography
GRF	Ground reaction force
LG	Lateral gastrocnemius
MG	Medial gastrocnemius
MTP	Metatarsophalangeal
PCSA	Physiological cross-sectional area
T	Terrestrial

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CHAPTER 3. Post-activation muscle potentiation and its relevance to cyclical behaviors

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3.0 Abstract

Muscle can experience post-activation potentiation (PAP), a temporary increase in force and rate of force development, when contractions are closely timed; therefore, cyclical behaviors are likely affected by PAP, as succeeding contraction cycles can lead to potentiation over several subsequent cycles. Here, we examined PAP during *in situ* cyclical contractions of the mallard lateral gastrocnemius (LG). Surface swimming, a cyclical behavior, was mimicked with work-loops utilizing *in vivo* LG length change and stimulation parameters. Tests were performed at mallards' preferred cycle frequency as well as at lower and higher frequencies. Like muscles from mammals, anurans, and arthropods, the mallard LG exhibited PAP with increases in peak force, average force rate, and net work. Staircase potentiation occurred over two or more work-loop cycles, resulting in gradual increases in PAP. The number of cycles needed to reach maximum work varied with cycle frequency, requiring more cycles at higher cycle frequencies. PAP occurred under *in vivo*-like stimulation parameters, suggesting a potentially important role of PAP in animal locomotion, especially in cyclical behaviors.

3.1 Introduction

The contraction history of a muscle can influence its performance. Post-activation potentiation (PAP) is one such history-dependent phenomenon where muscle force is higher for a given submaximal stimulation, if the muscle has been recently stimulated. The potentiated state may be the predominate condition for muscles *in vivo* [1]. PAP is caused by phosphorylation of the myosin regulatory light chain (MRLC), which increases the rate of cross-bridge formation [2], thereby increasing both the magnitude of force and rate of force development for submaximal activation [3,4]. PAP has been studied for more than a century (for review [5]). Much of the foundational work on PAP focused on isolated muscle fibers [4] or whole muscles [1,2,3,6] *in vitro*. PAP was also measured in humans as an increase in twitch force following a maximal voluntary contraction [7,8], demonstrating that voluntary activation could elicit PAP. More recently, increases in human performance during high-power behaviors following a tetanic contraction have been studied [5,9,10]; however, differences in timing of MRLC phosphorylation relative to improved exercise performance led to concerns that these studies measured a different phenomenon [5]. Thus, a gap in understanding remains between measurements of PAP and naturally-occurring contractile behaviors.

Cyclical behaviors are likely affected by PAP since conditioning by one cycle can lead to potentiation in subsequent cycles. MRLC phosphorylation levels remain high for up to a minute following a conditioning stimulus [2,11], and PAP can affect muscle forces for several minutes [1,7,8] or longer under non-fatiguing conditions [6]. Observations of PAP during low-frequency isometric contractions uncovered a phenomenon known as staircase potentiation – a gradual rise in muscle force over several contractions [3,6,12,13]. Staircase potentiation has been observed during twitch [3,6,13] and incompletely-fused tetanic contractions [12]. We, therefore, hypothesize that staircase potentiation may also affect force and work production during dynamic contractions.

Relatively few studies have examined PAP during dynamic contractions [2,11,14]. The work-loop technique is a powerful tool for mimicking dynamic muscle contractions and is particularly useful for studying cyclical behaviors [15]. Work is the area inside of a force versus length loop, so both increased force and force rate should together increase work output. An experiment examining a single work-loop in the potentiated state suggests PAP increases work output [2], and work has been observed to increase over several work-loops [15, 16, 17]. Although the term post-activation potentiation has been restricted to certain conditioning stimuli and responses [5,13], we define PAP as any increases in muscle force, rate of force development, or work following prior activation.

Here, we used the work-loop technique *in situ* to examine how PAP affects work output over repeated cycles in mallard lateral gastrocnemius (LG). The LG is an important ankle extensor during walking and surface swimming, and *in vivo* LG function during these behaviors has been described [18]. The current study differs from previous studies of PAP by measuring PAP while mimicking the *in vivo* LG length change and activation patterns observed during surface swimming, a cyclical behavior. Mallards use a cycle frequency of 2.6 Hz during surface swimming [18,19], but use a range of cycle frequencies during terrestrial locomotion [20]. To explore the effect of cycle frequency, we measured work across successive cycles at 1.0, 2.6, and 4.0 Hz. We tested three hypotheses: (H1) PAP increases muscle work output under *in vivo*-like conditions; (H2) because of the staircase effect [13], PAP builds over cycles, resulting in increased work with each subsequent cycle until it reaches some maximum; and (H3) cycle frequency affects the degree of potentiation.

3.2 Methods

All animal procedures were approved by Harvard University's Institutional Animal Care and Use Committee (protocol 20-09). Mallard ducks (*Anas platyrhynchos*, n=5) were anesthetized with 1-2% isoflurane:oxygen gas mixture using mask induction. To measure fascicle length,

sonomicrometry crystals (2 mm, Sonometrics Corporation) were implanted at each end of a mid-belly LG fascicle (Figure S3.1) and secured (5-0 silk suture, Ethicon). The muscle's origin remained intact, but all other connective tissue attachments were removed to allow free movement of the LG. To measure muscle force and impose length changes, kevlar thread was tied proximal to the tendon's fibrocartilage pad and connected to a muscle ergometer lever arm (10 N, Aurora Scientific Inc.) modified by drilling a hole at half its length to increase its upper force limit (18.3 N). The animal was mounted on a moveable stage by clamping the femur mid-shaft and securing the foot to a flat plate (Figure S3.1); this setup ensured that the limb and muscle origin remained stationary and the muscle line of action was aligned with the ergometer. The muscle was stimulated (pulse duration: 0.2 ms, Grass S48, Grass Instruments, Warrick, RI) intramuscularly through medial and lateral pairs of 24 gauge, stainless steel wire electrodes separated proximally and distally as far possible (~3 cm) and sutured in place (Figure S3.1).

The voltage that induced maximal twitch force was determined, with voltage reduced (~2-3 V) to elicit submaximal force ($35 \pm 5\%$ maximum), resulting in dynamic forces likely similar to forces produced during slow swimming [18]. Using stimulation frequencies of 125 (n=3) or 250 Hz (n=2), we determined the submaximal isometric L_0 (length at which submaximal tetanic force is produced). We then used the work-loop technique [15] to measure muscle work while mimicking strain and activation patterns observed during surface swimming *in vivo* [18]. Specifically, ramp changes in muscle length were applied over 5-15 cycles with stimulation onset at -1.6% phase, stimulation duration of 26%, and lever arm excursion of 9 mm (Figure 3.1), which corresponded to fascicle length changes of 5.4 ± 0.9 mm and strains (maximum: 0.03 ± 0.04 , minimum: -0.21 ± 0.04) consistent with *in vivo* strains observed during surface swimming [18, KTB personal observations]. Keeping the length change, activation phase, and duty factor constant, we tested three cycle frequencies (1.0, 2.6, and 4.0 Hz) in a random order with a 5 min rest between series, consistent with previous PAP work-loop experiments [2]. Because of in-series compliance, minimum strains decreased over cycles with increased force production, but these differences were small (change in strain from first to last cycle ~0.02 for 2.6 & 4 Hz and ~0.001 at 1 Hz). Isometric force production at L_0 was monitored throughout the experiment to ensure that force decreases were <10%.

Post-mortem dissections confirmed sonomicrometry crystal alignment. Resting LG fascicle and inter-crystal lengths were measured and the ratio of these was used to calculate time-varying fascicle lengths (time-varying segment lengths * resting fascicle length/inter-crystal distance). Force and length data were filtered (low-pass, 1st order, dual pass Butterworth, 20 Hz cutoff; Figure

3.1C,D) before plotting length versus force to calculate resulting loop areas (i.e., net work, Figure 3.1E). Peak force and the average rate of force development ($F_{\text{rate}} = \text{change in force} / \text{duration from stimulation onset to maximum force}$) were measured. 2nd order polynomials were fitted to net work, peak force, and F_{rate} versus cycle number for each frequency to calculate (1) the maximum net work, peak force, or F_{rate} and (2) the cycle when the maximum occurred. Percent change in net work, peak force, and F_{rate} were calculated as: $((\text{maximum} - \text{cycle 1 value}) / \text{cycle 1 value}) * 100$. Percent change data are available on Dryad (doi:10.5061/dryad.69p8cz8z4).

Statistical tests were performed in R (v. 3.6.0), with mixed-effects linear models used to predict the percent change in net work, peak force, and F_{rate} and the maximum work cycle number. Individual was a random effect and cycle frequency, stimulation frequency, and strain at peak force (taken from cycle 2) were added iteratively as fixed effects. Models were compared using ANOVA with $\alpha = 0.05$. Stimulation frequency and strain at peak force did not significantly improve the models ($p > 0.05$), so were not included in further analysis. Frequency significantly improved the models ($p < 0.05$) for all variables. Diagnostic plots for the selected model (frequency as a fixed effect and individual as a random effect) revealed assumptions of homoscedasticity and normal distribution of residuals were reasonable. We performed pairwise comparisons using the emmeans package and Tukey's adjustment. All reported values are mean $\pm$ s.e.m.

3.3 Results

We performed work-loop experiments to determine the degree of work, force, and F_{rate} PAP over 5-15 work-loop cycles at 1.0, 2.6, and 4.0 Hz. The number of work-loop cycles required to reach maximum work varied by frequency ($p < 0.001$, Figure 3.2A,B, Table 3.1). The maximum work cycle number significantly differed between 1 Hz and 2.6 Hz and 1 Hz and 4 Hz, but not between 2.6 and 4 Hz (Table 3.2).

Cycle frequency explained a significant amount of the variation in percent change (i.e., PAP) for net work (Figure 3.2A,C; $p = 0.001$), peak force (Figure 3.2D; $p < 0.001$), and F_{rate} (Figure 3.2E; $p = 0.001$). The percent change in net work was significantly different from zero at 4 Hz, but not 2.6 and 1 Hz (Table 3.1). Peak force and F_{rate} both significantly increased over work-loop cycles at 2.6 and 4 Hz, but not at 1 Hz (Table 3.1). Pairwise comparisons showed PAP in net work, peak force, and F_{rate} was significantly different between 2.6 and 4 Hz and 1 and 4 Hz, but not between 1 and 2.6 Hz (Figure 3.2; Table 3.2).

Table 3.1. Maximum work cycle number and mean percent change for net work, peak force, and F_{rate} by frequency. P-values<0.05 (bolded) indicate percent change is significantly different from zero.

Frequency		Max Work Cycle	Work (%)	Force (%)	F_{rate} (%)
1 Hz	mean $\pm$ s.e.m.	4 $\pm$ 1	16 $\pm$ 9%	10 $\pm$ 4%	29 $\pm$ 8%
	p-value		0.577	0.535	0.570
2.6 Hz	mean $\pm$ s.e.m.	8 $\pm$ 1	52 $\pm$ 14%	37 $\pm$ 11%	64 $\pm$ 20%
	p-value		0.051	0.016	0.026
4 Hz	mean $\pm$ s.e.m.	10 $\pm$ 1	115 $\pm$ 35%	69 $\pm$ 17%	117 $\pm$ 27%
	p-value		<0.001	<0.001	0.002

Table 3.2. P-values for a Tukey-adjusted pairwise-comparison for maximum work cycle number and percent change in net work, peak force, and F_{rate} by frequency. Bolded p-values indicate a significant difference (<0.05).

Comparison	Max Work Cycle	Work	Force	F_{rate}
1 Hz vs. 2.6 Hz	0.011	0.234	0.065	0.072
2.6 Hz vs. 4 Hz	0.288	0.045	0.048	0.046
1 Hz vs. 4 Hz	0.001	0.004	0.001	0.002

Figure 3.1. Work-loop experiments. (A) Ramp length change pattern applied via the lever arm and stimulation timing. Stimulation offset (1.6%) and duration (26%) are expressed as % of cycle and scaled to cycle frequency. Example (B) lever position and stimulation, (C) fascicle length change from sonomicrometry, and (D) force from the ergometer during a work-loop series at 2.6 Hz. (E) Force versus length work (area inside the loop) loops at 2.6 Hz. Length is expressed as an absolute measure (mm) and as strain (relative to $L_{o, \text{submax}}$). All loops are counterclockwise (positive work). Loops progress from light to dark with each subsequent loop. Thicker lines indicate the period of activation.

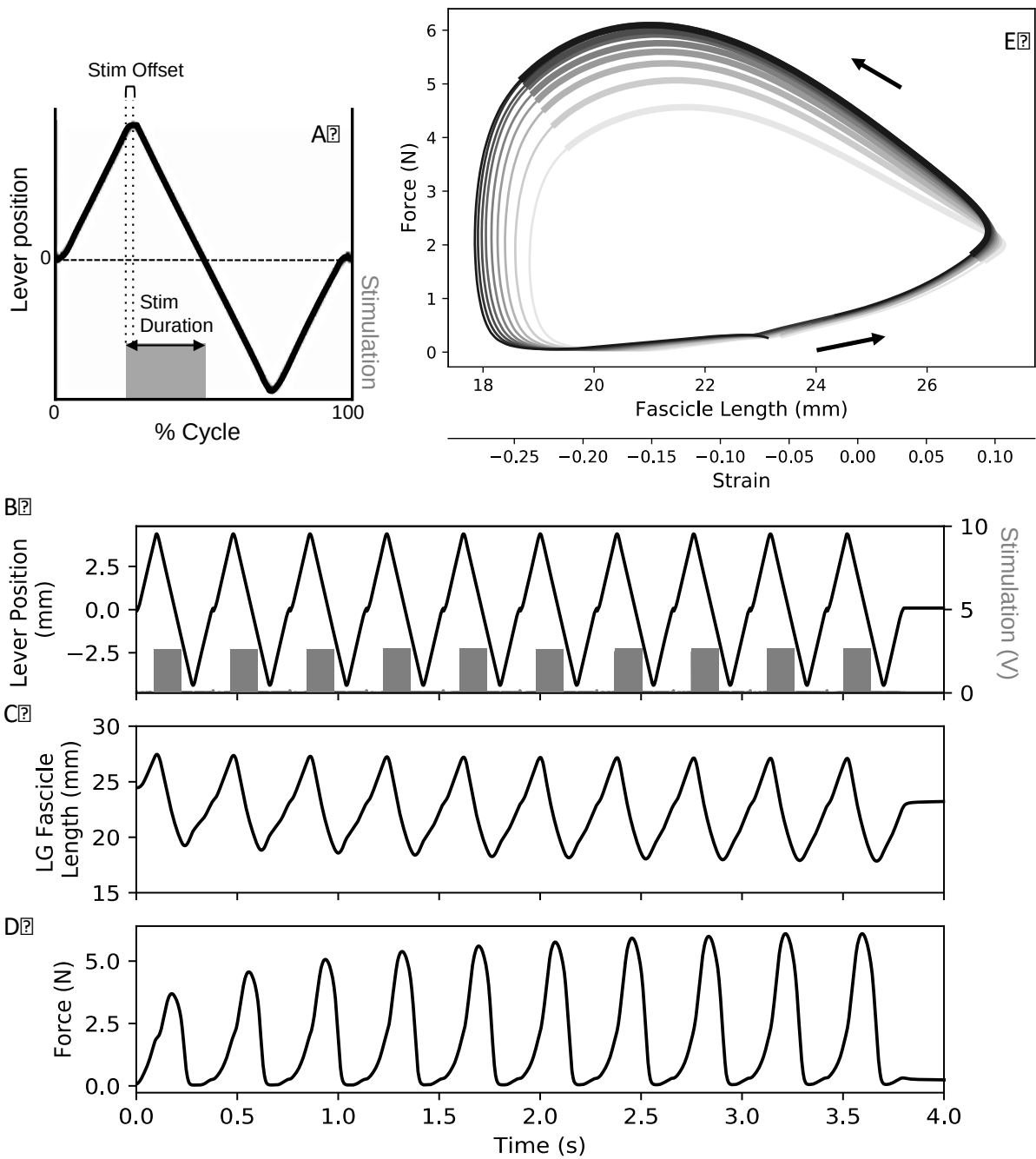
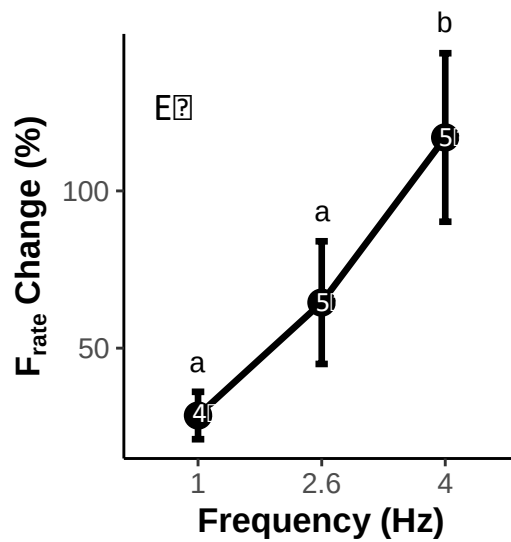
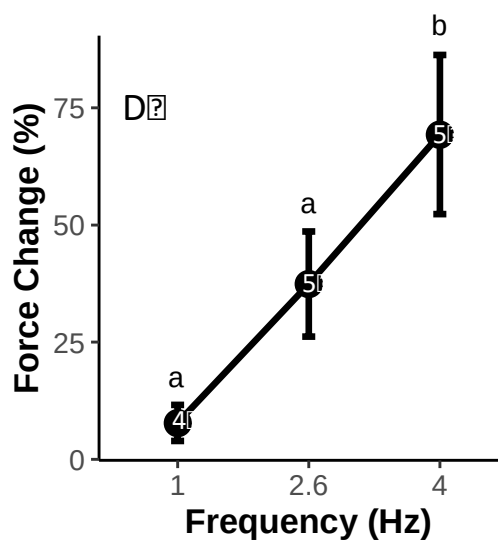
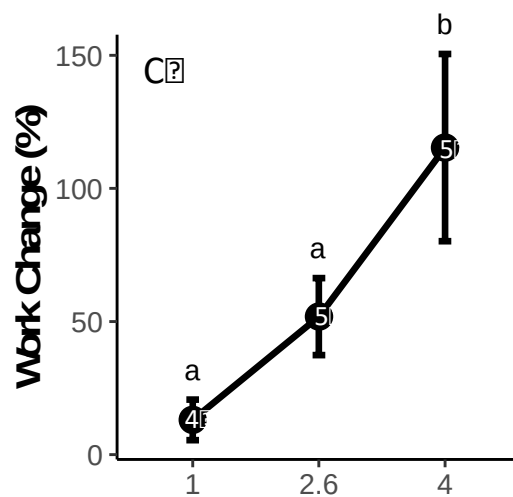
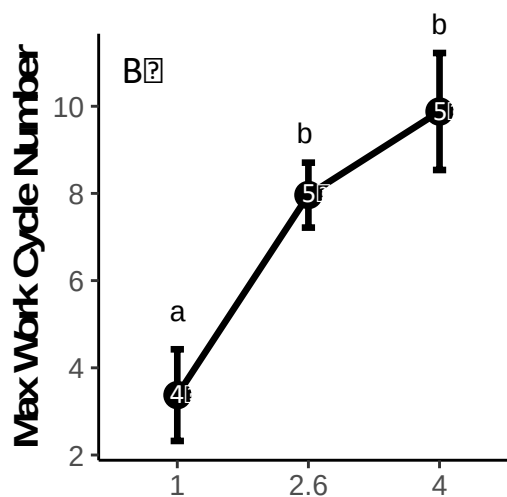
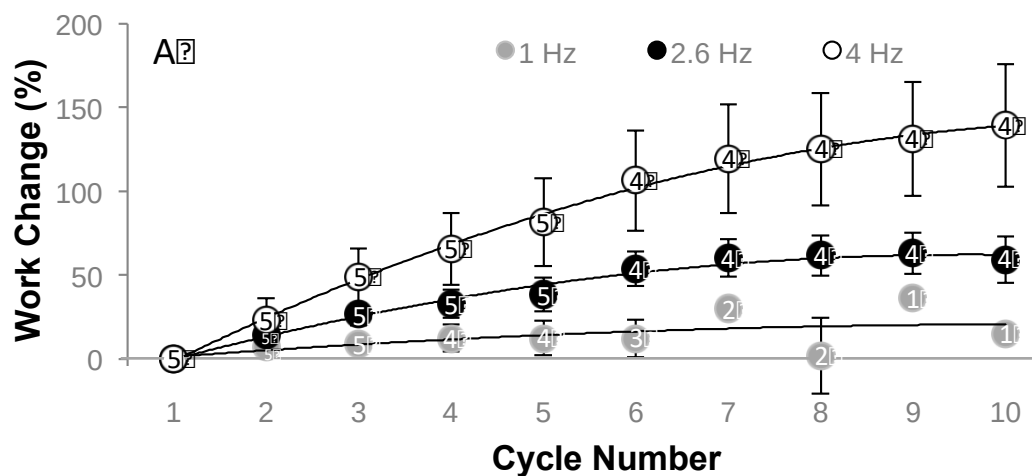


Figure 3.2. Post-activation potentiation occurred during work-loops in the mallard LG. (A) Percent change in net work versus cycle number at three frequencies: 1 Hz (gray, filled circles), 2.6 Hz (black, filled circles; mallards' preferred surface swimming cycle frequency), and 4 Hz (open circles). Solid lines are 2nd order polynomial fits. (B) Maximum work cycle number was significantly higher at 2.6 Hz and 4 Hz than at 1 Hz, but there was no difference between 2.6 and 4 Hz (Table 3.2). Percent change in (C) net work, (D) peak force, and (E) F_{rate} were significantly greater at 4 Hz than 1 Hz and 2.6 Hz, but not between 1 Hz and 2.6 Hz (Table 3.2). All points are averages $\pm$ s.e.m. Lower case letters (a,b) indicate groups for Tukey's pairwise comparison, with different letters assigned to groups that were significantly different ($p < 0.05$). Sample size is indicated by the number on each point.



3.4 Discussion

We examined the effect of PAP on mallard LG cyclical force and work performance, by imposing *in vivo* length change and activation data to mimic the contractile behavior of this muscle during surface swimming [18]. Under these conditions, PAP resulted in a 37% increase in peak force, a 64% increase in F_{rate} , and a 52% increase (n.s.) in net work (Figure 3.2, Table 3.1), suggesting PAP is a relevant and substantial contractile phenomenon at the mallard's preferred cycle frequency.

Past work provided evidence of PAP in mammals [1,2,3,4,7,8,17], anurans [6,21], fish [16], and insects [15]; that birds also experience PAP is not surprising. The mallard LG is a mixed fibered muscle, with mostly type II fibers (74-100%, depending on the sample location, [22]). Previous work has shown that muscles with large populations of type II fast fibers demonstrate larger increases in force and F_{rate} than type I slow-fibered muscles [8].

We observed increases in both force and F_{rate} (Figure 3.2, Table 3.1), consistent with previous findings [2,3,4]. Unlike isometric contractions where force and F_{rate} can be considered separately, force and F_{rate} are interrelated during dynamic contractions. Force drops when muscles shorten to lengths below L_o , so a greater F_{rate} will permit more force development prior to substantial shortening. Thus, we cannot draw independent conclusions about these factors here.

During a dynamic contraction, changes in force and F_{rate} should also correspond to a change in work (H1). We observed a significant increase in peak force and F_{rate} for both 2.6 and 4 Hz, but a significant increase in net work only at 4 Hz (although there was a tendency toward significance at 2.6 Hz). Work potentiation has previously been observed during a single work-loop of a mouse fast-fibered muscle (extensor digitorum longus) following a conditioning stimulus [2]. The current study builds on this finding by examining work potentiation over several cycles of the mallard LG. Our observation of gradual increases in net work, peak force, and F_{rate} over 4-10 cycles (Figures 3.1E, 3.2A) indicates that PAP is cumulative during dynamic contractions (H2). Our findings are consistent with (1) staircase potentiation, where force increases over several isometric stimulations [3,6,12,13], and (2) observations of work increasing over several cycles [15,16,17]. PAP can build and possibly be maintained over a large number of cycles for sustained locomotion.

At higher cycle frequencies, (1) net work, peak force, and F_{rate} potentiation was larger and (2) a larger number of cycles were needed to achieve this potentiation, supporting our hypothesis that cycle frequency would affect PAP (H3). Krarup [3] similarly observed that higher contraction frequencies elicited larger twitch force potentiation but, in contrast to our findings, found that peak potentiation was reached with fewer stimuli at higher contraction frequencies. The lack of a

significant effect of PAP on LG work at lower frequencies could be due to longer inter-cycle times, allowing the effects of PAP to diminish before the next cycle commences. Alternatively, the effects of fatigue due to longer contraction durations at lower frequencies could counteract the effects of PAP, causing work potentiation to be lower and to peak with fewer contractions. Because we maintained a constant duty factor of activation across frequencies, the total stimulation duration and number of spikes was higher at lower frequencies. PAP is thought to offset the effects of fatigue [7,23], possibly helping to maintain force production over a series of cycles. Thus, PAP may very well affect work output by mitigating the effects of fatigue and reducing the drop in force that might otherwise be observed.

The present study demonstrates that PAP occurs under conditions that mimic a cyclical swimming behavior and could elicit meaningful increases in muscle work, force, and F_{rate} under certain cycle frequencies. Further exploration is needed of how changes in force and work production resulting from PAP relate to locomotor performance in naturally-behaving animals.

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CHAPTER 4. Hindlimb skeletal anatomy and kinematics vary with swimming
behavior in ducks

4.0 Abstract

Hindlimb swimming has evolved several times throughout avian evolution, with swimming clades exhibiting a convergent suite of anatomical characters. However, swimming ability can come at a cost to terrestrial locomotion in the most specialized swimmers. Ducks have a range of swimming abilities, including highly terrestrial species, surface swimmers, foot-propelled divers, and wing-and-foot-propelled divers. In the first part of this chapter, I explored how swimming ability in ducks relates to hindlimb and pelvic anatomy. I hypothesized that ducks exhibit a gradient of anatomical traits, with the diving species showing anatomical traits that most resemble other hindlimb swimming specialists like grebes and loons and terrestrial and surface swimming species more closely resembling galliformes and other non-swimming birds. I found that while ducks do show differences in hindlimb skeletal anatomy (femur and tarsometatarsus proportional lengths, femur and tibiotarsus aspect ratios, and lateral cnemial crest size) among behavioral groups, only one of these traits (lateral cnemial crest size) is similar to a pattern seen in loons and grebes. The larger cnemial crest size observed in diving species likely serves to increase the moment arms for knee extensors and hip flexors, increasing stabilization of these joints during swimming, and to increase attachment area for muscles that power foot plantarflexion during the swimming power stroke. In the second part of this chapter, I examined hindlimb kinematics during terrestrial locomotion, surface swimming, and diving in a surface swimmer (mallard), foot-propelled diver (New Zealand scaup), and wing-and-foot-propelled diver (common eider). I found that although all three species increase stride frequency and length with speed during terrestrial locomotion, scaup and eiders walked using a higher body angle and eiders used a higher relative frequency. During surface swimming, kinematic patterns of stride frequency and length vs. velocity were remarkably similar across species, except eiders use lower frequencies than the other species. However, during diving, eiders swam at higher relative velocities and increased stride length faster than the other two species.

Some observed patterns may indicate advantages in swimming (i.e., faster swimming velocities and longer stride lengths in diving eiders) and costs in terrestrial locomotion (i.e., higher body angle and higher stride frequency) for more specialized swimmers. However, all three species performed similarly across behaviors with especially remarkable similarity between mallards and scaup in terms of relative velocities across all behaviors. That hindlimb anatomy and kinematics show some variation with swimming ability in closely related species may reflect the demands that swimming places on the body. However, all three species use similar top walking velocities and are capable of diving, which may point to the versatility of the duck body plan; with differences in swimming ability reflecting different kinematics (i.e., synchronous use of wings and feed observed in eiders) rather than anatomical changes that could compromise performance on land.

4.1 Introduction

Avian swimming is remarkable since many swimming birds are also capable of terrestrial locomotion and flight. However, swimming ability often seems to come at a cost in the most specialized swimmers, with repeated evolution of flight loss among wing-propelled swimmers and reduced ability to move terrestrially in foot-propelled swimmers (see Raikow, 1973; Johnsgard, 1987; Habib, 2010). Foot-propelled swimming tends to be associated with a particular suite of hindlimb skeletal traits. In fact, hindlimb measures alone can be used to identify avian swimming species and, in some cases, swimming technique (Zeffer *et al.*, 2003; Hinić-Frlog & Motani, 2010; Stoessel *et al.*, 2013). From an evolutionary perspective, specialization of the avian hindlimb for swimming can be attributed, in part, to the presence of multiple locomotor modules (wings and hindlimbs), which released selection pressure on the limbs faced by non-volant theropods (Gatesy & Middleton, 1997).

Almost all duck species (within Anseriformes) are proficient swimmers. However, ducks exhibit a range of locomotor specialization, despite their close evolutionary relationships, including

species with a range of diving abilities, many species that swim well but only at the surface, and some species that live almost entirely on land. However, it is unknown whether the relationships between hindlimb anatomy and swimming behaviors that have been observed across the avian tree also exist as a spectrum across the duck clade. In addition, although variation in walking and swimming ability have been described, there are few if any studies comparing the kinematics across behaviors and across duck tribes. In this study, I sought to examine the changes in anatomy and kinematics associated with variation in swimming behaviors across the duck evolutionary tree.

Previous work has focused on anatomical differences between swimmers and non-swimmers across the avian phylogeny. A suite of common skeletal adaptations associated with swimming have been resolved, including specialized pelvis shape, limb bone proportions, foot morphology, and muscle attachment points. These traits tend to be most extreme in swimming specialists like grebes and loons (Johnsgard, 1987), so are thought to be indicators of swimming ability. The enlarged digit length and the presence of foot webbing or lobes is the most striking adaptation for swimming. These adaptations increase the surface area of the foot, which is used as a drag-producing paddle, lift-producing hydrofoil, or both (Johansson & Lindhe Norberg, 2001; Johansson & Norberg, 2003). Differences in digit lengths and webbing area among groups of ducks with different swimming strategies has been previously described (Raikow, 1973), with more specialized diving species reported to exhibit larger foot areas relative to body size than dabbling (i.e., surface swimming) species. The pelvis of specialized divers tends to be narrow and elongate with a reduced preacetabular portion (i.e., more cranially positioned acetabula, Johnsgard, 1987; Ibáñez & Tambussi, 2012; Stoessel *et al.*, 2013; Anten-Housten *et al.*, 2017). A longer pelvis, with postacetabular expansion, should increase attachment area and muscle moment arms for extensor muscles acting at the hip. The hip and knee are stationary during surface swimming (Carr, 2008; Provini *et al.*, 2012) and seem to be stationary as part of the streamlined hull during diving

(Johnsgard, 1987; Clifton *et al.*, 2018). The elongate pelvis and increased size and mechanical advantage of the hip extensors is thought to be a specialization for swimming by increasing hip stabilization during force transmission from the distal leg (Hinić-Frlog & Motani, 2010; Clifton *et al.*, 2018). When limb bones are normalized by limb length, the femora of specialized swimmers are relatively longer and the tarsometatarsi are relatively shorter than nonswimmers (Gatesy & Middleton, 1997; Zeffer *et al.*, 2003).

Together, the short femur, cranial acetabulum, and narrow pelvis can allow swimmers to align the tibiotarsus with the body axis, improving swimming ability by streamlining the body shape (Johnsgard, 1987; Ibáñez & Tambussi, 2012; Clifton *et al.*, 2018) and/or by improving force transmission by bringing the posteriorly positioned feet closer to the body midline than ventrally positioned feet (Hinić-Frlog & Motani, 2010). However, posterior foot placement may also be associated with reduced terrestrial ability in specialized swimmers, as feet positioned behind the center of mass decreases stability while walking and requires an increased body angle or larger muscle moments to support body mass (Johnsgard, 1987).

Other skeletal adaptations of swimmers include larger cortical limb bone areas, decreasing buoyancy (Habib & Ruff, 2008), as well as relatively mediolaterally-flattened tarsometatarsi (Cracraft, 1982; Johnsgard, 1987; Lovvorn, 1990), which can also help to increase resistance to bending in one direction and possibly serve to increase the paddle area during the power stroke (Johnsgard, 1987) and decrease drag during the recovery stroke (Lovvorn, 1990). Swimming birds typically have enlarged cnemial crests and/or patellar fusion (Johnsgard, 1987), increasing moment arms of knee extensors and hip flexors and increasing attachment area for muscles acting at the ankle and foot (Clifton *et al.*, 2018). Thus, increasing cnemial crest size can contribute both to increased hip and knee stabilization during swimming and increased ability to power the ankle and foot motion during the swimming power stroke.

Although prior avian work has integrated locomotion studies across media (Johnston & Bekoff, 1992; Biewener & Corning, 2001; Provini *et al.*, 2012) and swimming abilities (Baudinette & Gill, 1985; Abourachid, 2001; Sato *et al.*, 2007; Carr, 2008), none have considered both terrestrial and swimming behaviors across duck species with different swimming strategies. During terrestrial locomotion, avian and human bipeds increase stride length and stride frequency to increase speed (Gatesy & Biewener, 1991). Among ducks, the relationship between swimming speed and frequency varies, with some ducks changing stride frequency with speed (Woakes & Butler, 1983; Baudinette & Gill, 1985) while others use a constant stride frequency across swimming speeds (Prange & Schmidt-Nielsen, 1970; Clark & Fish, 1994; Heath *et al.*, 2006). Interestingly, loons seem to increase speed by changing duty factor, not cycle frequency (Clifton & Biewener, 2018). Finally, given their posteriorly positioned feet, some specialized swimmers like loons and grebes are unable to walk digitigrade and others walk with a steeply inclined body angle to position the center of mass over the feet (Johnsgard, 1987; Clifton *et al.*, 2018).

Anseriformes, the avian order that includes ducks, geese, swans, and screamers, includes a wide range of swimming abilities (Raikow, 1973), from highly terrestrial species to diving specialists, with the majority of species falling into the intermediate space as excellent surface swimmers with good walking ability. Although the phylogenetic position of some duck species is still being resolved, most ducks can be divided into four tribes: (1) anatini (dabbling ducks), which swim primarily at the surface and spend significant time on land; (2) aythyini (diving ducks), which are foot-propelled diving birds that typically dive in shallow water; (3) mergini (sea ducks), which include foot- and wing-and-foot-propelled divers that spend much time at sea; and (4) tadornini (shelducks), a diverse group that includes surface swimmers and highly terrestrial species (sheldgeese).

In this chapter, I explore whether ducks exhibit the same anatomical adaptations (Raikow, 1973; Cracraft, 1982; Johnsgard, 1987; Lovvorn, 1990; Gatesy & Middleton, 1997; Zeffer *et al.*, 2003;

Hinić-Frlog & Motani, 2010; Ibáñez & Tambussi, 2012; Stoessel *et al.*, 2013; Anten-Housten *et al.*, 2017) and kinematic tradeoffs (Johnsgard, 1987; Provini *et al.*, 2012; Clifton *et al.*, 2018) with swimming ability as is observed across the avian tree. I do so by performing an anatomical analysis of the primary hindlimb skeletal elements and pelvis across Anseriformes, with a particular focus on ducks and their close allies. I expect to observe a gradient of morphologies associated with swimming abilities, with wing-and-foot-propelled divers (hereafter, wing-propelled) with the highest degree of specialization, followed by foot-propelled divers, surface swimmers, and lastly, terrestrial species. Specifically, I hypothesize that, relative to surface swimmers and terrestrial species, specialized swimmers (wing and foot-propelled divers) will have a: (Hypothesis [H]1) relatively shorter femur and longer tarsometatarsus (TMT); (H2) medio-laterally flattened TMT; (H3) cranially positioned acetabula and (H4) narrower pelvis; and (H5) enlarged cnemial crests.

I also examine hindlimb kinematics across behaviors (terrestrial locomotion, surface swimming, and diving) in a wing-propelled diver (eider), foot-propelled diver (scaup), and a surface swimming species (mallard), allowing direct comparison among these three groups. Because divers are expected to have pelvic and limb adaptations seen in other avian swimmers, I hypothesize that the eider and scaup walk with shorter stride lengths and more upright body posture due to having posteriorly positioned legs and, as a result, have a limited range of walking speeds for their body sizes. Surface swimming kinematics and speeds are hypothesized to be similar among all ducks since this is a behavior in which all ducks engage and speeds are limited by the hull speed of the animal (Prange & Schmidt-Nielsen, 1970). However, I hypothesize that the diving specialists will dive at higher speeds than mallards, with eiders using the fastest speeds and scaup diving at intermediate speeds.

4.2 Methods

4.2.1 Skeletal Sampling & Analysis

Sampling

Specimens were measured from ornithological skeletal collections at Harvard University's Museum of Comparative Zoology, Yale University's Peabody Museum, and the Smithsonian National Museum of Natural History. Outgroup sampling included galliformes (n=1) and from within anseriformes, included screamers (n=2), anserinae (n=4), and whistling ducks (n=1). In-group (i.e., duck) sampling focused on tribes anatini (n=16), aythyini (n=13), mergini (n=14), and tadornini (n=10) and species closely aligned with these groups (anatini adjacent: n=5, "diving" adjacent: n=4, aythyini adjacent: n=3, tadornini adjacent n=3). Some basal ducks (n=6) were also sampled. Overall the sample included a 52% species coverage and 35 out of 44 genera (80%) from within ducks (Winkler *et al.*, 2020).

Linear skeletal measures were measured using digital calipers to 0.01 mm accuracy. Where possible, both limbs were measured and averaged. Otherwise, limbs were measured twice and averaged such that all values represent the average of at least two measures. The linear skeletal measures and the measures calculated from them are listed in Table 4.1.

Behaviors were determined using the Birds of the World Handbook (Winkler *et al.*, 2020) species descriptions. Categories were "terrestrial" for birds that primarily forage on land, "surface" for birds that frequently swim but do not dive, "foot" for diving birds that do not use their wings for propulsion, and "wing" for diving birds that use their wings and feet for propulsion (Figure 4.1, Table S4.1-5).

Constructing a Tree

A phylogenetic tree of the species included in this study was produced using BirdTree.org (Jetz *et al.*, 2012; Jetz *et al.*, 2014). Using the Ericson All Species data set (Ericson *et al.*, 2006), BirdTree created

1000 trees (Rubolini *et al.*, 2015). A majority rule consensus tree was created using the phytools package (v. 0.7-47; Revell, 2012) in R (version 3.6.0). The resulting tree (Figure 4.1) was used to determine “Tribe”-level groupings, which included the tribes Anatini, Aythyini, Mergini, and Tadornini. For the purposes of the analyses carried out here, others were grouped as Tadornini-adjacent, Aythyini-adjacent, Diving-adjacent, basal ducks, or outgroup, as indicated (Table S4.1-5).

Table 4.1. Skeletal measures taken from ornithological specimens

Bone	Measure	Description
Pelvis	L _{syn}	Synsacrum length, measured midline
	L _{acet}	Preacetabular length of synsacrum; midline distance between anterior of synsacrum and center of acetabulum
	L _{interacet}	Inter-acetabular distance: width between acetabula
Femur (F)	L _F	Length; from trochanter to lateral condyle—maximum length
	W _{F, ML}	Midshaft mediolateral width
	W _{F, AP}	Midshaft anteroposterior width
Tibiotarsus (TT)	L _{TT}	Length; longest distance between proximal articular surface & condyles
	W _{TT, ML}	Midshaft mediolateral width
	W _{TT, MLprox}	Proximal mediolateral width, includes fibular crest
	W _{TT, AP}	Midshaft anteroposterior width
		Medial & lateral cnemial crests
	L _{cnem, med} , L _{cnem, lat}	Length along long-axis of TT
	H _{cnem, med} , H _{cnem, lat}	Height: largest distance between shaft of TT & tip of crest
Tarsometatarsus (TMT)	L _{TMT}	Length (maximum)
	W _{TMT, ML}	Midshaft mediolateral width
	W _{TMT, AP}	Midshaft anteroposterior width
Calculated	L _{limb}	Total limb length = L _F + L _{TT} + L _{TMT}

Figure 4.1. Majority rule consensus tree of species included in this analysis. This tree was constructed from 1000 trees downloaded from birdtree.org (Jetz *et al.*, 2012; Jetz *et al.*, 2014), and the majority rule consensus tree was produced using the phytools package (Revell, 2012) and plotted using ggtree (Yu *et al.*, 2017; Yu *et al.*, 2018; Yu, 2020) in R. Swimming behavior (tip labels) and tribe affiliation are indicated (also see Tables S4.1-5).



Principle Component Analysis

A principal component analysis (PCA) was conducted on hindlimb and pelvis measures. To eliminate the effect of body size, data were normalized by taking ratios (Table 4.2) instead of raw measures. These ratios aligned with the hypotheses outlined above. Since the outgroup and basal duck specimens dominated the PCA (Figure 4.2), they were excluded from further analysis.

Table 4.2. Normalized measurements for PCA

Normalized Measure	Calculation	Abbreviation	Hypothesis
F proportion	L_F/L_{limb}	F_percent	H1
TT proportion	L_{TT}/L_{limb}	TT_percent	
TMT proportion	L_{TMT}/L_{limb}	TMT_percent	H1
Femur aspect ratio	$W_{F, ML} / W_{F, AP}$	F_AR	H2
TT aspect ratio	$W_{TT, ML} / W_{TT, AP}$	TT_AR	
TT aspect ratio, proximal	$W_{TT, ML, prox} / W_{TT, AP}$	TTprox_AR	
TMT aspect ratio	$W_{TMT, ML} / W_{TMT, AP}$	TMT_AR	H2
Acetabulum position	Preacetabular Length/Synsacrum Length	Acetab_SynsacL	H3
Pelvis width	Interacetabular distance/Synsacrum length	Interacet_Synsac	H4
Relative cnemial crest dimensions	For both medial and lateral cnemial crests: L_{cnem}/L_{TT} H_{cnem}/L_{TT}	CnemMed_L_TTL CnemLat_L_TTL CnemMed_H_TTL CnemLat_H_TTL	H5

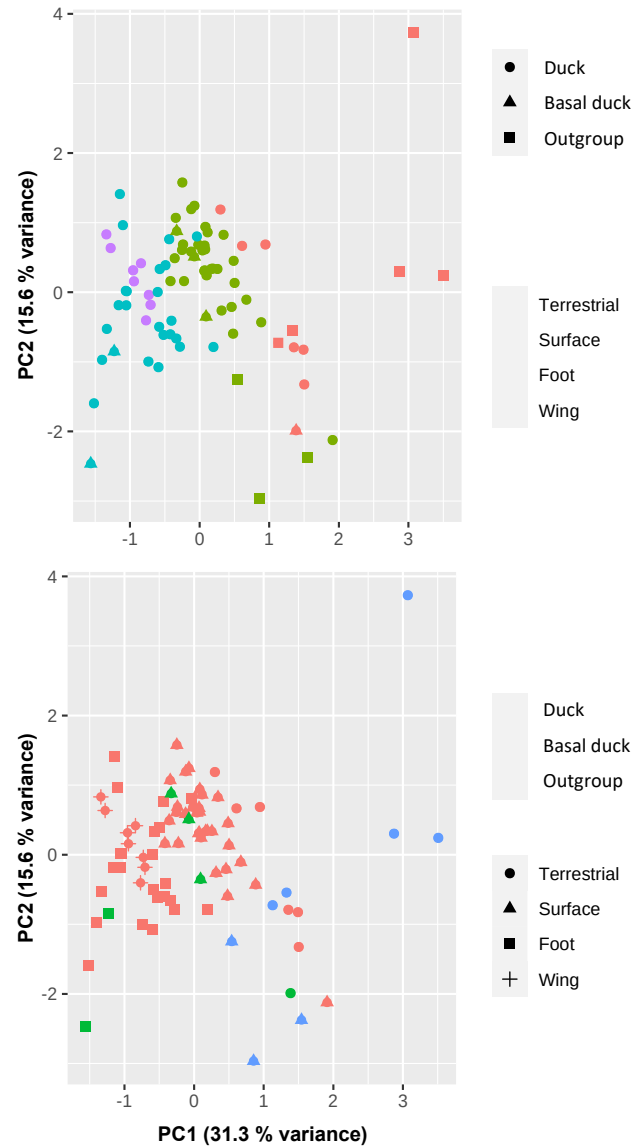


Figure 4.2. A PCA including all outgroups and basal ducks revealed that behavioral groups were separated along PC1 (color in A, shape in B), but outgroups and some species of basal ducks occupied a different morphospace than in-groups ducks (shape in A, color in B), so these groups were excluded based from further analysis. PC1 explained 31.3% and PC2 explained 15.6% of variance among all species.

The results of the PCA are shown in Table 4.3. Using the standard deviation cutoff of >1 , five PCs were retained for further analysis. The loadings for each ratio for the first five PCs are shown in Table 4.4. A multivariate analysis of variance (MANOVA) was conducted on PC1-5. Assumptions of this test were tested as outlined in Tabachnick & Fidell (1983) and using the R code

provided by Datanovia (2020). Assumptions tested and results of those tests were as follows: (1) No outliers: One extreme univariate outlier was removed from the dataset (*Aix sponsa*) with non-extreme outliers retained (n=6). There were no multivariate outliers, tested using the Mahalanobis distances. (2) Univariate normality: Q-Q plots were used to confirm normality along each PC for each behavioral group. (3) Multivariate normality: A multivariate Shapiro-Wilk test showed non-normality ($p < 0.05$), so nonparametric post-hoc tests were performed. (4) Homogeneity of variance: A Levene's test showed homogeneity of variance for all PCs except PC3. (5) Homogeneity of covariance: The Box's M test indicated that there was not equal covariance; therefore, the Pillai's statistic and Wilk's lambda were used for the MANOVA since these statistics are robust to violations of assumptions under different circumstances (Ateş *et al.*, 2019).

The Kruskal-Wallis test with Bonferroni-Holm adjustment was used to determine along which PCs the groups differed, and the Tukey's pairwise comparison was used to compare groups. Differences in specific skeletal measures among behavioral groups were tested using analysis of variance (ANOVA). Phylogenetically-corrected ANOVA (phyloANOVA) and MANOVA (phyloMANOVA) were performed using the phyloANOVA function in the *geiger* package (v. 2.0.7; Pennell *et al.*, 2014) in R. Post-hoc tests included pairwise t-tests with Bonferroni correction for multiple comparisons.

4.2.2 Kinematics

Animals

Kinematics of walking, surface swimming, and diving were collected from mallards (n=9, 3-4 per behavior; *Anas platyrhynchos*; Linnaeus, 1758) from tribe anatini, New Zealand scaup (n=6; *Aythya novaeseelandiae*; Gmelin, 1789) from tribe aythyini, and common eider (n=6; *Somateria mollissima*; Linnaeus, 1758) from tribe mergini. Mallards are medium-sized (0.7 -1.1 kg in this study) dabbling

ducks that frequently venture on land, feed primarily in the water through dabbling, and only dive occasionally (Winkler *et al.*, 2020). New Zealand scaup are small ducks (generally 0.55–0.73 kg) that forage by foot-propelled diving in shallow water (Winkler *et al.*, 2020). Eiders are large sea ducks (generally 1.3-2.6 kg) that feed by diving up to 10 m; eiders dive using their wings and feet and spend relatively little time on land, although they are more stable while walking than some other sea ducks (Winkler *et al.*, 2020). Mallards were either wild-caught (n=6, Massachusetts Division of Fisheries & Wildlife Permits 011.15SCB, 007.17SCB, and 093.18SCB and U.S. Fish & Wildlife Permit MB005348-0) or farm-raised (n=3). They were housed at the Concord Field Station (Bedford, MA) in indoor-outdoor enclosures with access to a small pool for swimming. Scaup and eiders were captive bred at the Livingston-Ripley Waterfowl Conservatory (Litchfield, CT) and were housed in outdoor habitats with terrestrial and aquatic components.

Data Collection

Mallards were trained to perform the desired behaviors in the laboratory. They were filmed while walking at a constant speed on a treadmill (0.4-1.8 ms⁻¹, n=33 trials from N=4 individuals). Mallard surface swimming (n=13 trials from N=4 individuals) and diving (n=8 trials from N=3 individuals) were filmed in a small acrylic tank (2.4 m x 0.6 m x 0.6 m depth) or in a pool (oval, 3.7 m x 7.3 m x 1.2 m water depth). Mallards were trained to cross from one end of the tank or pool to the other. Diving was elicited by placing a barrier just below the water's surface in the center of the tank or pool. Diving is a natural but not common behavior in mallards, so while the majority of ducks readily swam under the barrier, some did not and were excluded from the analysis.

Scaup and eider data collection occurred at the LRWC. The LRWC has a mission of waterfowl conservation; as experts in waterfowl biology, the LRWC staff work to maintain breeding populations of species that are rare and/or typically difficult to maintain in captivity. LRWC policies support their conservation mission by minimizing animal stress; thus, animals are rarely handled. In

compliance with their policies, these birds were not trained, and in order to reduce capture and handling stress, this sequence was completed only once per animal. Thus, sample sizes per animal and per species were limited for scaup and eiders. Birds were captured from their home enclosures and moved to the outdoor filming enclosure. For walking trials, birds were released at some distance from a pond on a grassy substrate. They were filmed while walking from the release point to the pond at self-selected speeds, with one trial captured per individual per LRWC policy (scaup: n=6 trials from 6 individuals, eiders: n=4 trials from 4 individuals). Surface swimming (scaup: n=5 trials, eider: n=8 trials) and diving (scaup: n=5 trials, eider: n=4 trials) were filmed in the large artificial pond (sloped bottom 1-2 m deep). Surface swimming and most diving sequences were spontaneous behaviors or were encouraged by adding food to the pond; however, a few sequences captured were escape behaviors as the ducks evaded recapture.

Kinematics were recorded using high speed videography (125 fps). Two IDT NR5-S1 cameras (Integrated Design Tools, Inc., Pasadena, CA, USA) equipped with Nikon AF Nikkor 24-85 mm zoom lenses (Nikon Inc., Melville, NY, USA) were used to permit 3D analysis. While filming the scaup & eiders in the pond, cameras were submerged in a custom, watertight camera box (Clifton & Biewener, 2018). A calibration with a known wand length was calculated using EasyWand5 (Theriault *et al.*, 2014) and points were digitized using DLTdv6 (Hedrick, 2008).

Analysis

For each trial and each behavior, velocity, frequency and stride length were measured. A constant point on the hull of the duck (such as a distinct feather or mark) was digitized to measure speed, except for mallard treadmill trials where speed was pre-determined. Average velocity (v) was the distance traveled by the body/trial duration and was calculated across all analyzed cycles. Frequency (f) was measured as 1/cycle time, where cycle time was measured from beginning of stance (walking) or power stroke (surface swimming and diving) from one cycle to the next. Stride

length (L_s) was the distance the body traveled during each cycle. For all behaviors, each trial represents the average for all strides of steady locomotion that were captured (n=1-10 strides). For mallard treadmill trials, average values for each speed were calculated by first finding the average for each individual and then averaging across individuals. For walking in the other two species (scaup and eiders) and for all swimming behaviors, speeds were self-selected, so the average for each trial is shown and no attempt was made to average across individuals.

Because body size differs considerable among these species, each of these measures was normalized for body size as in Gatesy & Biewener (1991) and Abourachid (2001). Bone lengths were obtained for each individual mallard by palpating the bones and measuring lengths using calipers. Touching the LRWC birds was not permitted, so distal elements (phalanges and tarsometatarsus) were measured for each individual while proximal elements (tibiotarsus and femur) measurements were taken from skeletal museum specimens. Hip height was estimated from bone lengths and calibrated images taken at mid-stance during terrestrial locomotion. A single average value was used across all individuals (mallard: 0.13 m, scaup: 0.11 m, eider: 0.17 m). These values were slightly lower than but similar to values used by others (mallard: 0.14 m, Abourachid, 2001, 0.17, Biewener & Corning, 2001; eider: 0.2 m, Hasley, 2013) and with measurements taken from X-ray on other individuals (mallard: 0.14 m, data not shown, X-ray video recorded as in Taylor-Burt & Biewener, 2020 but at midstance on a treadmill). Relative velocity (v_{rel}) was $v(gh)^{0.5}$, where g is gravitational acceleration (9.8 ms^{-2}) and h is hip height (m). Relative frequency (f_{rel}) was $f(h/g)^{0.5}$ and relative step length ($L_{s,rel}$) was L_s/h .

A planar analysis of body angle during terrestrial locomotion was performed using video frames from a single lateral view. Body angle was defined as the angle the body axis (the midline of the torso, from the midline of the breast to the tip of the tail) was raised from horizontal. Body angle varied with speed, so body angles were compared at the same relative velocities. Data were

limited for perfectly lateral views of scaup and eider terrestrial locomotion, so body angles were analyzed for two mallards at each of the relative velocities observed for the other two species. Each body angle was measured three times from a given frame and averaged to reduce digitizing error.

All statistical tests were performed in R (v. 3.6.3). Linear models were fit to the relative frequency and relative stride length data for each behavior, with species, relative velocity, and their interaction as independent variables. The significance of each independent variable was determined by ANOVA. All values reported for skeletal and kinematic analyses are averages $\pm$ S.D.

4.3 Results

4.3.1 Skeletal Analysis

PCA

The ratios used in the PCA are reported by specimen in Tables S4.1-5. The first five PCs had an eigenvalue > 1 and so were retained for further analysis (Table 4.3). The first PC explained 26% of the variance, and all five PCs explained 72% of the variance.

Table 4.3. PCA results; eigenvalues, proportion of variation explained, and cumulative variation explained.

	PC1	PC2	PC3	PC4	PC5
Eigenvalue	3.38	1.84	1.45	1.39	1.35
Proportion of variance	0.2602	0.1418	0.1113	0.1072	0.1040
Cumulative proportion	0.2602	0.4020	0.5133	0.6205	0.7245

The variable loadings for the PC1-5 are shown in Table 4.4, with the contributions that are larger than expected by chance (i.e., a contribution larger than $1/n$, where $n=13$ variables [see Table 4.2] and $1/n$ is the contribution expected if each variable contributes equally) in bold. Of particular

interest is that divers have lower PC1 values than non-divers (Figures 4.2A, 4.3A), indicating that they have relatively longer femora (since F_percent has negative PC1 loading) and shorter tarsometatarsi (since TMT_percent has a positive PC1 loading, Table 4.4). However, both tribe affiliation and swimming behaviors are separated along PC1 (Figure 4.3), highlighting the importance of phylogenetic correction when considering how skeletal anatomy is linked to swimming behaviors.

Table 4.4. PCA variable loadings for PC1-5 for each of the normalized hindlimb skeletal measurements

	PC1	PC2	PC3	PC4	PC5
F_percent	-0.8023	0.4678	0.1097	-0.047	0.1132
TT_percent	0.0929	-0.6045	0.5425	-0.3253	0.1209
TMT_percent	0.7778	-0.1337	-0.4289	0.2377	-0.1876
F_AR	0.6365	0.2631	0.1053	0.1099	0.4036
TT_AR	-0.5511	-0.4054	-0.1378	-0.1315	0.4605
TTProx_AR	0.0459	0.0273	0.5669	-0.3407	-0.5749
TMT_AR	-0.0411	0.4519	0.6302	0.297	0.3301
InterAcet_Synsac	0.332	0.6714	-0.0509	-0.3641	-0.2571
Acet_SynsacL	0.4726	0.4889	-0.1676	-0.4199	0.29
CnemMed_L_TTL	-0.4795	0.2758	0.0937	0.6157	-0.1661
CnemMed_H_TTL	-0.3414	-0.0122	-0.0865	-0.0387	-0.5248
CnemLat_L_TTL	-0.4906	0.1187	-0.177	-0.5516	0.1441
CnemLat_H_TTL	-0.7012	0.1508	-0.3605	-0.0339	-0.0321

Bolded values indicate contribution higher than expected by chance

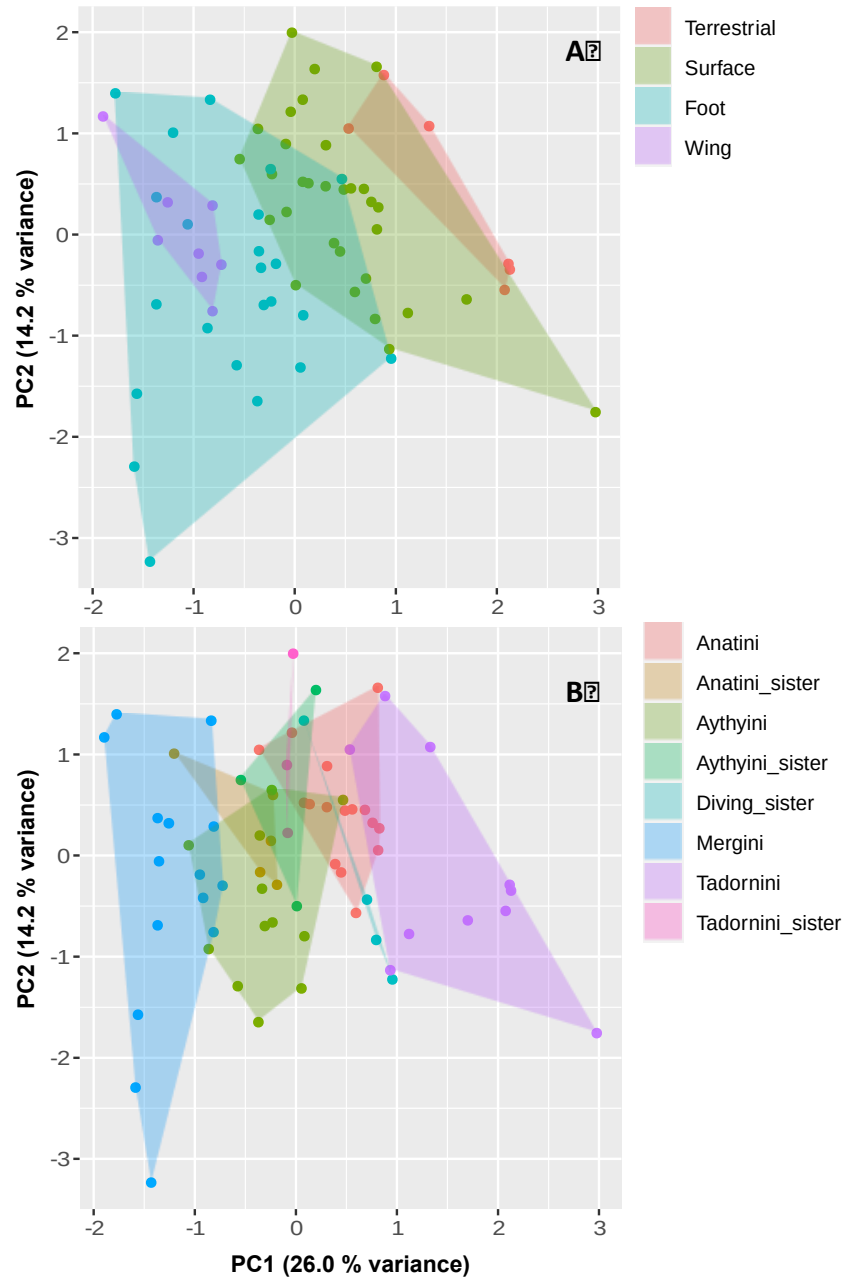


Figure 4.3. Both swimming behavior categories (A) and tribes (B) were separated along PC1.

26% of the sample variance was explained by PC1 and 14.2% by PC2. Both MANOVA and phyloMANOVA revealed significant differences among the behavioral groups (A), with post-hoc tests showing significant separation along PC1 but not PC2.

MANOVA & phyloMANOVA for PCs

The MANOVA on PC1-5 indicated that the skeletal measures differed among swimming behaviors (Wilk's and Pillai tests $p < 0.001$). The Kruskal-Wallis test with a Bonferroni correction revealed that groups were significantly different along PC1 ($p < 0.001$), PC3 ($p = 0.02$), and PC5 ($p = 0.02$), but not along PC2 ($p = 0.13$) and PC4 ($p = 0.49$). The phyloMANOVA also supported differences among behavioral groups ($p = 0.031$). A phyloANOVA with Bonferroni correction revealed that behavioral groups differed along PC1 ($p = 0.005$), but not along any other PCs ($p > 0.05$). Thus, variables with a strong loading on PC1, like the proportional lengths of the femur and TMT (F_percent, TMT_percent), aspect ratios of the femur and TT (F_AR, TT_AR), and the height of the lateral cnemial crest (CnemLat_H_TTL) help to separate behavioral groups even when accounting for phylogenetic relationships.

Factors that load on PC2 and PC4, like the relative width of the pelvis (InterAcet_Synsac) and the position of the acetabula (Acet_SynsacL) varied among ducks but not systematically among behavioral groups. Factors that load on PC3 and PC5, like the aspect ratio of the TMT (TMT_AR) and proximal TT (TTProx_AR) and height of the medial cnemial crest (CnemMed_H_TTL) varied among behavioral groups, but did not differ among these groups when considering the phylogenetic relationships, meaning closely related animals tend to have similarities along PC3 and PC5 because of their common ancestry, not necessarily their common swimming strategies.

Tukey-HSD pairwise comparisons for the non-phylogenetically corrected analysis showed differences among all groups along PC1 except between the two diving groups (foot and wing, Table 4.5). When including the phylogenetic correction, pairwise comparisons (paired t-tests with Bonferroni correction) showed similar results except the surface swimmers were not significantly different from terrestrial or foot-propelled diving species (Table 4.5). These results indicate that the differences observed between surface swimmers vs. terrestrial species and vs. foot-propelled divers

could be explained by their phylogenetic relationships, but the differences between terrestrial species and diving species cannot.

Table 4.5. Pairwise comparisons of behavioral groups for non-phylogenetically corrected (Non) and phylogenetically corrected (Phylo) comparisons along PC1.

	Surface		Foot		Wing	
	<i>Non</i>	<i>Phylo</i>	<i>Non</i>	<i>Phylo</i>	<i>Non</i>	<i>Phylo</i>
Terrestrial	0.048	0.990	<0.001	0.042	<0.001	0.018
Surface			<0.001	0.174	<0.001	0.048
Foot					0.133	1.000

Significant p-values are bolded.

ANOVA and phyloANOVA for select traits

Because there were significant differences among groups along PC1 even with phylogenetic correction, variables that loaded heavily along this axis (Table 4.4, bolded values) were used for further analysis. Specifically, these included the proportion of the hindlimb made up by the femur and tarsometatarsus (F_percent, TMT_percent, Figure 4.4), the relative height of the lateral cnemial crest (CnemLat_H_TTL, Figure 4.5), and the mid-shaft aspect ratio (ML vs AP diameters) of the femur (F_AR) and tibiotarsus (TT_AR, Figure 4.6). Groups with significantly different means (pairwise comparisons) are indicated with different letters in Figures 4.4-6 (i.e., all groups labeled “A” are significantly different from all groups labeled “B”, etc.).

Figure 4.4 includes a diagram showing the three segments of the hindlimb (F, TT, and TMT) as well as boxplots and a ternary plot, which both show the relative proportion of the hindlimb length that is made up by each segment. An ANOVA for the proportional lengths of the femur and TMT both revealed differences among groups ($p < 0.001$), with significant p-values ($p < 0.05$) for all pairwise comparisons of divers (foot and wing) with non-divers (surface and terrestrial). Specifically,

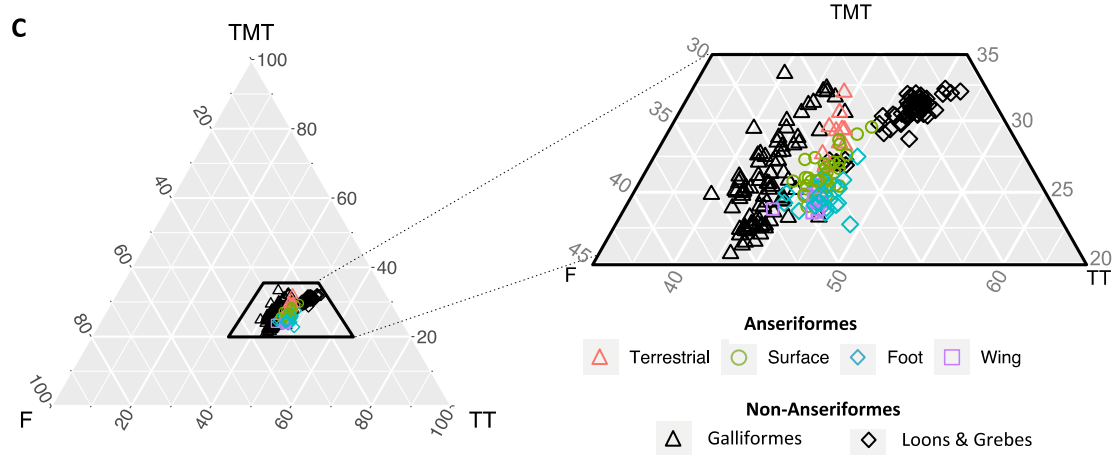
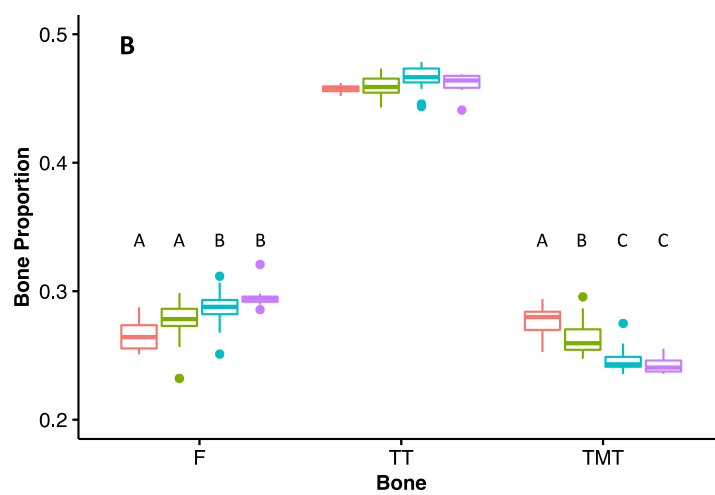
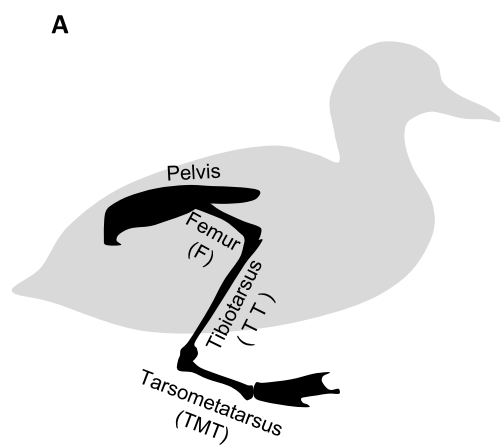
divers have longer femora and shorter TMT proportional to total limb length than surface swimmers and terrestrial species (see Table S4.1-5). Neither femur nor TMT proportions differed when comparing foot and wing-propelled divers. Femur proportions were also not different between surface swimmers and terrestrial species, but TMT proportions significantly differed between these two groups with surface swimmers having relatively shorter TMT than terrestrial species (Figure 4.4).

The dimensions for both the lateral and medial cnemial crests are shown in Figure 4.5. The relative height of the lateral cnemial crest loaded strongly on PC1, so this measure was selected for further analysis. ANOVA showed there were significant differences among groups ($p=0.006$). The only significant Tukey HSD pairwise comparisons were between wing-propelled divers and surface swimmers ($p=0.009$) and terrestrial species ($p=0.011$), with wing-propelled having proportionally taller lateral cnemial crests (Table S4.1-5). All other comparisons were not significant ($p>0.05$).

Aspect ratios (i.e., shape) of the hindlimb bones are shown in Figure 4.6. For all behavioral groups, the femur tends to be deeper ($W_{ML} / W_{AP} < 1$), the TT tends to be wider ($W_{ML} / W_{AP} > 1$) and the TMT is relatively uniform in dimensions ($W_{ML} / W_{AP} \sim 1$). The femur and TT aspect ratios loaded on PC1, so behavioral group means for these measures were compared using ANOVA. Both differed significantly among behavioral groups ($p<0.001$). Femur aspect ratios differed between the terrestrial group and all other groups, with the terrestrial group displaying a higher average femur aspect ratio (i.e., closer to one and less mediolaterally flattened) than all other groups (Figure 4.6, Table S4.1-5). No other comparisons of femur aspect ratios were significant. All pairwise comparisons of TT aspect ratios showed significant differences except between surface swimmers and terrestrial species. Wing-propelled divers had the highest TT aspect ratio (most anterior-posteriorly flattened TT), surface swimmers and terrestrial species had the lowest aspect ratio (i.e., roundest TT), and foot-propelled divers were intermediate (Figure 4.6, Table S4.1-5).

Figure 4.4. Proportion of the hindlimb made up by the F and TMT varies with swimming behavior.

(A) Diagram of the avian hindlimb showing the F, TT, and TMT. (B) Proportional lengths for the hindlimb long bones. F and TMT proportional lengths were significantly different among groups when compared using ANOVA ($p < 0.001$ for both) but not phyloANOVA (F: $p = 0.727$, TMT: $p = 0.823$). Groups with means that are significantly different (TukeyHSD pairwise comparisons, $\alpha = 0.05$) are indicated by different letters (A, B, C). Boxplots show the median (thick line), upper and lower quartiles, and highest and lowest values (vertical lines), excluding outliers. (C) A ternary plot of proportion of the hindlimb made up by each bone with an expanded view of the region of interest (black trapezoid; Galliform, loon, & grebe data from Gatesy & Middleton, 1997). Anseriformes occupy the morphospace between terrestrial Galliformes and foot-propelled diving specialists (loons & grebes), with diving ducks (wing & foot) falling farther from and non-diving ducks (surface & terrestrial) falling closer to the specialized foot-propelled swimmer morphospace. Ternary plot was created using the ggtern package in R (Hamilton & Ferry, 2018).



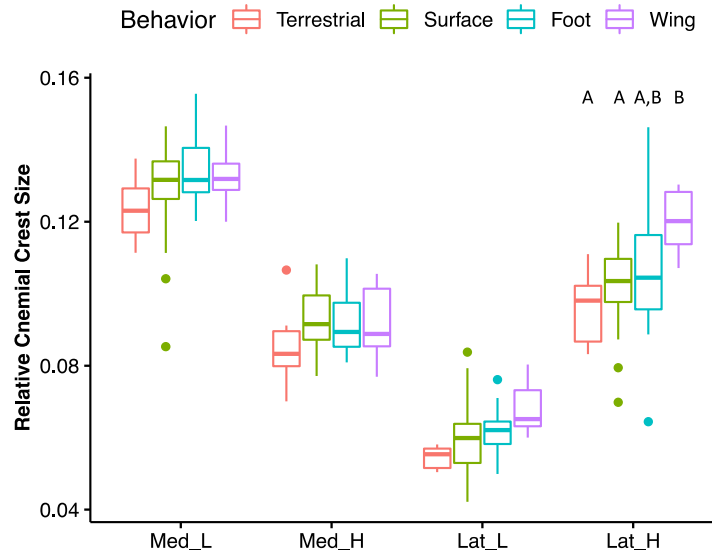


Figure 4.5. Dimensions normalized by TT length for the medial (Med) and lateral (Lat) cnemial crests. Length (L) was measured along the long axis of the TT and height (H) is maximum distance from the TT shaft to the tip of the crest. Lateral cnemial crest height was significantly different among behavioral groups when using ANOVA ($p=0.006$) but not phyloANOVA ($p=0.539$). Letters (A, B) indicate groups with significantly different means found using TukeyHSD pairwise comparisons ($\alpha=0.05$). Boxplots show the median (thick line), upper and lower quartiles, and highest and lowest values (vertical lines), excluding outliers.

Following phylogenetic correction (phyloANOVA), no differences remained among groups for the femur proportion ($p=0.727$), TMT proportion ($p=0.823$), lateral cnemial crest height ($p=0.539$), femur aspect ratio ($p=0.275$), and tibiotarsus aspect ratio ($p=0.055$).

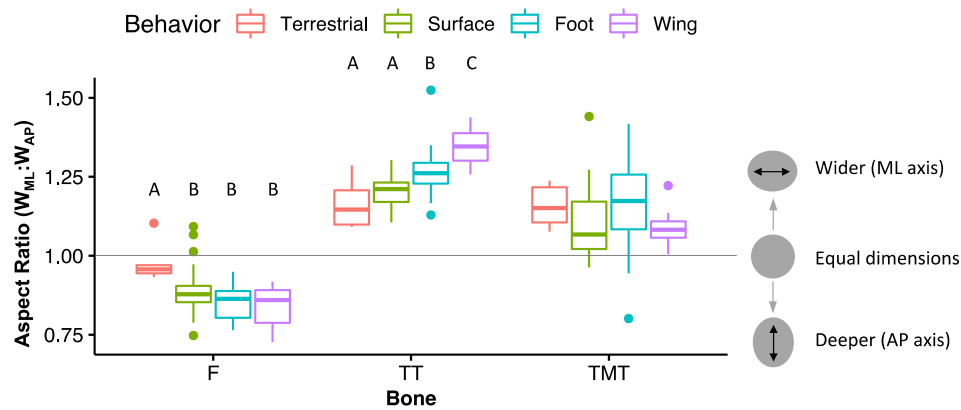


Figure 4.6. Aspect ratio for F and TT loaded on PC1 (see Table 4.4) and were significantly different among behavioral groups when compared using ANOVA ($p<0.001$ for both) but not phyloANOVA (F: $p=0.275$, TT: $p=0.055$). Aspect ratio is the ratio of the mediolateral and anterior-posterior diameters measured mid-shaft. An aspect ratio of one (indicated by gray horizontal line) indicates equal diameters in both directions (i.e., an approximately circular cross-section) while bones that deviate from one would have an elliptical cross-section, as represented by the gray shapes. Letters (A, B, C) indicate groups with significantly different means found using TukeyHSD pairwise comparisons ($\alpha=0.05$). Boxplots show the median (thick line), upper and lower quartiles, and highest and lowest values (vertical lines), excluding outliers.

4.3.2 Kinematics

Cycle frequency and stride length vs. velocity relationships are shown in Figure 4.7 and their normalized counterparts (see Methods) in Figure 4.8. These results are summarized in Table 4.6.

Terrestrial Locomotion

Eiders moved at absolutely higher speeds than the other two species during terrestrial locomotion (Figure 4.7A,B), but the ranges overlapped when normalized for body size (Figure 4.8A,B). During terrestrial locomotion, relative frequency and relative stride length both increased

with relative velocity (Figure 4.8A,B, Table 4.6). The relationship with relative velocity did not differ among species for relative stride length, but did differ among species for relative frequency (Figure 4.8A), with eiders tending to use higher relative frequencies for a given relative velocity than mallards ($p=0.018$) and scaup ($p=0.024$); but no significant difference was found between mallards and scaup (0.803).

Table 4.6. Relationship between relative frequency and stride length across velocities and species for terrestrial locomotion, surface swimming, and diving (linear model $y \sim v_{rel} * \text{species}$)

Dependent variable	Independent variable	Terrestrial	Surface Swimming	Diving
f_{rel}	v_{rel}	<0.001	0.241	0.299
	species	0.002	0.004	0.524
	species : v_{rel}	0.229	0.241	0.159
$L_{s,rel}$	v_{rel}	<0.001	<0.001	0.009
	species	0.060	0.363	0.001
	species : v_{rel}	0.672	0.811	0.009

Significant p-values are in bold.

Because body angle varied with speed, body angles were averaged across trials and individuals (scaup: $n=3$ trials, $N=3$ individuals; eider: $n=2$ trials, $N=2$ individuals; mallard: $n=6$ trials, $N=2$ individuals) and also compared at the same relative velocity (1.4). On average, mallards adopted a much lower body angle ($11 \pm 5^\circ$) than scaup ($32 \pm 11^\circ$) and eiders ($29 \pm 5^\circ$) when moving over ground. At a relative velocity of 1.4, scaup exhibited the highest body angle (40°), followed by eiders (26°), and mallards with the lowest body angle (11°). No statistical analyses were performed due to low sample sizes for these comparisons.

Surface Swimming

Surface swimming speed ranges of the three species overlapped, except the range of surface swimming speeds used by mallards extended above the speeds used by the other two species (Figure 4.7C,D). Relative frequency did not change with relative swimming velocity, but average relative frequency differed among species (Figure 4.8C, Table 4.6). Eiders tended to use lower relative frequencies than mallards ($p < 0.001$) and scaup ($p = 0.013$), but there was no significant difference between scaup and mallards ($p = 0.764$). Relative stride length increased with relative velocity during surface swimming, but this relationship did not differ among species (Table 4.6).

Diving

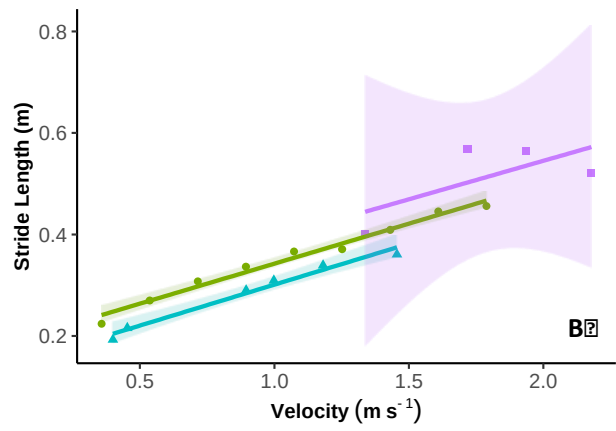
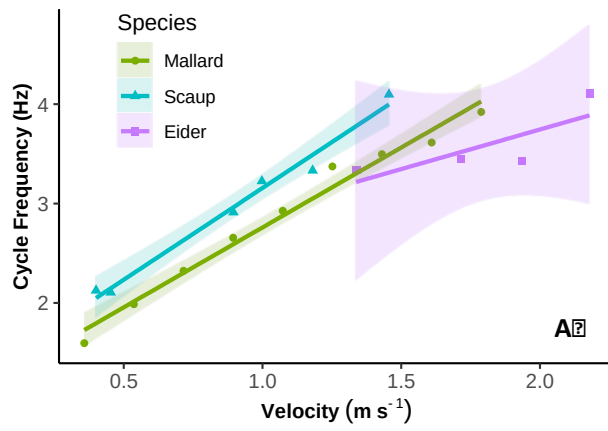
During underwater diving, each of the species used different swimming techniques. In order to submerge themselves, all three species spread the toes and drew the foot forward in a “reverse” power stroke to help angle the body downward in the water. Only the scaup preceded this “reverse” power stroke of the feet with a typical, caudal power stroke to first push the body out of the water (caudal stroke) and then draw the head down (reverse power stroke). Once submerged, scaup only used their feet in a synchronous motion unless turning, displaying the least diversity in kinematic patterns while underwater. Mallards descended using only their wings but navigated horizontally underwater and turned upward using only their feet in an asynchronous paddling that resembled surface swimming. Like scaup, ascent was primarily passive for mallards, but during deep dives in the pool, mallards often used their feet intermittently and their wings for one stroke as they neared the surface. Eiders always used their wings to submerge themselves, but once underwater, they varied their swimming technique, showing more diversity than either mallards or scaup. Eiders dove using only their feet, their feet and wings moving all four limbs synchronously, both wings and feet with wing movement asynchronous with foot movement, and, rarely, with only their wings. When eiders used their feet, they were always moved synchronously. Ascent in eiders was typically active

using a variety of kinematic patterns. The trials included in this analysis for eiders represent both foot-only swimming (the slower two trials) and wing-and-foot-propelled swimming (the faster two trials, Figures 4.7E,F & 4.8E,F). Notably, the arc of foot motion was more lateral in scaup than in eiders and mallards, which moved their feet in a cranial-caudal arc.

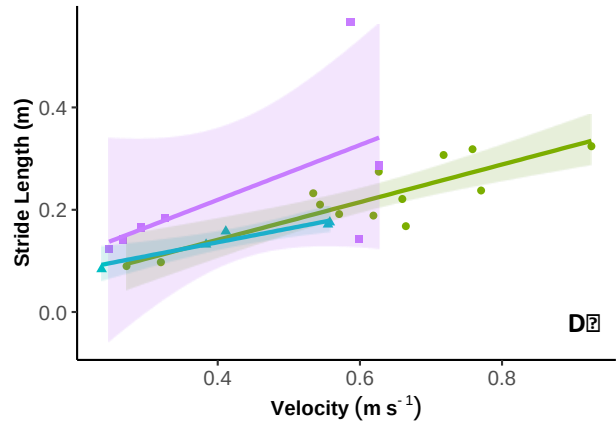
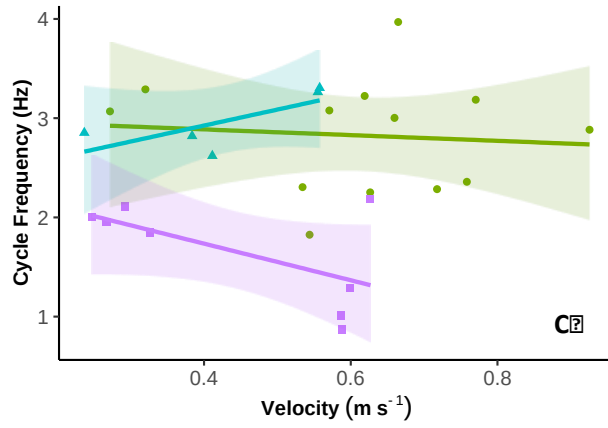
During diving, eiders swam at both higher absolute (Figure 4.7E,F) and relative (Figure 4.8E,F) velocities. As with surface swimming, relative frequency did not change with speed during diving, but relative stride length did (Table 4.6). There was no significant difference among species for relative frequency, but there were significant species differences for stride length, with the relationship between stride length and relative velocity demonstrating a significantly different intercept (eider vs. scaup: $p=0.034$, eider vs. mallard: $p=0.005$) and slope (eider vs. scaup: $p=0.034$, eider vs. mallard: $p=0.003$) between eiders and the other species. The slope of this relationship was significantly different from zero for eiders ($p<0.001$). However, neither intercept ($p=0.147$) nor slope ($p=0.300$) for stride length vs. relative velocity differed between scaup and mallards, and the slope was not significantly different from zero for either species (scaup: $p=0.116$, mallard: $p=0.688$).

Figure 4.7. Cycle frequency and stride length vs. velocity for terrestrial locomotion (A, B), surface swimming (C, D), and diving (E, F). Each point is the average across several cycles ($n=1-10$) for each trial except for mallard terrestrial data, where velocity was controlled by the treadmill and could be averaged across individuals at each speed. Dark lines indicate the linear relationship between frequency or stride length and velocity for each species, with the shaded region indicating standard error.

Terrestrial



Surface Swimming



Diving

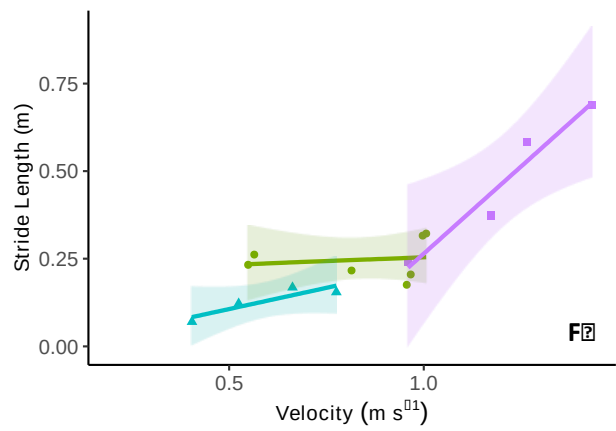
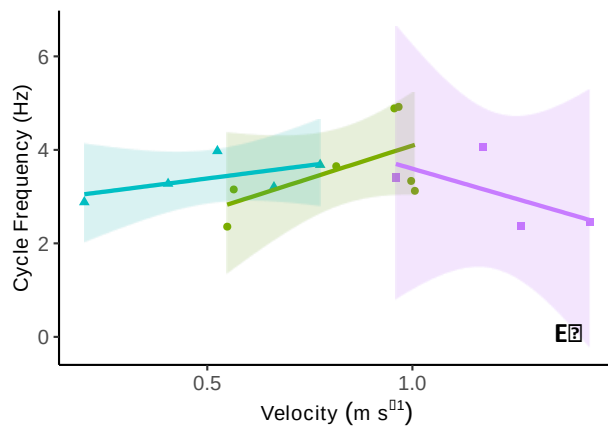
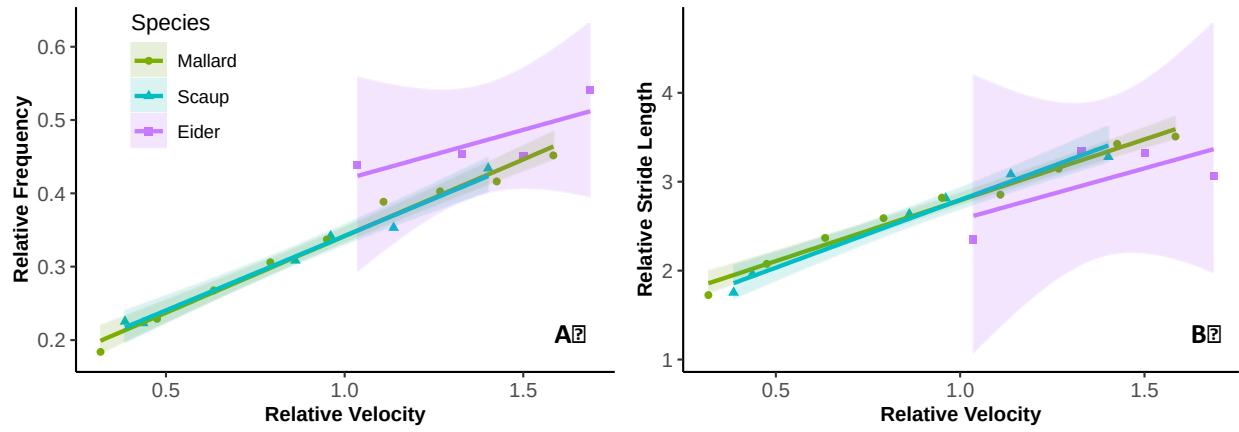
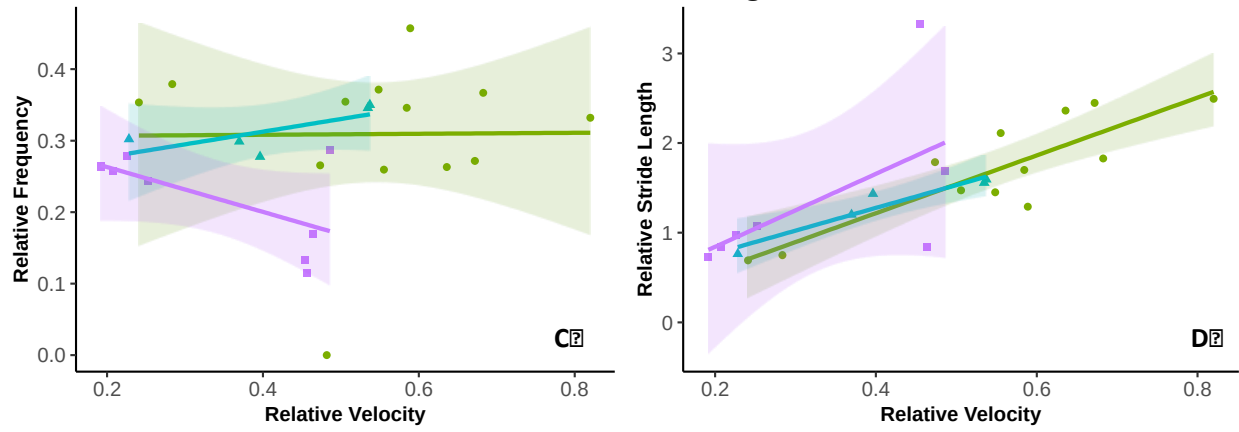


Figure 4.8. Relative frequency and relative stride length vs. relative velocity for terrestrial locomotion (A, B), surface swimming (C, D), and diving (E, F). Each point is the average across several cycles ($n=1-10$) for each trial except for mallard terrestrial data, where velocity was controlled by the treadmill and could be averaged across individuals at each speed. Dark lines indicate the linear relationship between frequency or stride length and velocity for each species, with the shaded region indicating standard error. Linear models were fit with relative velocity, species, and their interaction. Relative frequency increased with relative velocity for terrestrial locomotion but not surface swimming or diving. Although the slopes are not significantly different, relative frequencies used by eiders are higher during terrestrial locomotion and lower during surface swimming than mallard and scaup. There were no species differences for the frequency-velocity relationship for diving. Relative stride length increased with relative velocity for all three behaviors except for scaup and mallards during diving. There were significant differences in slope for the stride length-velocity relationship among species only for diving, where eiders had a higher slope than the other two species, whose stride length vs. velocity slopes were not different from zero. See Table S4.6 and Results text for more detail.

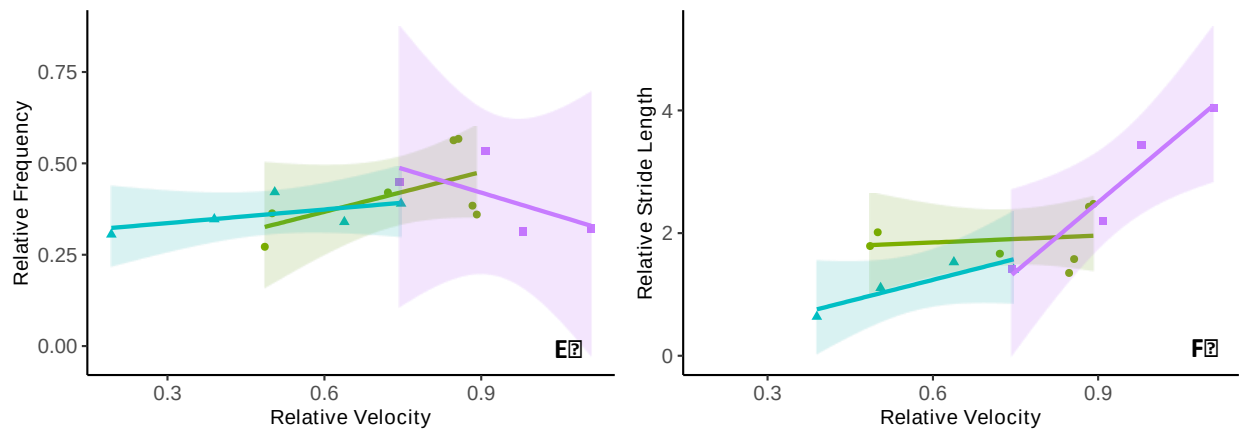
Terrestrial



Surface Swimming



Diving



4.5 Discussion

The swimming abilities of duck species range from highly terrestrial and to diving specialists. In this study, hindlimb and pelvic skeletal anatomy and hindlimb kinematics during terrestrial locomotion, surface swimming, and diving were observed across the duck phylogeny. I hypothesized that swimming ability in ducks would be associated with the same suite of hindlimb skeletal adaptations observed across the avian tree (Raikow, 1973; Cracraft, 1982; Johnsgard, 1987; Lovvorn, 1990; Gatesy & Middleton, 1997; Zeffer *et al.*, 2003; Hinić-Frlog & Motani, 2010; Ibáñez & Tambussi, 2012; Stoessel *et al.*, 2013; Anten-Housten *et al.*, 2017), with a gradient of hindlimb morphologies reflecting the range of swimming abilities in ducks. I also hypothesized that diving species would use shorter strides and walk at relatively slower speeds for their body size while using faster swimming speeds during diving compared with dabbling species.

4.5.1 Hindlimb Skeletal Anatomy & Swimming Behavior

Hindlimb and pelvic skeletal anatomy was compared using ratios of measured anatomical lengths to remove the effect of body size (Table 4.2). The ratios were analyzed with PCA. PC1, which explained 26% of the variance in the data set, was the only axis that separated behavior groups following phylogenetic correction (Table 4.3). The outgroups and some basal duck species occupied a different morphospace than the duck tribes included in this analysis, although some basal duck species overlapped with the in-group duck species (Figure 4.2B). Despite these differences, the spread of behavioral groups of the full sample (Figure 4.2A) resembles the spread of the in-group species by behavior (Figure 4.3A), supporting the conclusion that behavioral groups are separated along PC1, even among distantly related groups like screamers and Galliformes.

Measures that loaded heavily on PC1 and were selected for further analysis included the proportional lengths of the femur and TMT, shapes of the femur and TT, and height of the lateral cnemial crest (Table 4.4). TMT length and cnemial crest height were also useful for distinguishing

swimming birds across the avian phylogeny (Hinić-Frlog & Motani, 2010). Other measures, including the shape of the pelvis and relative position of the acetabulum loaded on PC2 and PC4, but behavioral groups did not differ along these axes, indicating that although variation exists in these morphological traits among ducks, they do not vary with swimming behavior. Thus, my hypotheses about variation in pelvic anatomy in relation to swimming behavior (H3 and H4) were not supported. PC3 and 5 also explained the variation among swimming behaviors and included strong loading from variables like 'TMT' shape and medial cnemial crest height; however, swimming groups were not significantly different along these axes when accounting for phylogeny.

Proportional lengths of the femur and 'TMT', shapes of the femur and 'TT', and height of the lateral cnemial crest were compared across behavioral groups using ANOVA and phyloANOVA. Although group differences were observed with ANOVA for all traits, patterns did not always support my hypotheses. When limb elements are normalized to limb length (recall, $L_{limb} = L_F + L_{TT} + L_{TMT}$), clades of specialized swimmers typically have shorter femora and longer 'TMT' relative to many non-swimming clades (Gatesy & Middleton, 1997; Zeffner *et al.*, 2003); whereas galliformes, which are highly terrestrial, have long femora and short 'TMT' relative to their limb length (Gatesy & Middleton, 1997). The proportional femur length is longer and proportional 'TMT' length is shorter in diving ducks than non-divers (Figure 4.4), exactly opposite the hypothesized pattern (H1). Species with exceptionally long legs, like waders, and cursorial birds, such as the ostrich, tend to achieve these long lengths by lengthening the 'TMT', and less so the femur (Gatesy & Middleton, 1997). Longer limbs are related to a lower cost of transport during terrestrial locomotion (Pontzer, 2007). Terrestrial ducks have longer L_{limb} than any of the swimming categories (terrestrial: 0.27 ± 0.05 m, surface: 0.18 ± 0.06 m, foot: 0.17 ± 0.04 m, wing: 0.17 ± 0.03 m). This pattern held even when limb length was normalized for body size by dividing by synsacrum length, a measure correlated with body mass across birds and within galloanseres (Stoessel *et al.*, 2013; see also Anten-Houston *et al.*,

2017; terrestrial: 2.7 ± 0.1 , surface: 2.4 ± 0.2 , foot: 2.2 ± 0.2 , wing: 2.3 ± 0.1). Thus, although the proportional length of the femur was high in swimming ducks compared to terrestrial ducks, this was driven by an elongation of L_{limb} and the 'TMT', in particular, in terrestrial species, and not by longer femora in diving birds. Body size-normalized femur length (L_F/L_{syn}) was actually lower in swimmers (surface: 0.66 ± 0.04 , foot: 0.63 ± 0.06 , wing: 0.68 ± 0.03) than non-swimmers (terrestrial: 0.72 ± 0.05). 'TT' proportional length did not load on PC1 and seems to vary little among ducks (Figure 4.4). That 'TT' proportion seems constant among ducks is not surprising given that 'TT' proportion changes very little across the entire avian tree (Gatesy & Middleton, 1997; Stoessel *et al.*, 2012).

Although lateral cnemial crest height loaded on PC1, medial cnemial crest height did not. Wing-propelled diving ducks had significantly longer lateral cnemial crests than non-divers, providing some support to my hypothesis that swimming specialists would have larger cnemial crests (H5). In grebes and loons, the medial and lateral cnemial crests fuse into a single process, which also fuses with the patella in loons (Cracraft, 1982; Johnsgard, 1987). Although the cnemial crests in ducks are well developed, they remain separate structures. The lateral cnemial crest serves as the insertion of hip flexors and knee extensors and the origin of some digital flexors and other muscles acting on the ankle and foot (Clifton *et al.*, 2018). The larger lateral cnemial crest may increase the moment arm of the hip flexors and knee extensors, perhaps helping with joint stabilization during the power stroke and powering the recovery stroke. In addition, the larger lateral cnemial crest offers more surface area for muscle attachment of the digital flexors, fibularis longus, and tibialis cranialis. The digital flexors are enlarged in specialized swimmers, like loons and grebes, and are thought to contribute to ankle and metatarsophalangeal joint motion and controlling foot shape during the power stroke (Clifton *et al.*, 2018).

The aspect ratio (mediolateral width: anterior-posterior width) of the femur was significantly lower and the aspect ratio of the T^{*}T was significantly higher in diving ducks than terrestrial ducks, indicating these bones have a less circular cross-section in diving species (Figure 4.6). Contrary to my hypothesis (H2), TMT shape did not contribute to separation among swimming behaviors, with an aspect ratio close to one for all groups, indicating a circular cross-section (Figure 4.6). Although fluid loading and streamlining are thought to play a role in anterior-posterior (or dorso-ventral) flattening (i.e., aspect ratio > 1) of the humerus in penguins (Habib & Ruff, 2008; Habib, 2010) and the mediolateral flattening (i.e., aspect ratio < 1) in the TMT in grebes (Johnsgard, 1987; Lovvorn, 1990), fluid dynamics are not likely to play a role in flattening of either the femur or T^{*}T since these bones are held close to the body during swimming. However, it is possible that flattening these bones allows for a more streamlined hull.

Skeletal loading patterns by the muscles seem more likely to contribute to the differences in bone shape. Non-circular cross-sections are often associated with predictable, uni-directional bending loads whereas circular cross-sections are often associated with unpredictable bending and/or torsional loading. Torsional loading is thought to dominate over axial and bending loads in avian hindlimb long bones, at least during terrestrial locomotion (Main & Biewener, 2007). Since the femur and T^{*}T are largely held stationary during surface swimming (Carr, 2008; Provini *et al.*, 2012) and are thought to have lower range of motion in swimming specialists (Clifton *et al.*, 2018), loading may be more predictable for the femur and T^{*}T in swimmers and permit strengthening in a given direction. In addition, mallard ducks use very crouched postures compared to more terrestrial avian species (Daley & Bird-Jeffery, 2018). If all avian swimming species use crouched limb postures during walking, bending loads may be more important for these animals (Gatesy & Biewener, 1991; Main & Biewener, 2007). Additional studies that assess the loading of these bones are needed to

elucidate what adaptive significance may lie behind different cross-sectional shapes among these behavioral groups.

Although ANOVA revealed significant differences existed among behavioral groups for relative femur and TMT lengths, lateral cnemial crest size, and shapes of the femur and TT, none of these effects remained significant after accounting for phylogenetic history as assessed by phyloANOVA. This result is difficult to interpret given that behavioral groups significantly differed along PC1 when accounting for phylogenetic history using the phyloMANOVA. It seems that this combination of traits is sufficient to resolve differences among behavioral groups even when phylogeny is accounted for, but that the differences are not sufficiently large that they can be detected when factors are considered individually.

4.4.2 Kinematics & Swimming Behavior

Comparisons of the walking, surface swimming, and diving kinematics of mallards, scaup, and eiders exhibited patterns consistent with other birds (Gatesy & Biewener, 1991; Abourachid, 2001). As expected, both stride frequency and stride length increased with speed during terrestrial locomotion for all three species (Figures 4.7A,B, 4.8A,B, Table 4.6). I hypothesized that hindlimb specialization for swimming in scaup and eiders would result in less stable walking, requiring higher body angles, slower walking speeds, shorter strides, and higher stride frequencies. The velocity range assessed for mallards represented the full range that could be elicited on the treadmill. Walking speeds in scaup and eiders were self-selected by the animals, given constraints imposed by studying them at the LRWC.

The three species used overlapping relative velocities during terrestrial locomotion. Although the range of relative velocities were similar for the scaup and mallards, surprisingly eiders selected speeds that were at the higher end of the ranges observed for the other two species during terrestrial

locomotion (Figure 4.8A,B). The experimental setup may have encouraged rapid terrestrial movement, as the scaup and eiders were moving away from the LRWC staff and toward the pond, a place of relative safety for ducks. Eiders frequently moved their wings and performed hops while walking, indicating that they were near their top over-ground speeds. No significant differences existed among species for the relationship between relative terrestrial velocity and stride length (Figure 4.8B, Table 4.6), although eiders did use significantly higher frequencies for a given relative velocity (Figure 4.8A, Table 4.6). Gatesy & Biewener (1991) reported that larger terrestrial birds tended to use higher relative frequencies and shorter relative stride lengths, likely because smaller birds have more crouched postures, allowing them to extend stride length more easily. Although eiders are larger than mallards and scaup, ‘functional leg length’ (calculated as hip height/ L_{limb} , where hip height was estimated from photographs at mid-stride, see Methods) seems to be similar across all three species (~ 0.7). Examining hindlimb kinematics of more species may help to elucidate whether the patterns observed here are a result of size or reflect differences in hindlimb anatomy.

Scaup and eiders used higher body angles during terrestrial locomotion at similar relative velocities than mallards. Together, the higher body angle, the use of wings by the eiders during some walking trials, and one observation of a scaup scooting on its belly rather than walking (trial not included in analysis) suggest that stability during terrestrial locomotion is reduced in divers. However, whether this truly represents a compromise between swimming specialization and walking ability remains unclear.

During surface swimming and diving, cycle frequency did not change with relative velocity for any of the species (Figure 4.8C,E, Table 4.6). This pattern is consistent with previous studies on surface swimming in mallards (Prange & Schmidt-Nielsen, 1970; Clark & Fish, 1994) and diving in eiders (Heath *et al.*, 2006). During surface swimming, however, eiders used a lower cycle frequency than the other two species. Eiders have a similar paddle area as mallards for their body size and

smaller foot area for body size compared to foot-propelled divers (Raikow, 1973). Consequently, foot size seems unlikely to account for this difference. Since stride frequency did not change, stride length necessarily increased with relative velocity, with no differences observed among species. Mallards used a broader range of surface swimming speeds than the other ducks, but this may be an effect of motivation as mallards were encouraged to swim across the pool or tank by researcher approach while scaup and eider used self-selected swimming speeds. Eiders used higher relative speeds during diving and increased their stride length faster with increasing speed than the other two species. The differences in speed could again be explained by motivation, as scaup, which dove at the lowest speeds, dove voluntarily; mallards, which dove at intermediate speeds, dove to avoid researchers but only after training; and eiders, which dove at the highest speeds, only did so as an escape behavior in all analyzed trials.

Nevertheless, some differences in swimming speed can also likely be attributed to swimming technique during diving. Eiders use both their wings and feet during diving, permitting both to contribute to hydrodynamic force production. Mallards also use both their wings and feet; although these are typically used serially. Scaup used only their feet for swimming. Foot motion patterns also differ among species. Loons use lateral sweeps of the feet to swim (Johnsgard, 1987; Clifton & Biewener, 2018) while other swimmers, including most ducks, use cranial-caudal motions with the feet held ventrally (Johansson & Norberg, 2003). Here, mallards and eiders used the cranial-caudal pattern while the scaup employed more lateral motion of the feet than the other two species, suggesting that wing use during diving may not be compatible with more laterally positioned feet.

4.6. Conclusions

Ducks exhibit a wide range of swimming techniques and specialization. Differences in hindlimb skeletal and pelvic anatomy seemed to fall along a gradient of swimming ability, from

terrestrial to surface swimmers to divers. Traits that were useful in separating behavioral groups included proportional lengths of the femur and TMT, midshaft aspect ratios of the femur and TT, and lateral cnemial crest height. Of these, only the lateral cnemial crest height aligned with patterns seen in other clades of swimming specialists. Other traits that are hallmarks of swimming specialists, including a narrow, elongated pelvis, did not separate behavioral groups in ducks. Finally, only minor indications of a tradeoff between anatomical specialization and terrestrial and diving kinematics were observed. Specifically, during terrestrial locomotion, divers walked with more steeply inclined body angles than the surface swimming mallards, and eiders used higher cycle frequencies and augmented walking by flapping their wings. During diving, eiders swam at higher relative velocities than the other two groups; in large part, this likely was influenced by motivational cues but could also be attributed to swimming technique. Although some tradeoffs between anatomical specialization for swimming and ability to move on land may exist among ducks, all three species studied here were strong swimmers and capable of comparable terrestrial speeds. Thus, perhaps a better interpretation of these results is that the body plan of ducks is capable of supporting a wide range of behaviors such that performance differences can be supported by changes in kinematics (i.e., synchronous use of wings and feet in eiders) rather than large changes in anatomy that might compromise the ability to move between land and water.

4.7. References

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CHAPTER 5. Summary & Conclusions

Despite their close phylogenetic relationships, ducks exhibit a range of swimming and terrestrial behaviors, with varying degrees of specialization for swimming. While some species are highly terrestrial, most are strong swimmers at the surface. Some groups have further specialized to be foot- or wing-and-foot propelled divers. Other clades of avian swimmers have evolved a set of hindlimb morphologies and kinematic patterns for swimming. This specialization, however, seems to reduce their ability to move on land. Because all birds return to land at least occasionally, the hindlimb anatomy, kinematics, and muscle function represent a compromise among their behaviors.

In the work presented in this dissertation, I sought to explore the differences in duck hindlimb anatomy, kinematics, and muscle function across behaviors and species. This work was motivated by the question: how are ducks able to use their hindlimbs to power many different behaviors? Integrating hindlimb anatomy, kinematics, muscle function, and evolutionary history is an important step to understanding how animals perform different

behaviors in disparate environments. I tried to understand (1) how kinematics and muscle function change between environments (terrestrial and aquatic) when performing a similar behavior (takeoff, Chapter 2); (2) how muscle properties observed *in situ* may relate to real behaviors (Chapter 3); and (3) how anatomy and kinematics differ among species with different swimming modes (Chapter 4). I found evidence that muscle function and kinematics are modulated between environments (Chapters 2 & 4), which may present challenges for tuning muscle properties across behaviors. I also found evidence that force production during walking and surface swimming could be affected not only by force-length and force-velocity properties, but also by contraction history-dependent properties (specifically, post-activation potentiation, Chapter 3). I found that differences in hindlimb anatomy among species mirror the gradient in swimming behaviors observed in ducks: from terrestrial to increasingly specialized swimmers (Chapter 4). Finally, a comparative kinematic analysis indicated that specialized swimmers may experience advantages during swimming relative to less specialized swimmers, but that may also come at a cost during terrestrial locomotion (Ch.4).

5.1 Chapter Summaries

Chapter 2: Mallards change both their kinematics and LG muscle function with takeoff medium. During terrestrial takeoff, the hip, knee, ankle, and metatarsophalangeal (MTP) joint all extend to launch the mallard into the air. During aquatic takeoff, the wings slap the water's surface and the tail is moved ventrally synchronously with ankle extension and knee flexion. The LG powers both ankle extension and knee flexion, so it undergoes larger excursions and higher shortening velocities during aquatic takeoffs than terrestrial. Because muscles often perform best (producing highest forces and power) under a limited

range of lengths and shortening velocities, the wider ranges of lengths and higher velocities required during aquatic takeoffs may represent a challenge for tuning the LG to moving both on land and in water.

Chapter 3: I found that PAP affected LG function, resulting in a gradual increase in muscle force, rate of force development, and work production over several cycles. The degree of PAP and number of cycles over which it built depended on cycle frequency, with a larger effect of PAP at higher cycle frequencies. PAP could help muscle to produce higher forces for a given activation level, potentially decreasing the cost of force production and/or counteracting the effects of fatigue for cyclical behaviors like walking and swimming.

Chapter 4: In the first part of Chapter 4, I conducted an analysis of duck hindlimb and pelvic skeletal anatomy. I found that some hindlimb structures differed among behavioral groups, including the proportional length of the femur and tarsometatarsus, the shape of the femur and tibiotarsus, and the size of the lateral cnemial crest. These differences did tend to follow the gradient of swimming ability, with divers being more similar to one another than to nondivers. However, only the increased size of the cnemial crest is consistent with the anatomical specialization seen in other avian swimming specialists. During swimming, the hip and knee are held stationary while the distal limb is moved as a paddle through the water. The larger lateral cnemial crest can contribute both to increased stabilization at the knee (by increasing moment arms for the muscles acting at this joint) and to powering distal limb movement (by increasing attachment surface for the digital flexors). One complication of this study is that behavioral groups tend to be related to taxonomic position. When the above traits were considered together, hindlimb anatomy was significantly different among behavioral groups, even when accounting for phylogenetic relationships (phyloMANOVA); however, when each trait was considered individually in a

phylogenetically-corrected context (phyloANOVA), there were no differences among behavioral groups.

In the second part of Chapter 4, I compared hindlimb kinematics during walking, surface swimming, and diving in mallards (surface swimmers), scaup (foot-propelled divers), and eiders (wing-and-foot-propelled divers). When cycle frequency, stride length, and velocity were normalized for body size, patterns of relative cycle frequency, stride length, and speed were remarkably similar with a few exceptions. For all three species, stride frequency increased with relative velocity during walking but not during surface swimming and diving. Stride length increased with speed for all three behaviors. Notable differences among species include: (1) Eiders used lower relative frequencies during surface swimming and higher relative frequencies during walking, (2) swam at higher relative speeds during diving, and (3) increased stride length to move at faster speeds more during diving than the other two species. (4) Eiders and scaup used a higher body angle than mallards during terrestrial locomotion. (5) Scaup used lateral foot motions while eiders and mallards used cranial-caudal foot motion. Some of these differences may indicate a compromise between swimming specialization and terrestrial locomotion (higher cycle frequency during walking in eiders, higher body angle during walking in eiders and scaup) while other differences may reflect differences in wing use (i.e., lateral foot motion may not be compatible with wing use and eiders increasing stride length rapidly with speed during diving may be attributable to their simultaneous use of wings and feet). Overall, all three species achieved similar locomotor performance for all three behaviors, perhaps indicating the versatility of the duck body plan.

APPENDIX A. Supplemental Information for Chapter 2

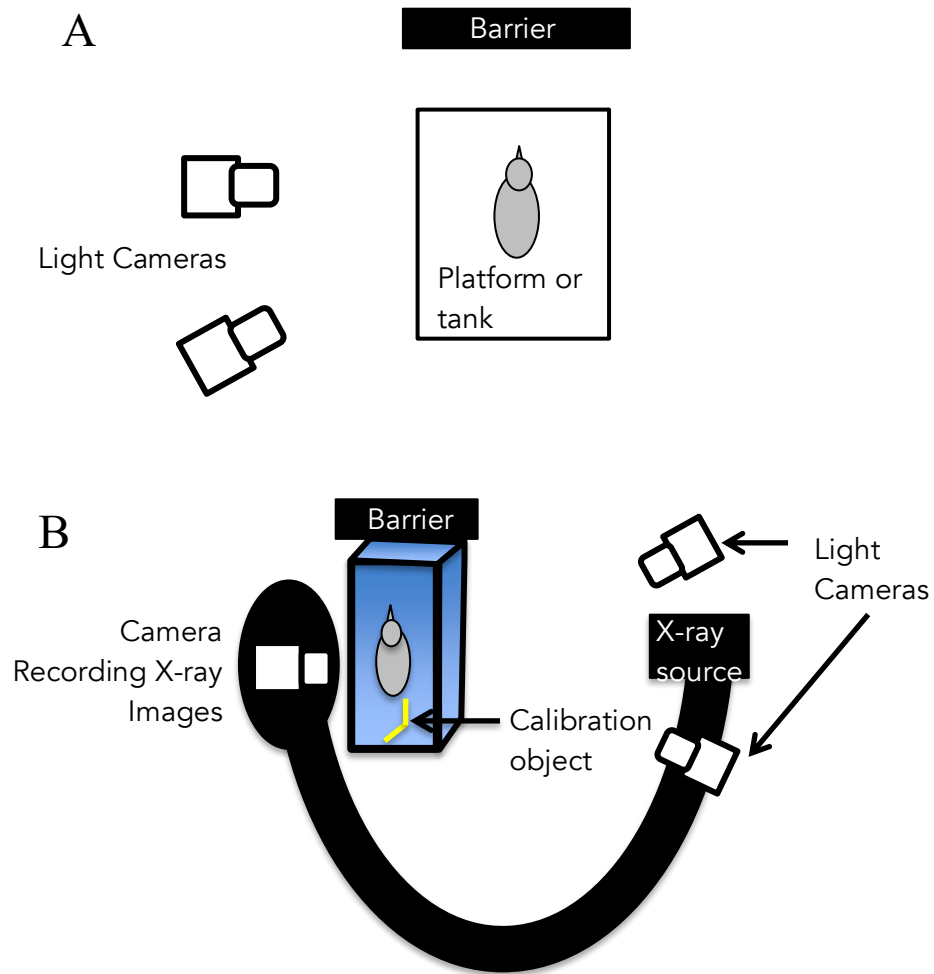


Figure S2.1. Methods for filming joint angle changes during aquatic and terrestrial takeoffs. (A) Light video camera only setup. This setup was used to measure ankle and MTP joint movement. Ducks were recorded at 250 fps as they took off from platform or tank over a barrier. (B) X-ray video setup used to measure hip and knee joints. For terrestrial takeoffs, ducks took off from a platform and hip and knee were measured from X-ray video. For aquatic takeoff, ducks took off from a tank. Hip angles were measured from the X-ray video. Knee angles were calculated using a 2D light and X-ray setup.

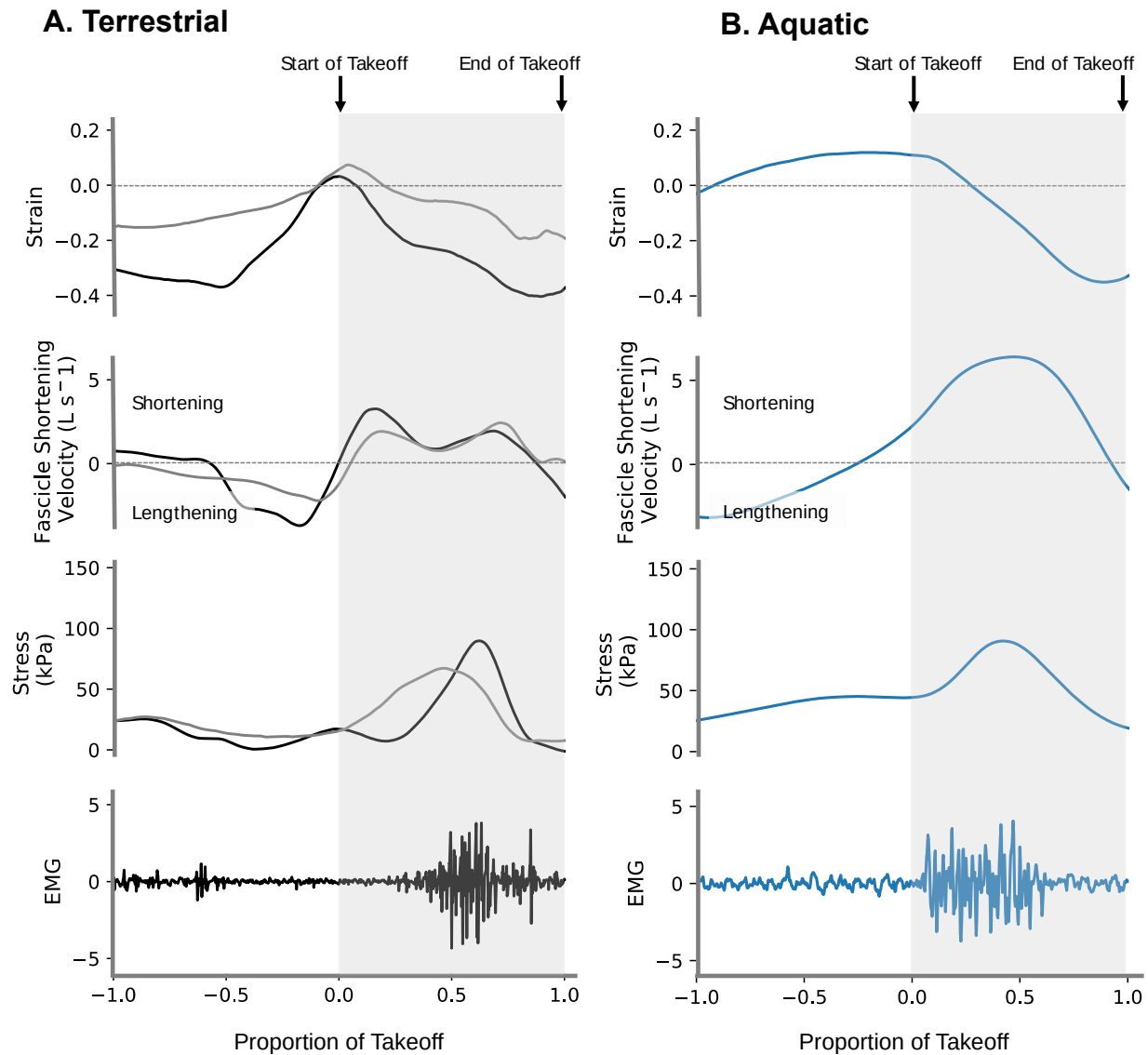


Figure S2.2. Example fascicle strain, shortening velocity, muscle stress, and EMG recorded from the LG of one duck during a (A) terrestrial (black) and (B) aquatic (blue) takeoff. Example fascicle length, fascicle velocity, and stress during terrestrial takeoff are shown for a second individual (gray) to illustrate the variation in timing of force production. Data are plotted against the proportion of takeoff, with the start and stop times of takeoff determined from kinematics (see methods for more detail). The gray box indicates the takeoff power stroke. Negative velocity indicates muscle lengthening and positive velocity indicates muscle shortening. Pennation angle data were not available for these animals; however, average patterns across ducks, including for pennation angle, are shown in Fig. 2.4.

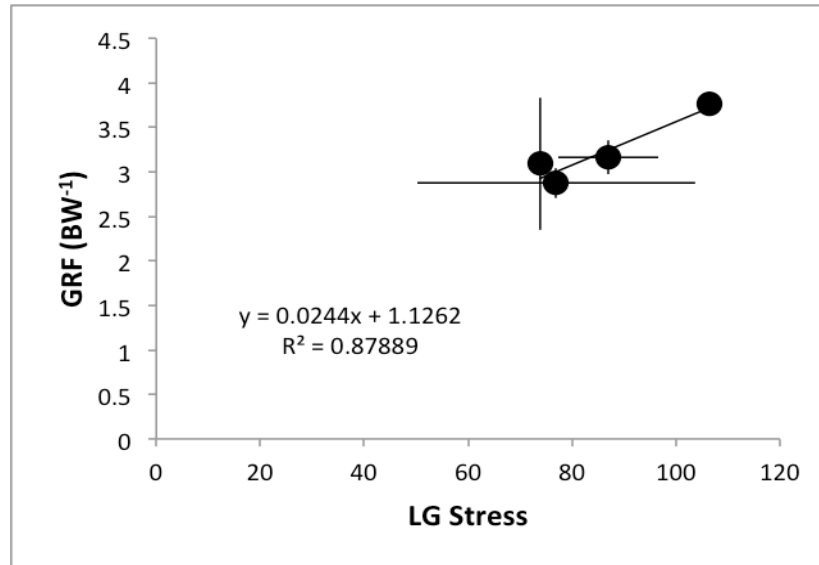


Figure S2.3. Preliminary force plate analysis results. Ground reaction force (GRF) vs. LG stress measured during terrestrial takeoffs (N=4 animals, n=10 trials). Points are individual averages. GRF was measured as the vector sum of vertical and fore-aft forces (mediolateral forces were assumed to be negligible). Stress was calculated as described in the Methods section. The magnitude of GRF was normalized by body weight (BW). The mean of the individual averages for peak GRF was 3.2 ± 0.2 BW⁻¹. The corresponding stresses were 86 ± 7 kPa. The ratio of LG force (N) to GRF (N) was 0.59 ± 0.07 . Despite a relatively strong correlation ($r^2=0.88$), there was not a significant relationship between LG stress and GRF ($p=0.06$).

Movie 1. Example terrestrial and aquatic takeoff in light video setup (Fig. S2.1). Note: two cameras were used at oblique angles to provide 3D kinematics and so may not be perfectly perpendicular to the animal's motion.

Table S2.1. Animal information & experimental use

Sex	Origin	Body Mass (kg)	LG Mass (g)	LG PCSA (cm ²)	Light Kinematics	X-ray Kinematics	Fascicle Length	LG Force	Pennation Angle	EMG
1 F	Wild	0.868			T(3), A(3)					
2 F	Wild	0.841	3.75	1.76	T(3), A(3)		T(2), A(3)		T(2), A(3)	T(2)
3 M	Wild	1.03				T(2), A(2)				
4 M	Wild	1.005	5.01	2.48	T(3), A(3)	T(2), A(2)	T(3)	T(3)*	T(3)	T(3)
5 F	Wild	0.984	4.24	1.57			T(3), A(3)	T(2), A(3)	T(2), A(3)	T(1)
6 M	Farm	0.843			T(3), A(3)	T(2), A(2)				
7 F	Wild	0.712	2.72	1.05			T(2), A(2)			T(2)
8 F	Wild	0.88	4.32	1.89	T(3), A(3)		T(2)	T(3)	T(2)	
9 M	Farm	0.834	4.01	1.68			T(3), A(3)	T(3), A(3)	T(1), A(2)	T(3), A(3)
10 F	Farm	1.008	6.45	2.86			A(3)	A(3)	A(2)	A(3)
11 F	Farm	0.996	5.86	2.72				T(3), A(2)		
12 M	Wild	1.137	4.37	1.67			T(3), A(3)		T(3), A(3)	

T = terrestrial takeoff, A = aquatic takeoff
Numbers in parentheses indicate the number of trials.
* Timing only

APPENDIX B. Supplemental Information for Chapter 3

Figure S3.1. *In situ* experimental setup to measure force and length in the mallard lateral gastrocnemius (LG). (A) Schematic showing the *in situ* rig. The animal was placed on a stage that could be translated along a track to change the distance between the animal and muscle lever. (B) Larger view of a portion of the *in situ* rig (blue box). The femur was clamped mid-shaft and the foot was anchored to a plate to hold the leg and muscle origin stationary. The LG tendon was severed below the fibrocartilage pad, and a Kevlar thread was secured above the pad and connected to a muscle ergometer lever arm. The medial gastrocnemius (MG) tendon was severed and the muscle was freed from connective tissue attachments to surrounding tissues except for the point of origin on the femur. A pair of sonomicrometry crystals was inserted along the fascicle to measure a segment of fascicle length ($L_{\text{fas, seg}}$). An effort was made to measure as much of the fascicle as possible. We assumed the shortening in the segment was representative of shortening along the full length of the fascicle. Post-mortem ‘resting’ measurements of the segment ($L_{\text{fas, seg, rest}}$) and the full fascicle length ($L_{\text{fas, rest}}$) permitted us to correct sonomicrometry measurements of segment length ($L_{\text{fas, seg}}$) to fascicle lengths ($L_{\text{fas, calculated}} = L_{\text{fas, seg}} * L_{\text{fas, resting}} / L_{\text{fas, seg, rest}}$). Two pairs of stainless steel stimulating electrodes were inserted through the muscle belly with one polarity as far proximal and the other as far distal as possible. Although only one pair is shown, two pairs were inserted in medial and lateral sagittal planes.

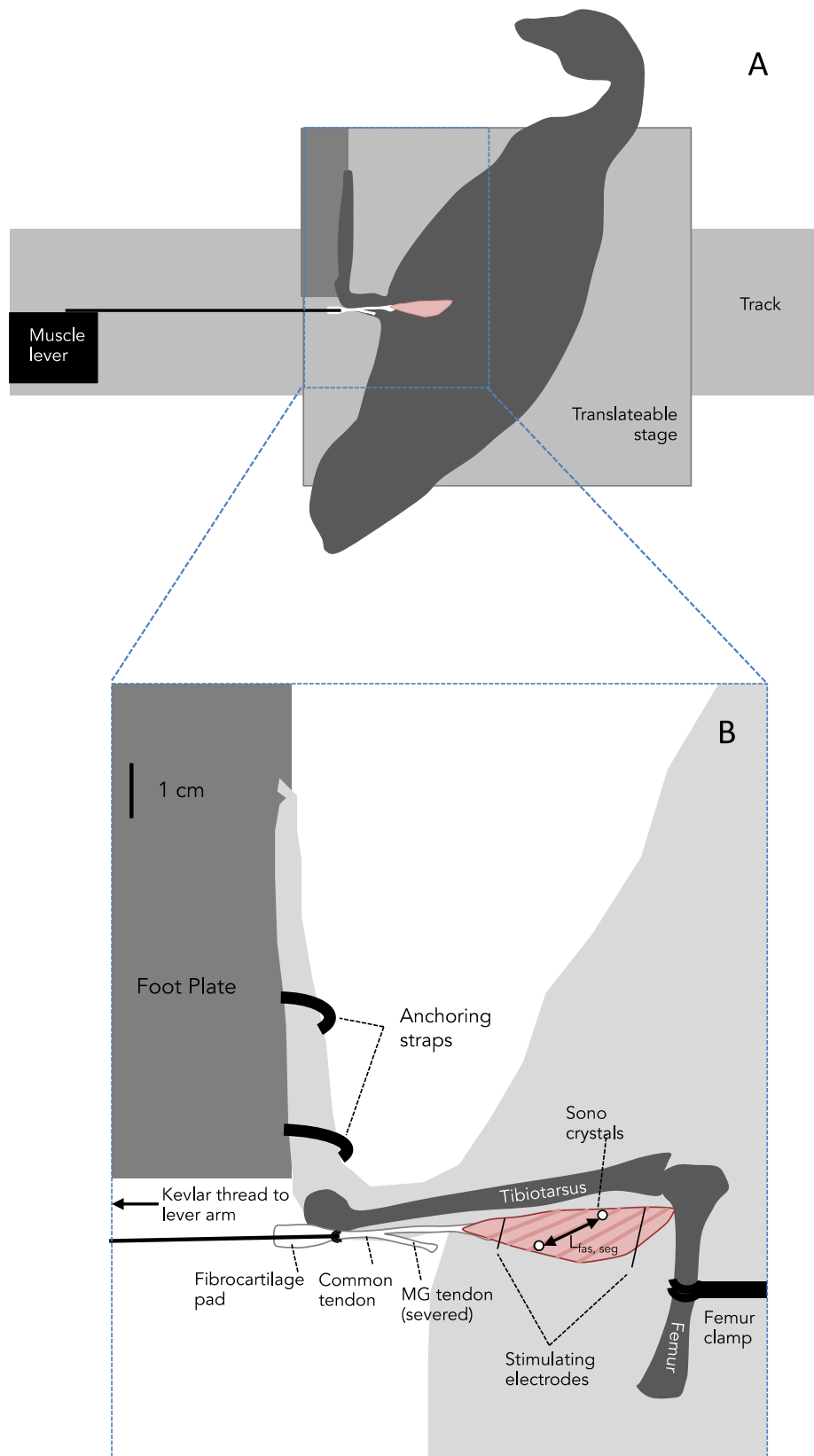


Table S3.1. Data used to create models for percent change in work, force, and force rate & used to create Figure 3.3.

Individual	Frequency	Stim_frequency	Strain_at_peak_Force	MaxWork_Cycle	%ChangeW	%ChangeF	%ChangeFrRate
1	1	250	-0.0278195	6.958789	27.02027	9.9987	43.25
2	1	250	-0.209023	2.372288	2.44306	3.3588	24.4683
3	1	125	-0.0822603	4.406271	35.72382	21.7355	37.2505
4	1	125					
5	1	250	-0.1017354	2.134228	0.03714	3.7738	9.2604
1	2.6	250	-0.0364544	9.227767	69.0968	40.0188	62.5418
2	2.6	250	-0.2456486	6.530817	32.1414	25.7234	46.6662
3	2.6	125	-0.1257148	9.058485	89.1458	64.9619	116.2618
4	2.6	125	-0.0985852	5.783729	6.9861	1.35	3.2083
5	2.6	250	-0.1028482	9.206497	61.8178	54.9595	93.6995
1	4	250	-0.0705575	9.277488	201.4121	105.2095	162.979
2	4	250	-0.2562605	9.858548	67.9507	50.9002	83.6916
3	4	125	-0.1049216	10.372731	190.8486	96.2594	132.2143
4	4	125	-0.0799206	5.73682	20.4025	12.1826	30.4337
5	4	250	-0.0816711	14.146463	96.148	81.9381	175.6327

Table S3.2. Percent Change in Work by Cycle--used to create Figure 3.2A

Cycle	Frequency	Fractional Change by Animal					% Change		
		Animal 1	Animal 2	Animal 3	Animal 4	Animal 5	Average	n	sem
1	1	0	0	0	0		0	4	0
2	1	0.15	0.04	0.17	0	0	6.99	5	3.65
3	1	0.18	0.01	0.25	-0.01	-0.01	8.60	5	5.36
4	1	0.23	0.00	0.29		-0.03	12.38	4	8.04
5	1	0.29	-0.04	0.32		-0.07	12.18	4	10.41
6	1	0.14	-0.08	0.31			12.07	3	11.33
7	1	0.28		0.31			29.74	2	1.52
8	1	0.24		-0.21			1.65	2	22.71
9	1	0.36					36.01	1	
10	1	0.15					15.14	1	
1	2.6	0	0	0	0	0	0	5	0
2	2.6	0.16	0.19	0.27	0.03	0.07	14.58	5	4.32
3	2.6	0.28	0.28	0.46	0.04	0.27	26.46	5	6.58
4	2.6	0.31	0.29	0.59	0.06	0.38	32.55	5	8.42
5	2.6	0.41	0.30	0.68	0.07	0.45	38.31	5	9.95
6	2.6	0.61	0.26	0.75		0.52	53.52	4	10.33
7	2.6	0.72	0.30	0.81		0.56	60.05	4	11.23
8	2.6	0.74	0.28	0.85		0.59	61.59	4	12.25
9	2.6	0.71	0.30	0.89		0.62	62.93	4	12.29
10	2.6	0.61	0.23	0.91		0.62	59.24	4	13.97
1	4	0	0	0	0	0	0	5	0
2	4	0.52	0.25	0.48	0.08	-0.18	23.20	5	13.00
3	4	0.95	0.41	0.81	0.14	0.13	48.83	5	16.89
4	4	1.24	0.50	1.09	0.17	0.29	65.62	5	21.48
5	4	1.55	0.59	1.31	0.20	0.43	81.38	5	26.05
6	4	1.67	0.55	1.48		0.55	106.33	4	29.92
7	4	1.86	0.63	1.64		0.65	119.36	4	32.31
8	4	1.91	0.60	1.76		0.74	125.04	4	33.77
9	4	1.93	0.65	1.87		0.80	131.25	4	33.98
10	4	2.10	0.67	1.94		0.86	139.29	4	36.65
11	4		0.63			0.90	76.55	2	13.41
12	4		0.61			0.93	77.06	2	15.77
13	4		0.59			0.94	76.17	2	17.46
14	4		0.61			0.95	78.13	2	17.36
15	4		0.51			0.96	73.74	2	22.26

APPENDIX C. Supplemental Information for Chapter 4

Table S4.1. Skeletal ratios for terrestrial ducks with averages and standard deviations (s.d.).

TERRESTRIAL DUCKS																
Museum ID	Species	Tribe	Sex	Proportional Length			Aspect Ratio				Synsacrum		Med Cnem		Lat Cnem	
				F	TT	TMT	F	TT	TTprox	TMT	AR	Acet	L	H	L	H
MCZ343047	Chloephaga hybrida	Tad	M	0.29	0.46	0.25	0.94	1.09	1.44	1.23	0.34	0.5	0.12	0.11	0.06	0.1
YPM107144	Chloephaga melanoptera	Tad	U	0.26	0.46	0.28	1.1	1.29	1.21	1.12	0.29	0.51	0.11	0.08	0.06	0.08
MCZ342847	Chloephaga picta	Tad	F	0.25	0.46	0.28	0.97	1.18	1.44	1.1	0.31	0.53	0.12	0.07	0.05	0.1
YPM84631	Chloephaga poliocephala	Tad	F	0.27	0.46	0.27	0.93	1.21	1.38	1.24	0.42	0.54	0.14	0.08	0.06	0.11
YPM105106	Chloephaga rubidiceps	Tad	F	0.27	0.45	0.28	0.95	1.1	1.38	1.08	0.36	0.52	0.13	0.09	0.05	0.1
YPM142074	Neochen jubata	Tad	U	0.25	0.46	0.29	0.97	1.11	1.35	1.18	0.28	0.47	0.13	0.08	0.05	0.08
AVERAGE				0.27	0.46	0.28	0.98	1.16	1.36	1.16	0.33	0.51	0.12	0.09	0.05	0.1
s.d.				0.01	0.0	0.01	0.06	0.08	0.08	0.07	0.05	0.03	0.01	0.01	0.0	0.01

Abbreviations: femur (F), tibiotarsus (TT), tarsometatarsus (TMT), aspect ratio (AR, interacetabular length/synsacrum length), preacetabular length/synsacrum length (Acet), Medial cnemial crest (Med Cnem), Lateral cnemial crest (Lat Cnem), length (L), height (H)
Sex: Female (F), Male (M), Unknown (U)
Duck Tribes: Anatini (Ana), Anatini-adjacent (Ana_A), Aythiini (Ay), Aythiini-adjacent (Ay_A), Diver adjacent (ID_A, i.e., outside of clade that includes Tadornini, Aythiini, and Mergini), Mergini (Mer), Tadornini (Tad), Tadornini-adjacent (Tad_A)

Table S4.2. Skeletal ratios for surface swimming ducks with averages and standard deviations (s.d.)

SURFACE SWIMMING DUCKS																
Museum ID	Species	Tribe	Sex	Proportional Length			Aspect Ratio				Synsacrum		Med Cnem		Lat Cnem	
				F	T'T	TMT	F	T'T	T'Tprox	TMT	AR	Acet	L	H	L	H
MCZ341895	Anas acuta	Ana	M	0.27	0.47	0.26	0.84	1.22	1.53	1.06	0.36	0.47	0.13	0.11	0.05	0.08
YPM112306	Anas americana	Ana	M	0.28	0.47	0.25	0.91	1.27	1.41	1.01	0.42	0.51	0.13	0.09	0.07	0.11
MCZ340299	Anas bahamensis	Ana	U	0.27	0.47	0.26	0.89	1.18	1.6	1.25	0.35	0.49	0.13	0.1	0.06	0.11
YPM84775	Anas castanea	Ana	M	0.27	0.46	0.27	0.83	1.19	1.37	1.03	0.38	0.45	0.13	0.09	0.06	0.1
MCZ340235	Anas crecca	Ana	U	0.27	0.47	0.25	0.88	1.13	1.5	0.99	0.41	0.52	0.12	0.09	0.08	0.09
MCZ346054	Anas discors	Ana	F	0.28	0.46	0.27	0.75	1.22	1.41	1.13	0.38	0.51	0.14	0.09	0.06	0.1
YPM84444	Anas falcata	Ana	F	0.28	0.46	0.26	0.88	1.14	1.4	1.02	0.34	0.45	0.13	0.1	0.06	0.1
YPM84430	Anas formosa	Ana	M	0.28	0.46	0.26	0.88	1.22	1.57	1.11	0.37	0.49	0.13	0.08	0.05	0.1
MCZ343238	Anas georgica	Ana	F	0.29	0.45	0.26	0.87	1.28	1.52	1.07	0.32	0.54	0.13	0.09	0.06	0.1
MCZ347544	Anas laysanensis	Ana	U	0.3	0.46	0.25	0.95	1.16	1.43	1.11	0.34	0.49	0.15	0.09	0.05	0.11
MCZ341914	Anas platyrhynchos	Ana	U	0.28	0.47	0.25	1.01	1.23	1.24	1.24	0.46	0.52	0.14	0.09	0.06	0.1
YPM84705	Anas puna	Ana	F	0.28	0.47	0.25	0.92	1.18	1.52	1.17	0.36	0.49	0.13	0.09	0.06	0.09
YPM84764	Anas rhynchotis	Ana	F	0.29	0.45	0.26	0.87	1.26	1.37	1.26	0.36	0.47	0.14	0.1	0.06	0.11
MCZ335525	Anas rubripes	Ana	U	0.28	0.46	0.25	0.88	1.22	1.44	1.03	0.4	0.49	0.13	0.08	0.05	0.1
MCZ341890	Anas sibilatrix	Ana	U	0.28	0.47	0.25	0.97	1.16	1.55	1.1	0.41	0.48	0.13	0.11	0.07	0.11
MCZ341872	Anas strepera	Ana	F	0.28	0.46	0.26	0.9	1.2	1.34	1.15	0.36	0.49	0.11	0.09	0.05	0.1

Table S4.2 (Continued)

Museum ID	Species	Tribe	Sex	Proportional Length			Aspect Ratio				Symsacrum		Med Cnem		Lat Cnem	
				F	TT	TMT	F	TT	TTprox	TMT	AR	Acet	L	H	L	H
YPM141962	Amazonetta brasiliensis	Ana_A	M	0.29	0.45	0.26	0.86	1.14	1.6	1.13	0.32	0.43	0.14	0.1	0.06	0.11
YPM103837	Specularnas specularis	Ana_A	F	0.28	0.46	0.26	0.79	1.22	1.5	1.05	0.32	0.47	0.14	0.08	0.06	0.11
USNM612612	Caïrina scutulata	Ay_A	M	0.3	0.44	0.26	0.9	1.11	1.36	1.23	0.3	0.5	0.14	0.09	0.04	0.11
YMP142578	Marmaronetta angustirostris	Ay_A	F	0.27	0.46	0.27	0.83	1.29	1.45	0.96	0.33	0.45	0.15	0.1	0.06	0.09
USNM292391	Pteronetta hartlaubii	Ay_A	F	0.3	0.45	0.25	0.89	1.22	1.29	1.17	0.3	0.48	0.13	0.09	0.06	0.12
MCZ347445	Callonetta leucophrys	Dive_A	M	0.28	0.45	0.27	0.85	1.19	1.37	1.27	0.36	0.49	0.14	0.09	0.08	0.11
MCZ339522	Chenonetta jubata	Dive_A	F	0.26	0.46	0.27	0.86	1.15	1.43	1.23	0.3	0.45	0.13	0.09	0.05	0.1
USNM490276	Sarkidiornis melanotos	Dive_A	M	0.27	0.46	0.27	0.92	1.3	1.36	0.97	0.27	0.52	0.1	0.08	0.05	0.12
MCZ342883	Alopochen aegyptiaca	Tad	F	0.23	0.47	0.3	1.09	1.18	1.26	1.03	0.26	0.49	0.12	0.08	0.05	0.07
YPM109957	Tadorna ferruginea	Tad	U	0.26	0.46	0.29	0.86	1.25	1.46	0.99	0.34	0.42	0.12	0.1	0.05	0.1
MCZ347540	Tadorna tadorna	Tad	F	0.26	0.46	0.28	0.87	1.24	1.4	0.98	0.32	0.5	0.13	0.1	0.05	0.1
YPM110846	Tadorna variegata	Tad	M	0.26	0.47	0.28	0.86	1.13	1.49	1.02	0.35	0.49	0.12	0.08	0.05	0.09

Table S4.2 (Continued)

Museum ID	Species	Tribe	Sex	Proportional Length			Aspect Ratio				Symsacrum		Med Cnem		Lat Cnem	
				F	TT	TMT	F	TT	TTprox	TMT	AR	Acet	L	H	L	H
YPM141906	Aix galericulata	Tad_A	M	0.28	0.44	0.27	0.85	1.19	1.44	1.06	0.38	0.45	0.13	0.1	0.06	0.12
MCZ341472	Aix sponsa	Tad_A	U	0.29	0.45	0.25	0.84	1.21	1.48	1	0.35	0.5	0.09	0.1	0.08	0.11
MCZ341901	Carina moschata	Tad_A	U	0.29	0.45	0.25	1.07	1.23	1.33	1.44	0.33	0.51	0.13	0.09	0.07	0.12
AVERAGE				0.28	0.46	0.26	0.89	1.2	1.43	1.11	0.35	0.48	0.13	0.09	0.06	0.1
s.d.				0.01	0.01	0.01	0.07	0.05	0.09	0.11	0.04	0.03	0.01	0.01	0.01	0.01

Abbreviations: femur (F), tibiotarsus (T^T), tarsometatarsus (TMT), aspect ratio (AR, interacetabular length/symsacrum length), preacetabular length/symsacrum length (Acet), Medial cnemial crest (Med Cnem), Lateral cnemial crest (Lat Cnem), length (L), height (H)
Sex: Female (F), Male (M), Unknown (U)
Duck Tribes: Anatini (Ana), Anatini-adjacent (Ana_A), Aythiini (Ay), Aythiini-adjacent (Ay_A), Diver adjacent (D_A, i.e., outside of clade that includes Tadornini, Aythiini, and Mergini), Mergini (Mer), Tadornini (Tad), Tadornini-adjacent (Tad_A)

Table S4.3. Skeletal ratios for foot-propelled diving ducks with averages and standard deviations (s.d.)

FOOT-PROPELLED DIVING DUCKS																
Museum ID	Species	Tribe	Sex	Proportional Length			Aspect Ratio				Synsacrum		Med Cnem		Lat Cnem	
				F	T:T	TMT	F	T:T	TTprox	TMT	AR	Acet	L	H	L	H
MCZ342204	Tachyeres brachypterus	Ana_A	F	0.29	0.47	0.24	0.85	1.19	1.52	1.26	0.31	0.5	0.14	0.09	0.07	0.15
YMP104149	Tachyeres patachonicus	Ana_A	M	0.29	0.47	0.24	0.85	1.27	1.52	1.17	0.32	0.45	0.13	0.09	0.06	0.09
YPM104235	Tachyeres pteneres	Ana_A	M	0.29	0.46	0.25	0.95	1.35	1.55	1.25	0.28	0.44	0.13	0.08	0.06	0.1
YPM84588	Aythya affinis	Ay	F	0.29	0.47	0.24	0.86	1.28	1.34	1.08	0.28	0.39	0.13	0.08	0.06	0.1
MCZ341912	Aythya americana	Ay	U	0.28	0.48	0.24	0.91	1.25	1.43	1.32	0.26	0.46	0.12	0.09	0.06	0.09
YPM84748	Aythya baeri	Ay	M	0.29	0.46	0.24	0.87	1.19	1.54	1.42	0.28	0.43	0.13	0.1	0.06	0.09
MCZ341868	Aythya collaris	Ay	U	0.29	0.47	0.25	0.88	1.13	1.52	1.02	0.4	0.48	0.12	0.09	0.06	0.1
YPM102616	Aythya ferina	Ay	U	0.29	0.47	0.24	0.89	1.3	1.51	1.38	0.28	0.41	0.13	0.09	0.05	0.1
MCZ341016	Aythya fuligula	Ay	U	0.29	0.46	0.25	0.86	1.24	1.53	1.04	0.39	0.46	0.13	0.09	0.06	0.11
YPM109891	Aythya marila	Ay	M	0.29	0.47	0.24	0.85	1.25	1.49	1.1	0.27	0.43	0.13	0.1	0.06	0.11
YMP110831	Aythya novaeseelandiae	Ay	F	0.29	0.47	0.24	0.87	1.26	1.48	1.23	0.3	0.43	0.14	0.09	0.07	0.12
MCZ347021	Aythya nyroca	Ay	F	0.28	0.48	0.24	0.91	1.31	1.4	1.21	0.26	0.45	0.13	0.1	0.06	0.06
YPM107585	Aythya valisineria	Ay	U	0.28	0.47	0.24	0.87	1.29	1.51	1.32	0.26	0.47	0.14	0.08	0.06	0.09
MCZ342988	Netta peposaca	Ay	U	0.29	0.47	0.24	0.8	1.25	1.4	1.09	0.3	0.46	0.13	0.08	0.05	0.1
YPM105129	Netta rufina	Ay	F	0.27	0.47	0.25	0.8	1.28	1.39	1.14	0.26	0.41	0.13	0.09	0.05	0.11
MCZ345094	Merganetta armata	D_A	F	0.25	0.47	0.27	0.89	1.17	1.59	1.21	0.3	0.41	0.14	0.09	0.06	0.09

Table S4.3 (Continued)

Museum ID	Species	Tribe	Sex	Proportional Length			Aspect Ratio				Synsacrum		Med Cnem		Lat Cnem	
				F	TT	TMT	F	TT	TTprox	TMT	AR	Acet	L	H	L	H
YPM84304	Bucephala albeola	Mer	M	0.31	0.44	0.25	0.9	1.26	1.36	1.21	0.31	0.43	0.16	0.08	0.06	0.1
MCZ347155	Bucephala clangula	Mer	M	0.31	0.45	0.24	0.81	1.19	1.27	1.16	0.32	0.43	0.15	0.11	0.06	0.13
MCZ347140	Lophodytes cucullatus	Mer	F	0.31	0.46	0.24	0.8	1.3	1.39	1.29	0.31	0.4	0.15	0.09	0.05	0.11
YPM102122	Melanitta nigra	Mer	U	0.28	0.47	0.25	0.76	1.35	1.28	0.97	0.22	0.45	0.14	0.1	0.07	0.13
YMP84661	Mergellus albellus	Mer	M	0.29	0.46	0.25	0.77	1.22	1.35	1.12	0.25	0.39	0.15	0.1	0.06	0.12
MCZ340318	Mergus merganser	Mer	U	0.28	0.46	0.26	0.77	1.52	1.37	0.94	0.2	0.42	0.13	0.1	0.08	0.11
MCZ341891	Mergus serrator	Mer	M	0.27	0.47	0.26	0.77	1.29	1.36	0.8	0.2	0.33	0.13	0.1	0.06	0.14
AVERAGE				0.29	0.47	0.25	0.85	1.27	1.44	1.16	0.29	0.43	0.13	0.09	0.06	0.11
s.d.				0.01	0.01	0.01	0.05	0.08	0.09	0.15	0.05	0.04	0.01	0.01	0.01	0.02

Abbreviations: femur (F), tibiotarsus (TT), tarsometatarsus (TMT), aspect ratio (AR, interacetabular length/synsacrum length), preacetabular length/synsacrum length (Acet), Medial cnemial crest (Med Cnem), Lateral cnemial crest (Lat Cnem), length (L), height (H)

Sex: Female (F), Male (M), Unknown (U)

Duck Tribes: Anatini (Ana), Anatini-adjacent (Ana_A), Aythiini (Ay), Aythiini-adjacent (Ay_A), Diver adjacent (D_A, i.e., outside of clade that includes Tadornini, Aythiini, and Mergini), Mergini (Mer), Tadornini (Tad), Tadornini-adjacent (Tad_A)

Table S4.4. Skeletal ratios for wing-and-foot-propelled diving ducks with averages and standard deviations (s.d.)

WING-AND-FOOT-PROPELLED DIVING DUCKS																
Museum ID	Species	Tribe	Sex	Proportional Length			Aspect Ratio				Synsacrum		Med Cnem		Lat Cnem	
				F	T ^T	TMT	F	T ^T	T ^T prox	TMT	AR	Acet	L	H	L	H
MCZ346497	Clangula hyemalis	Mer	F	0.29	0.47	0.24	0.73	1.28	1.43	1.08	0.33	0.5	0.13	0.09	0.07	0.13
MCZ336960	Histrionicus histrionicus	Mer	U	0.29	0.46	0.26	0.79	1.38	1.33	1.09	0.33	0.47	0.13	0.08	0.08	0.11
MCZ341493	Melanitta fusca	Mer	U	0.32	0.44	0.24	0.83	1.31	1.34	1.1	0.32	0.41	0.15	0.09	0.07	0.12
MCZ347150	Melanitta perspicillata	Mer	U	0.29	0.47	0.24	0.88	1.35	1.52	1	0.27	0.52	0.13	0.1	0.06	0.13
USNM17470	Polysticta stelleri	Mer	M	0.3	0.46	0.25	0.9	1.44	1.46	1.02	0.31	0.48	0.13	0.11	0.06	0.11
YPM84687	Somateria fischeri	Mer	F	0.3	0.47	0.24	0.92	1.41	1.27	1.22	0.3	0.51	0.13	0.1	0.06	0.13
MCZ342590	Somateria mollissima	Mer	M	0.29	0.47	0.24	0.77	1.34	1.28	1.07	0.29	0.49	0.12	0.08	0.07	0.11
YPM84699	Somateria spectabilis	Mer	M	0.29	0.46	0.25	0.89	1.26	1.36	1.14	0.28	0.48	0.14	0.09	0.06	0.13
AVERAGE				0.3	0.46	0.24	0.84	1.35	1.37	1.09	0.3	0.48	0.13	0.09	0.07	0.12
s.d.				0.01	0.01	0.01	0.07	0.06	0.09	0.07	0.02	0.03	0.01	0.01	0.01	0.01

Abbreviations: femur (F), tibiotarsus (T^T), tarsometatarsus (TMT), aspect ratio (AR, interacetabular length/synsacrum length), preacetabular length/synsacrum length (Acet), Medial cnemial crest (Med Cnem), Lateral cnemial crest (Lat Cnem), length (L), height (H)
Sex: Female (F), Male (M), Unknown (U)
Duck Tribes: Anatini (Ana), Anatini-adjacent (Ana_A), Aythiini (Ay), Aythiini-adjacent (Ay_A), Diver adjacent (D_A, i.e., outside of clade that includes Tadornini, Aythiini, and Mergini), Mergini (Mer), Tadornini (Tad), Tadornini-adjacent (Tad_A)

Table S4.5. Skeletal ratios for outgroups and basal ducks with averages and standard deviations (s.d.). Arranged by swimming behavior.

OUTGROUPS & BASAL DUCKS																
Museum ID	Species	Tribe	Sex	Proportional Length			Aspect Ratio				Synsacrum	Med Cnem		Lat Cnem		
				F	TT	TMT	F	TT	TTprox	TMT	AR	Acet	L	H	L	H
Terrestrial																
YPM84783	Cereopsis novae-hollandiae	Anser	U	0.26	0.45	0.29	1.04	1.16	1.3	1.11	0.3	0.49	0.14	0.08	0.06	0.11
YPM102986	Plectropterus gambensis	Basal	U	0.25	0.46	0.29	0.93	1.28	1.27	1.06	0.22	0.47	0.11	0.07	0.05	0.09
MCZ3441442	Gallus gallus	Gall	U	0.28	0.43	0.28	0.96	1.14	1.18	1.43	0.71	0.62	0.05	0.1	0.05	0.08
MCZ346993	Anhima cornuta	Scrc	F	0.25	0.45	0.31	1.04	1.03	1.43	1.52	0.35	0.57	0.07	0.07	0.06	0.1
MCZ340307	Chauna chavaria	Scrc	U	0.24	0.44	0.32	1.3	1.13	1.23	1.35	0.34	0.63	0.08	0.1	0.05	0.1
MCZ340273	Dendrocygna autumnalis	Whist	M	0.26	0.45	0.3	0.98	1.22	1.32	1.17	0.23	0.5	0.12	0.1	0.04	0.1
MCZ347953	Branta canadensis	Anser	F	0.27	0.46	0.27	1	1.29	1.34	1.12	0.26	0.43	0.11	0.08	0.05	0.1

Table S4.5 (Continued)

Museum ID	Species	Tribe	Sex	Proportional Length			Aspect Ratio				Synsacrum		Med Cnem		Lat Cnem	
				F	TT	TMT	F	TT	TTprox	TMT	AR	Acet	L	H	L	H
Surface Swimmers																
YPM84584	Coscoroba coscoroba	Anser	M	0.24	0.47	0.29	1.27	1.35	1.4	1.18	0.26	0.48	0.11	0.07	0.05	0.11
MCZ340335	Cygnus cygnus	Anser	U	0.27	0.47	0.27	1.1	1.31	1.18	1.12	0.2	0.4	0.11	0.06	0.05	0.09
MCZ341374	Nettapus auritus	Basal	U	0.29	0.46	0.25	0.97	1.11	1.26	1.14	0.34	0.42	0.14	0.08	0.06	0.1
USNM347644	Nettapus pulchellus	Basal	F	0.3	0.46	0.24	0.82	1.07	1.56	1.32	0.28	0.49	0.14	0.09	0.05	0.11
USNM555594	Stictonetta naevosa	Basal	M	0.28	0.46	0.26	0.92	1.18	1.53	1.27	0.27	0.5	0.14	0.1	0.06	0.1
Foot-Propelled Divers																
MCZ341437	Oxyura jamaicensis	Basal	U	0.29	0.47	0.24	0.87	1.29	1.28	1.31	0.23	0.43	0.15	0.08	0.07	0.14
MCZ342067	Biziura lobata	Basal	U	0.28	0.49	0.23	0.94	1.27	1.3	1.53	0.15	0.34	0.13	0.08	0.07	0.14

Abbreviations: femur (F), tibiotarsus (TT), tarsometatarsus (TMT), aspect ratio (AR, interacetabular length/synsacrum length), preacetabular length/synsacrum length (Acet), Medial cnemial crest (Med Cnem), Lateral cnemial crest (Lat Cnem), length (L), height (H)

Sex: Female (F), Male (M), Unknown (U)

Outgroup groups: Anserinae (Anser), Basal ducks (Basal), Screamers (Scre), Galliformes (Gall), Whistling ducks (Whist)