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RESEARCH ARTICLE

Wading through water: effects of water depth and speed on the drag and kinematics of walking Chilean flamingos, *Phoenicopterus chilensis*

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ABSTRACT

Wading behaviours, in which an animal walks while partially submerged in water, are present in a variety of taxa including amphibians, reptiles, mammals and birds. Despite the ubiquity of wading behaviours, few data are available to evaluate how animals adjust their locomotion to accommodate changes in water depth. Because drag from water might impose additional locomotor costs, wading animals might be expected to raise their feet above the water up to a certain point until such behaviours lead to awkward steps and are abandoned. To test for such mechanisms, we measured drag on models of the limbs of Chilean flamingos (*Phoenicopterus chilensis*) and measured their limb and body kinematics as they walked and waded through increasing depths of water in a zoo enclosure. Substantial drag was incurred by models of both open- and closed-toed feet, suggesting that flamingos could avoid some locomotor costs by stepping over water, rather than through it, during wading. Step height was highest while wading through intermediate water depths and while wading at a faster speed. Stride length increased with increasing water depth and velocity, and the limb joints generally flexed more while moving through intermediate water depths. However, movements of the head and neck were not strongly correlated with water depth or velocity. Our results show a wide range of kinematic changes that occur to allow wading birds to walk through different water depths, and have implications for better understanding the locomotor strategies employed by semi-aquatic species.

KEY WORDS: Biomechanics, Locomotion, Bird, Semi-aquatic, Limb

INTRODUCTION

The locomotor modes that animals use are influenced considerably by the surrounding environment. Aquatic environments expose the body to different forces from terrestrial environments; for example, the weight that the limbs must support while in the water is reduced (Ashley-Ross and Bechtel, 2004; Ashley-Ross et al., 2009, 2013; Zug, 1971). Water also imposes more drag than air when moving at comparable speeds (Vogel, 2003). As a result, many species will change their locomotor behaviour when moving between these different environments (i.e. switching from walking on land to swimming in water), leading to changes in limb and body movements, muscle activation and bone loading (Blob et al., 2008; Gillis and Blob, 2001; Nishizawa et al., 2013; Rivera and Blob, 2010; Young and Blob, 2015). Other species, such as turtles, salamanders

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and wading birds, do not make dramatic changes in locomotor mode between habitats, and instead use walking locomotion both in water and on land (Ashley-Ross and Bechtel, 2004; Ashley-Ross et al., 2009; Powell, 1987; Willey and Blob, 2004; Zug, 1971). When bottom-walking species such as turtles and salamanders walk while wholly submerged, their bodies will experience considerable buoyancy. In contrast, some birds, primates and large mammals commonly exhibit wading behaviours, in which the limbs move through the water, but the feet contact the submerged substrate and the body is either partly or entirely supported above the surface of the water (Barela et al., 2006; Halsey et al., 2014; Powell, 1987). Wading species may experience little buoyancy but still incur drag while moving the limbs through the water.

Birds such as cranes, herons, ibis and flamingos commonly exhibit wading behaviours, spending their lives in or around shallow water and walking through that water while seeking resources such as prey or nesting sites (Hartman, 1961; Pickens et al., 2017; Powell, 1987; Velasquez, 1992). Previous studies have focused on different aspects of terrestrial avian bipedalism and the challenges that birds may face on the ground (Andrada et al., 2013, 2015; Daley, 2006; Gatesy and Biewener, 1991). However, there is little information on how wading through water may influence avian locomotion. Do wading birds change their limb kinematics to accommodate the additional drag that deep waters impose?

Previous studies of wading limb kinematics have focused primarily on quadrupeds (Barnicoat and Wills, 2016; Coughlin and Fish, 2009) or hominin bipeds (Barela et al., 2006; Halsey et al., 2014). There are several mechanical differences between quadrupedalism and bipedalism (Nakatsukasa et al., 2004), as well as between avian (digitigrade) and hominin (plantigrade) bipedalism (Alexander, 2004). For example, human bipedalism is suggested to be stiffer and more energy efficient, whereas avian bipedalism is regarded as more compliant and stable (Gatesy and Biewener, 1991; Mederreg et al., 2003). In this context, a gap still remains in understanding the impact of wading on avian locomotor kinematics, and thus there is a lack of information on an important behaviour used by many aquatic birds. Moreover, as birds wade through various depths, greater contrasts from terrestrial locomotor kinematics may emerge. Direct measurement of wading kinematics in birds is needed to evaluate the impact of aquatic environments on avian walking.

Several kinematic changes can be predicted to occur when transitioning from terrestrial walking to wading through water, as changes in environmental conditions demand changes in energy expenditure and kinematics (Coughlin and Fish, 2009). For example, step height might increase with increasing water depth to avoid potential costs of drag on the limbs through shallow water. However, in especially deep waters, step height may eventually decrease as it will become kinematically awkward or unstable to

continue elevating the limbs above deep water with increasingly exaggerated steps. The point of this change will likely be near the height of the ankle joint during stance phase, as movements to bring the limb above this height could become kinematically unstable. Adjusting the kinematics of the ankle, knee or hip joints could control step height, where folding the legs and increasing the flexion of any of these angles would result in a higher step. In addition, because of a predicted increase in step height in shallow waters, stride length is likely to decrease, resulting in shorter steps as a way to increase stability despite the use of higher steps in shallow water. Decreasing stride length while walking has previously been suggested as a method to reduce fall risk and increase stability in humans (Espy et al., 2010). However, stride length might be expected to increase again as water depth increases and step height is reduced. This reduction in upward movements while increasing forward movements could increase stability and provide the individual with better balance. Stride velocity might also decrease as water depth increases, as a result of the increased energetic costs associated with fast wading (Halsey et al., 2014).

In addition to changes in limb kinematics, some animals display changes in the position of the head and neck relative to the body during changes in speed, direction or incline in order to stabilize the body or aid visual fixation (Menz et al., 2003; Mulavara et al., 2002). When moving through shallow water with increased drag and minimal effects of buoyancy, wading birds might improve their stability by drawing their head closer to their centre of mass. Birds with elongate necks might orient the beak vertically, or even tuck the tip of the beak closer to the body than the eye, with the horizontal distance between the head and body minimized when moving in shallow water. In contrast, head angles might be more horizontal and the horizontal distance between the head and body greater in terrestrial and deep-water strides, because increased buoyancy in deep water (and reduced drag experienced during terrestrial strides) could allow for greater freedom of the position of the head.

Here, we evaluated the impact of changing water depth on the locomotion of a representative wading bird, the Chilean flamingo (*Phoenicopterus chilensis*). Because we predicted that locomotor patterns might change, partly as a result of the effects of drag in the water, we evaluated the extent to which drag on the feet might influence flamingo kinematics by measuring drag imposed by flowing water on physical models of flamingo limbs. We predicted that drag on the feet would depend on (1) velocity and (2) the frontal area of the leg (tarsometatarsus) and toes moving through the water, so that drag would be greatest at higher speeds and when the webbed toes are held open (Rivera, 2008). However, we also predicted that even in low-drag scenarios, it would still be advantageous to make kinematic adjustments to reduce drag. Drag of a foot skimming the surface might exceed that of a fully submerged foot (Fish et al., 1991), providing an advantage for using step heights that either

cleared or stayed below the surface. In the context of our drag data, we measured the limb and body kinematics of flamingos walking and wading through increasing depths of water, testing for kinematic adjustments indicating that wading birds would initially make an effort to step over the water, but eventually resort to moving the leg through water, despite the cost of drag. Together, our comparisons provide some of the first quantitative kinematics for a novel but widespread locomotor mode, and evaluate potential mechanisms that may contribute to changes in locomotor patterns across environmental conditions.

MATERIALS AND METHODS
Drag modelling
To evaluate the drag experienced by the limbs of flamingos, we conducted flow tank trials (Rivera, 2008) on two plastic models of legs (tarsometatarsi and toes) made from moulds of a study skin specimen of an adult flamingo from Clemson University's Bob and Betsy Campbell Museum of Natural History (CU 1354). In one model, the toes were spread, exposing the webbing of the foot, and in the other model, the toes were folded together (Fig. 1). A 4 mm diameter steel rod (approximately one-third of the antero-posterior diameter of the limb) was inserted into the proximal end of each model. Shaft collars were used to mount the rod of each model into a 1 kg capacity bending beam load cell (EEB-1, Transducer Technologies, Inc., Temecula, CA, USA). The load cell was connected to a Vishay conditioning bridge amplifier (model 2120B, MicroMeasurements Group, Raleigh, NC, USA) and data were collected at 1000 Hz (Rivera, 2008) using custom data acquisition code written in LabVIEW (v.6.1, National Instruments Corp., Austin, TX, USA). The flow tank (120 cm×33 cm×33 cm working area) was filled to a water depth of 20 cm, and models were suspended in the centre of the flow tank to avoid wall effects. For two sets of trials, the models (one closed-toed and one open-toed) were submerged to a depth of 10 cm, fully covering the foot and a portion of the distal tarsometatarsus; in a third set of trials, the closed-toed model was partially submerged, covering only half of the foot and simulating the foot being dragged across the surface of the water. We collected three replicate trials for each combination of speed and toe configuration. Each trial contained an initial 5 s segment without flow to establish a baseline value, and then a 30 s segment in which flow velocity was set to one of four values by dial control (0.083, 0.151, 0.232 or 0.292 m s^{-1}). Speeds were chosen to provide repeatable increments on the dial control of the flow tank, up to a maximum that was near the body speeds of wading birds and below speeds which would have caused waves to form on the surface of the water. Our higher recording speeds were closest to what birds may actually experience, though leg speeds were likely higher and could incur greater drag than what could be replicated in the lab. For each speed–configuration combination, the
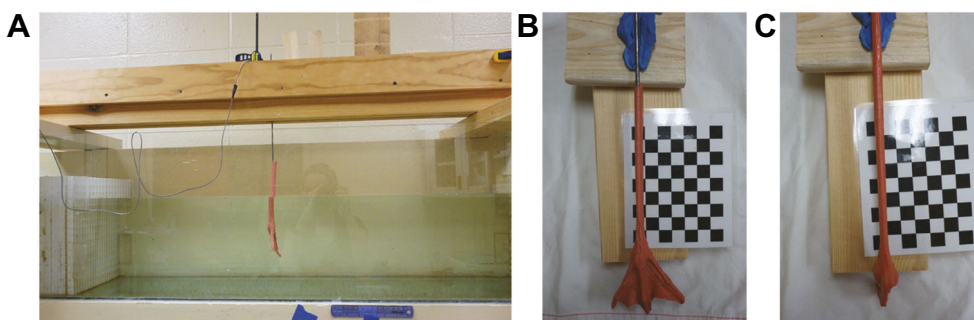

Fig. 1. Flamingo limb casts used for drag trials. (A) Flamingo limb cast mounted in the flow tank used to collect drag measurements. Water depth is 20 cm. (B) Cast model of a flamingo limb with spread digits and webbing. (C) Cast model of a flamingo limb with closed digits and webbing. Scale grid in B and C: 1 cm.

trial with the maximum value of drag was determined and retained for analysis.

Experimental animals

Five adult Chilean flamingos, *Phoenicopterus chilensis* Molina 1782 (1 female, 4 males), were housed and cared for by the staff of Greenville Zoo (Greenville, SC, USA). Flamingos were fed commercial pellet feed and had constant access to water. Filming sessions were conducted between August 2018 and September 2019. Each session lasted a maximum of 2 h, and at least a week elapsed between filming sessions. All procedures were approved by the Clemson University IACUC (AUP2017-078) and the Greenville Zoo veterinary staff (zoo veterinarian Nikolay Kapustin).

Measurement of walking and wading kinematics

All filming was conducted in the flamingo enclosure, which included a still water lagoon surrounded by dry land (Fig. 2). Video was captured at 60 Hz using three manually synchronized GoPro Hero 4 cameras (GoPro, Inc., San Mateo, CA, USA) placed in a triangular formation to allow the different camera viewing angles necessary for 3D kinematic data to be collected (Fig. 2C). Zookeepers guided all five birds to walk or wade in front of the

camera array (Movie 1). Handling and marking of the birds was not allowed, but each flamingo could be identified by leg bands, unique colour patterns or natural markings. A locomotor cycle ($N=131$ for ankle angle data, $N=145$ for all other variables; ∼30 trials per bird) was defined as beginning when the hindlimb furthest from the array was fully protracted and ending at the next point of full protraction of the same limb (Fig. 3).

Fisheye distortion from the GoPro cameras was corrected in Adobe Premiere Pro CC 2018 (Adobe Systems, Inc., San Jose, CA, USA). A 42 cm×32 cm×30 cm calibration object (LEGO, Billund, Denmark) was used to calibrate the 3D space (Mayerl et al., 2016). This calibration object occupied approximately 50% of the distance of the stride length of the flamingos. The object had 17 points with measured X, Y and Z distances which were input to the DLTDataViewer calibration software with the corresponding calibration object image in the video field of view. Strides were only digitized from videos in which the calibration object was fully in view between multiple cameras and where the calibration object covered the location of the stride. Videos shown by calibration software to have excessive calibration residuals were excluded from analysis. Ten anatomical landmarks were tracked on each bird (Fig. 2A) using DLTDataViewer5
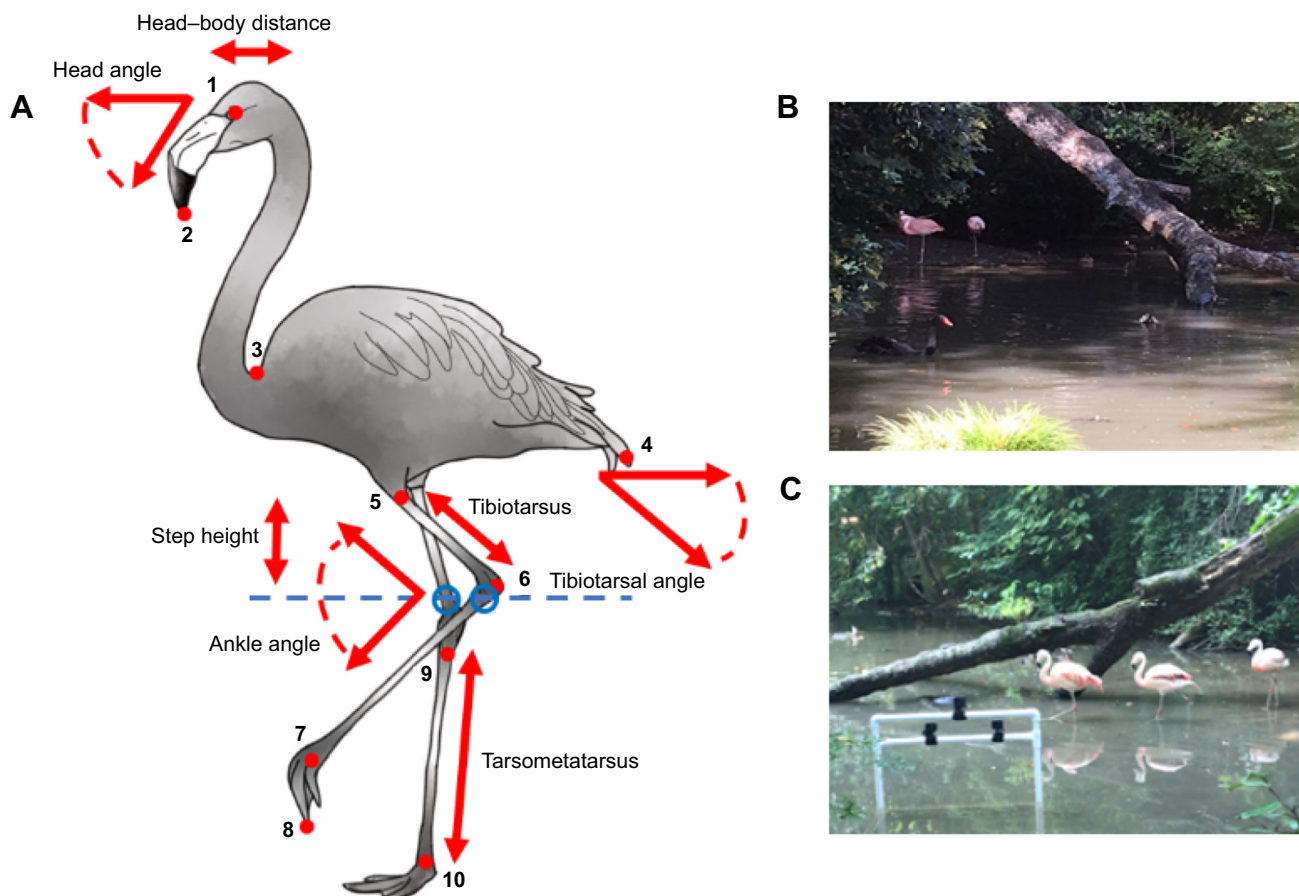

Fig. 2. Illustrations of anatomical landmarks and filming arena for flamingos. (A) Lateral view illustration of a flamingo, showing anatomical landmarks tracked on each bird: 1, eye; 2, tip of beak; 3, dorsal base of the neck; 4, tail tip; 5, proximal unfeathered edge of the near tibiotarsus; 6, posterior portion of the ankle of the near limb; 7, metatarso-phalangeal joint of the near limb; 8, tip of phalanges of the near limb; 9, bottom of ankle joint of the far limb; 10, metatarso-phalangeal joint of the far limb, or where the water depth met the leg in aquatic strides. The terms 'near' and 'far' refer to the distance of the limb from the camera. Illustrations displaying how head–body distance and head, ankle and tibiotarsal angle were calculated are shown in red. Example of deep water covering the ankle joint is indicated by the blue dashed line, with points of limb intersection with the water indicated by open blue circles. (B) Photograph of the flamingo enclosure at Greenville Zoo (Greenville, SC, USA), showing portions of the enclosure used for filming terrestrial strides and strides in water. (C) Filming apparatus of three GoPro cameras placed in a triangular formation for 3D video collection set in front of wading flamingos.
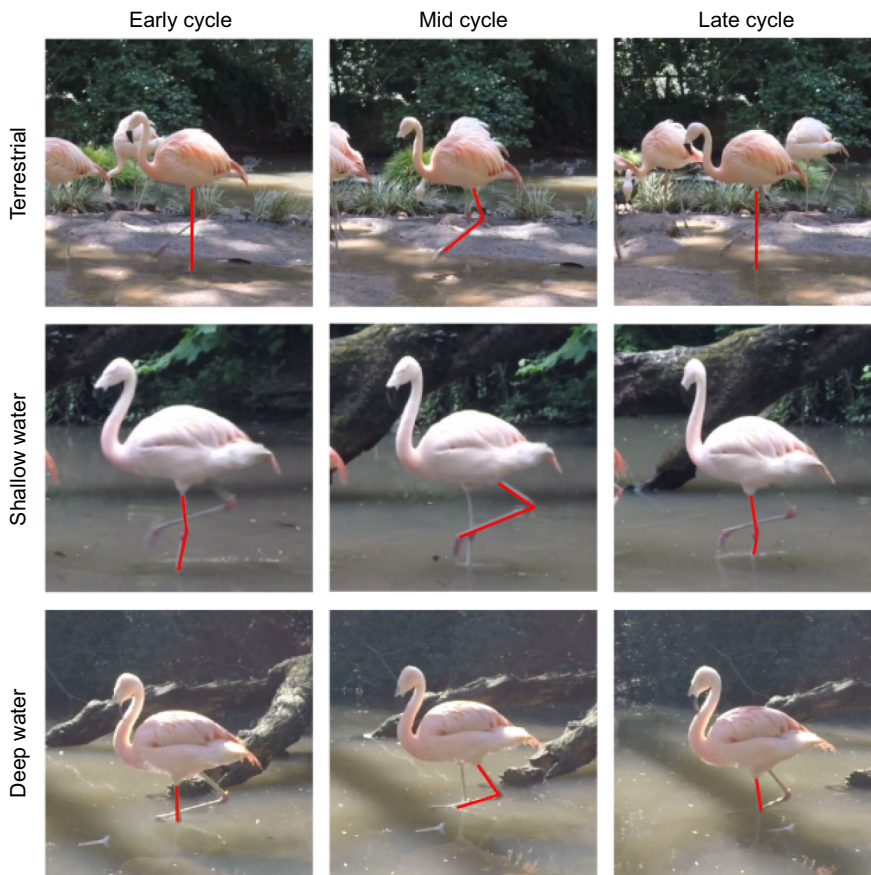
Fig. 3. Beginning, middle and end of one flamingo stride cycle in three different water depths. From top to bottom: terrestrial, shallow water (water below the ankle joint) and deep water (water at or above the ankle joint). Red lines overlay the focal limb for kinematic analysis in each sequence.

(Hedrick, 2008) and digitized 3D data were input into custom Matlab (Mathworks, Natick, MA, USA) routines to calculate kinematics and performance of each stride. These points included the eye, tip of the beak, the dorsal base of the neck where it meets the body, the tip of the tail, the proximal unfeathered edge of the tibiotarsus on the limb nearest to the camera, the middle of the ankle of the near limb, the metatarso-phalangeal joint of the near limb, the tips of the phalanges on the near limb, the bottom of the ankle on the limb farthest from the camera, and the metatarso-phalangeal joint on the far limb (when visible in shallow water or on land; see Fig. 2A). In deep water (depths that covered the ankle joint up to the mid-tibiotarsus), these points included the eye, tip of the beak, the dorsal base of the neck where it meets the body, the tip of the tail, the proximal unfeathered edges of the near and far tibiotarsus, the ankles of both limbs when visible, and where both limbs met the water surface.

Step height was initially measured as the vertical (Z) distance between point 5 in stance phase and point 6 at its maximal height during swing phase, with values normalized by the head length of each bird (see below). In this format, smaller distances indicate higher steps. However, for the purposes of graphical depiction, step height values were further standardized by dividing all values by the original maximum value (1.16), and then subtracting those values from 1. This allowed step height to be depicted on a more intuitive scale of 0 to 1 (i.e. as a percentage of head length), with higher values indicating higher steps. Other calculated kinematic variables included stride length (measured as the total horizontal distance that point 4 moved in a stride), average stride velocity for the limb cycle (measured as the stride length divided by the limb cycle duration), the angle of the tibiotarsus segment to the horizontal plane (a proxy for knee angle as the knee itself is covered by feathers; calculated as

the angle between a horizontal vector and a vector defined by points 5 and 6), ankle angle (the minimum angle created by the two vectors created from points 5–6 and 6–7), the head extension and flexion angles (i.e. the angle between a horizontal vector and a vector formed from points 1 and 2), and the minimum and maximum horizontal distance between the head and the body (calculated as the horizontal distance between points 1 and 5; Fig. 2A). Hip angle could not be evaluated, because the view of the hip was obstructed by feathers. To facilitate comparisons of kinematic profiles for locomotor cycles of different absolute durations, the calculated variables were processed through a quantic spline to smooth and interpolate the data to 101 values. These values represent 0–100% of the stride cycle, where 0 indicates full protraction of the limb farthest from the camera.

Statistical analyses of kinematics

To account for minor differences in size between the flamingos, water depth and all variables that were based on length measurements (step height, stride length and velocity) were normalized by size measurements from each bird. Water depth was normalized with reference to limb segment length, which was measured from calibrated video of each flamingo during terrestrial steps because handling of the birds was not allowed. Water depth was evaluated during the video frame of each stride when the limb segment emerged vertically from the water. For steps in shallow water, depth was considered as a fraction of tarsometatarsus length (i.e. the length between landmarks 6 and 7 in Fig. 2A), ranging from 0 for terrestrial steps, to 1.0 for depths that reached the ankle. For deeper water that covered the ankle, the submerged fraction of the unfeathered tibiotarsus length (i.e. the length between landmarks 5 and 6 in Fig. 2A) was added to 1.0 (representing the fully submerged

tarsometatarsus). In this way, water depth was considered on a scale of 0 to 2.0. Ankle angle data were not collected for strides that occurred in water depths that covered the ankle joint. Normalization for other variables was based on the length between an individual's eye and beak tip (i.e. a metric reflecting head length), which was consistently visible in all videos, regardless of water depth.

Limb and head kinematics were assessed as functions of water depth, water depth^2 and velocity. Water depth^2 was considered as a component of a quadratic relationship, testing our prediction of a parabolic relationship between kinematic variables and water depth, such that limb kinematics may be most similar on land and in the deepest water compared with shallow water kinematics. To test for significant differences between individual birds, the R package 'cvequality' was used (v.0.1.3, https://github.com/benmarwick/cvequality). No variation was detected between individual birds for any of the head and limb kinematics measured (Fig. S1). Because variation between individuals was not significant, we did not use linear mixed models.

We used R (http://www.R-project.org/) to evaluate the impact of water depth and velocity as main effects on flamingo kinematics. Velocity was evaluated via linear regression models, and water depth via quadratic regression models because of our prediction that step height would be greatest at intermediate depths. Interactions between velocity and water depth terms were also considered.

An exception to these approaches was made for the analysis of ankle angle. Quadratic terms of water depth were not considered for the ankle because its angle could not be measured for the deepest water steps in which it was always submerged, and a parabolic relationship would be unlikely to be present across the more limited range of water depths we could evaluate. Model selection was conducted using Akaike's information criterion (AIC) with the R package 'MuMIn' (v.1.43.17, https://CRAN.R-project.org/package=MuMIn).

All R statistical analyses are available in Supplementary Materials and Methods.

RESULTS

Drag on model flamingo feet
The different toe configurations of the flamingo model had considerably different frontal areas ($1.67×10^{-3}$ m^2 for the open-toed model, $4.75×10^{-4}$ m^2 for the closed-toed model, $3.81×10^{-4}$ m^2 for the partially submerged closed-toe model: Fig. 1B,C). As the speed of flow increased, drag increased substantially in all three models. Comparing fully submerged models, drag for the open-toed model exceeded that of the closed-toed model by 33% at the highest flow speed, but substantial drag was still present even in the closed-toed model (Fig. 4). Drag for the partially submerged model exceeded that for the other two models across the three slower flow speeds and was also higher than that of the closed-toed model at the highest flow speed, nearly reaching the magnitude of the open-toed model.

General patterns of flamingo stride kinematics
There was no change in gait between environmental conditions (Fig. 3), allowing us to develop a general characterization of the flamingo step cycle. The ankle was held in an extended position between 140 and 150 deg at the beginning of the step cycle, during mid-stance phase (Figs 3 and 5A). Once the limb was lifted to swing phase, the ankle flexed to a minimum of 70–80 deg before returning to its maximum extension (Figs 3 and 5A).

Although the knee was often hidden beneath the body feathers, we could measure approximate movements of the knee by tracking the angle of the distal tibiotarsus relative to the horizontal. At the beginning of the step cycle (during mid-stance), the tibiotarsus was directed nearly perpendicular (80–90 deg) to the horizontal (Figs 3 and 5B). As the limb was elevated for swing phase, this angle gradually decreased to a minimum of 40–50 deg, slightly earlier than maximal ankle flexion. As the limb proceeded to swing forward, the tibiotarsus could exceed its angle at the start of the cycle, extending to a maximum of 90–100 deg before returning to its starting position perpendicular to the horizontal and oriented directly under the body (Figs 3 and 5B).

Effects of water depth and speed on flamingo kinematics
Water depth had no correlation with velocity (P=0.9633, s.e.m. ±0.5516, R^2<0.0001). In this context, we felt it was appropriate to consider potential interactions between water depth and velocity and independent water depth and velocity terms, as explanatory factors in our statistical analyses of other kinematic variables for flamingos.

Summaries of the best models for explaining variation in flamingo kinematics are reported in Table 1, with all models compared reported in Tables S1 and S2. The best model to explain variation in stride length (P<0.0001, R^2=0.8249) indicated that it was significantly affected by the interaction between water depth and velocity (P=0.0029). Stride length increased with increasing velocity, though the slope of this relationship varied across changes
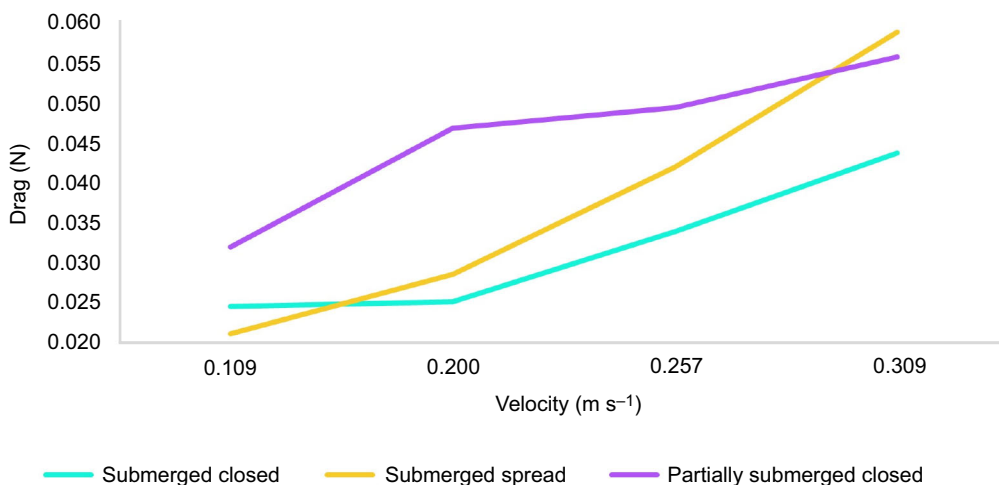

Fig. 4. Maximal magnitudes of drag experienced by models of distal flamingo limbs across four speeds of flowing water. Teal trace, submerged model with toes folded closed (see Fig. 1C); yellow trace, submerged model with toes spread apart (see Fig. 1B); purple trace, model with toes folded closed and only partially submerged in the water. As flow speed increased, the model with open toes experienced much greater drag than the model with submerged closed toes; however, considerable drag was still experienced by the closed-toe model. In the partially submerged closed-toe model, drag also increased in higher velocity flow, and at lower speeds experienced greater drag than the submerged models.
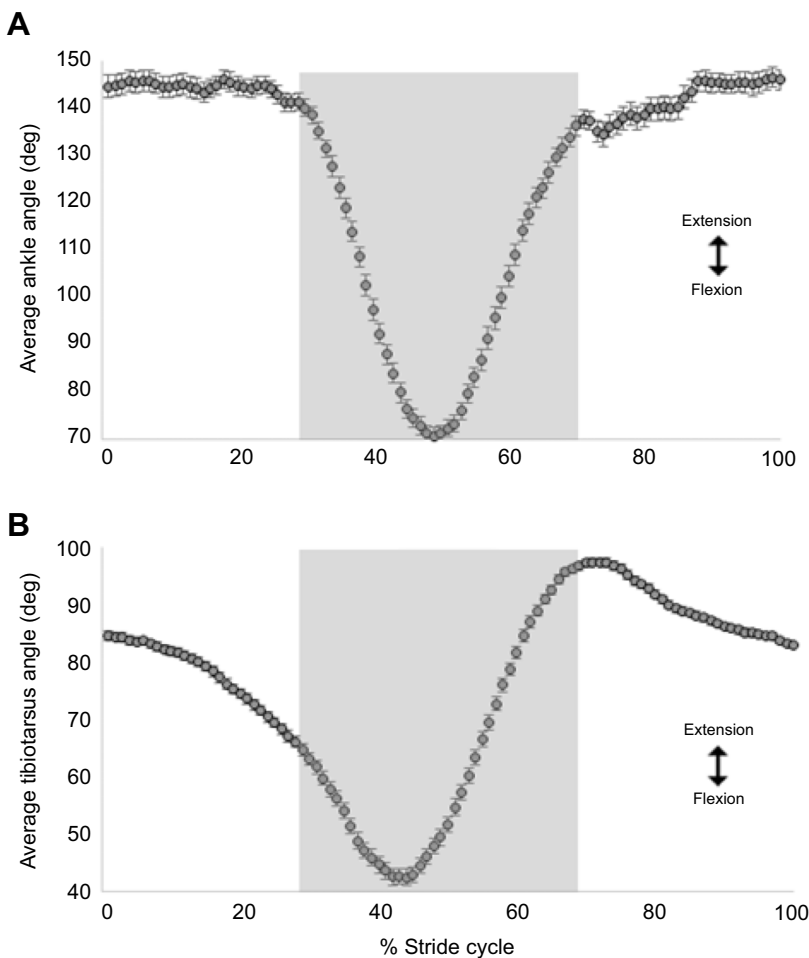
Fig. 5. General patterns of limb joint kinematics during the flamingo step cycle. All strides were normalized to the same duration, and each point and its error bars represent the mean and s.e.m. for the interpolated value of the angle at 1% increments through the stride cycle. Grey background indicates limb in swing phase. (A) Average ankle angle across all land and shallow water strides (ankle angle could not be evaluated in deep water). Ankle angle was smallest near the mid-point of the cycle, in the middle of swing phase. (B) Average angle of the tibiotarsus to the horizontal across strides from all water depths. Tibiotarsus angle was smallest during swing phase at mid-cycle, followed by the maximum extension at approximately 70% through the stride cycle.
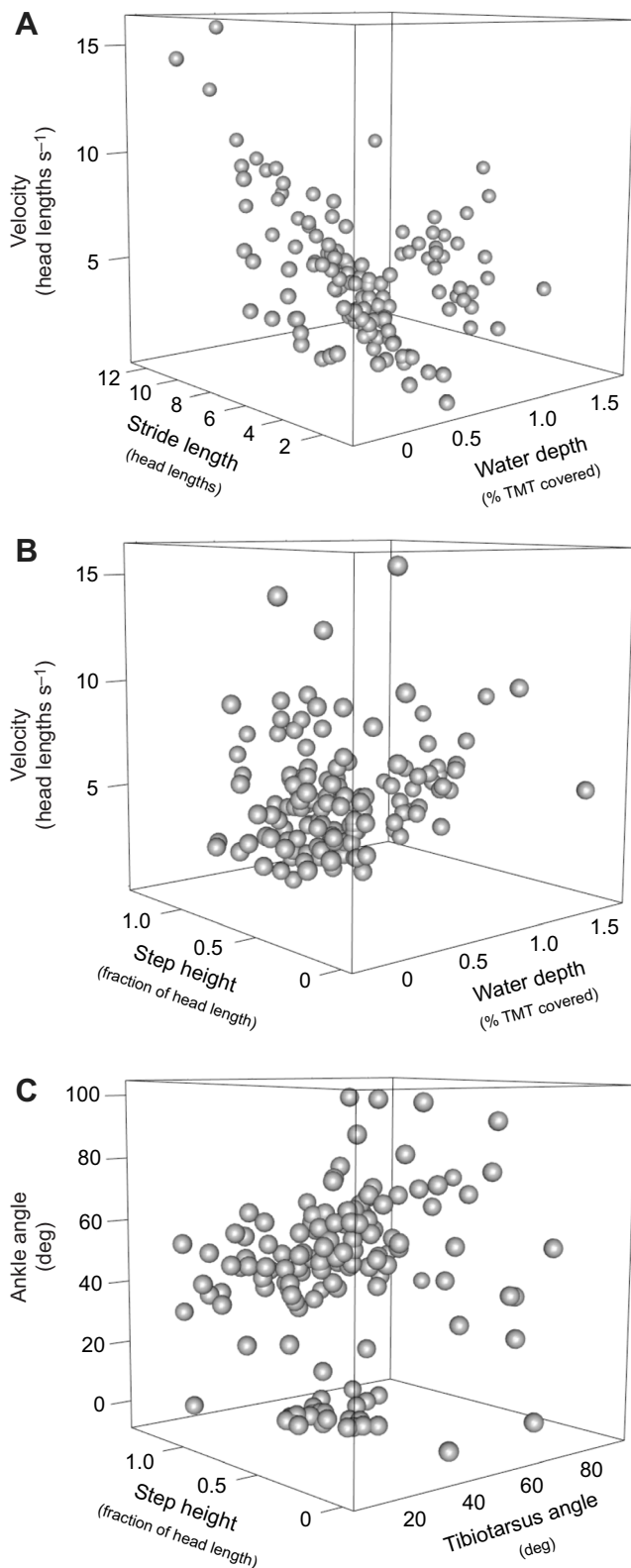
in water depth (Fig. 6A; Movie 2). The best model to explain variation in step height ($P=0.0010$, $R^2=0.0894$) included water depth^2 ($P=0.0083$), water depth ($P=0.0036$) and velocity ($P=0.0204$). Flamingos took the highest steps at intermediate water depths, but also showed a slight decline in step height as their velocity increased (Fig. 6B). Flamingos can control the height of their steps at several hindlimb joints, including the ankle, knee and

hip. Although we could not evaluate hip angles, we did identify likely impacts of water depth and step velocity on ankle and knee movements. The best model to explain variation in ankle kinematics ($P<0.0001$, $R^2=0.3187$) indicated that the minimum ankle angle (i.e. angle of greatest flexion, or folding of the joint) was significantly affected by the interaction between water depth and velocity ($P=0.002$), with the ankle bending more as water depth and

Table 1. Best fitting models for explanation of variation in kinematic variables as functions of water depth and speed for flamingo locomotion

Variable	Best fitting model	d.f.	AICc	Delta	Weight	P and adj R^2
Stride length	WaterDepth^2+WaterDepth+Velocity+WaterDepth^2 ×Velocity+WaterDepth×Velocity	7	390.1	0.00	0.820	$P<0.0001$ $R^2=0.8249$
Step height	WaterDepth^2+WaterDepth+Velocity	5	18.8	0.00	0.523	$P=0.0010$ $R^2=0.0894$
Step height (explained by angle)	AnkleAngle+TibiotarsusAngle	4	14.1	0.00	0.700	$P=0.0007$ $R^2=0.1054$
Minimum ankle angle	WaterDepth+Velocity+WaterDepth×Velocity	4	933.8	0.00	0.665	$P<0.0001$ $R^2=0.3187$
Minimum tibiotarsus angle	WaterDepth^2+WaterDepth+Velocity	5	1169.2	0.00	0.354	$P<0.0001$ $R^2=0.2201$
Minimum distance head–body	WaterDepth^2+WaterDepth+Velocity	5	310.0	0.00	0.399	$P<0.0001$ $R^2=0.2039$
Maximum distance head–body	WaterDepth^2+WaterDepth+Velocity	5	806.4	0.00	0.525	$P=0.8072$ $R^2=-0.0142$
Minimum head angle	WaterDepth^2+WaterDepth+Velocity	5	1082.0	0.00	0.474	$P=0.0006$ $R^2=0.0970$
Maximum head angle	WaterDepth^2+WaterDepth+Velocity+WaterDepth×Velocity	6	1077.3	0.00	0.584	$P=0.0152$ $R^2=0.0574$

See Tables S1 and S2 for results from all models compared.

Fig. 6. 3D plots of variation in locomotor variables from flamingo walking and wading steps. All non-angular measurements were normalized by body size as described in Materials and Methods. (A) Relationship between stride length, water depth (percentage of tarsometatarsus length, % TMT) and velocity. Stride length increased with increasing velocity, with a slope that varied across changes in water depth. (B) Relationship between step height, water depth and velocity. Step height is plotted as a fraction of head length as described in Materials and Methods, with larger values indicating higher steps. Step height was highest at intermediate water depths and lower in deep water and on land; it also decreased with increasing velocity. (C) Relationship between step height, ankle angle and the angle of the tibiotarsus to the horizontal (a proxy for knee angle). Again, smaller values indicate higher steps. There was no significant relationship between step height and tibiotarsus angle, though smaller ankle angles result in higher steps.

water. However, despite these patterns, our models did not show a significant effect of tibiotarsus angle on step height, but rather that there was a significant effect of ankle angle on step height, wherein more bending at the ankle resulted in a higher step height ($P=0.0007$, $R^2=0.1054$; Fig. 6C).

Head movements also varied with water depth and locomotor velocity, though generally higher AIC values suggested weaker relationships than those found for patterns of leg movements (Table 1; Table S2). The best model to explain variation in the minimum distance between the head and body ($P<0.0001$, $R^2=0.2039$) showed effects of water depth ($P=0.0005$) and velocity ($P<0.0001$), where the minimum distance became larger (i.e. the head was held further away from the body) as water depth and velocity increased. The maximum distance between the head and body was not significantly affected by water depth or velocity ($P=0.8072$, $R^2=-0.0142$). The best model to explain the minimum angle of the head to the horizontal ($P=0.0006$, $R^2=0.0970$) showed a significant relationship with water depth^2 ($P=0.0325$) and water depth ($P=0.0017$), in which the head was oriented closer to the horizontal at intermediate water depths and gradually was directed more vertically in deeper water. The best model to explain the maximum angle of the head to the horizontal ($P=0.0152$, $R^2=0.0574$) showed a significant effect of the interaction of water depth and velocity ($P=0.0015$), where the head was directed more vertically, or even had the bill tucked in so that its tip was posterior to the eye, as velocity and water depth increased.

DISCUSSION

We hypothesized that wading animals might employ a variety of kinematic changes as they move through different depths of water to limit increasing costs of drag on the limbs as water becomes deeper. Our measurements from models of flamingo feet (Figs 1 and 4) suggest that drag could be sufficient for such kinematic adjustments to be advantageous for wading birds. Flamingos typically move their feet forward through the water with the toes folded closed, and that configuration reduced drag by 25% from the open-toed configuration. However, even with the toes folded closed, drag averaging 0.09 N was measured at flow speeds near 0.3 m s^{-1}. These levels may be comparable to drag reported in various species of diving ducks, where larger diving duck species experience proportionally higher drag forces, and drag forces increase as velocity increases (Lovvorn et al., 1991). Such issues could be exacerbated during higher velocity strides when the limb might be subject to even greater drag while moving through the water, especially as the limb is breaking the surface of the water, rather than being completely submerged (Lyttle et al., 1998). Indeed, when modelling drag of a partially submerged foot in the closed-toed position used by flamingos during swing phase, drag was considerably higher than when the foot was fully submerged (Fig. 4),

velocity increased. Additionally, the best model to explain variation in the angle of the tibiotarsus to the horizontal (a proxy for knee kinematics; $P<0.0001$, $R^2=0.2201$) showed that this angle was significantly affected by water depth^2 ($P<0.0001$) and water depth ($P<0.0001$), with the tibiotarsus becoming most horizontal at intermediate water depths and becoming less horizontal in deeper

possibly due to phenomena such as spray drag that occur on objects moving through the water surface (Fish et al., 1991). This result suggests that it would be advantageous for flamingos to use steps that maximize movements either fully above or fully below the water.

To combat the potential for increased drag on the limbs during wading, our results suggest that, through depths up to ankle height, flamingos may attempt to elevate their feet above the water surface to: (1) limit the extent of the limb that incurs drag, and (2) limit the time over which drag is imposed. Step height was greatest when wading through intermediate depths, and lowest when moving on land and through the deepest water. Exaggerated lifting of the tarsometatarsus in deep water might have imposed costs of efficiency or balance that exceeded costs due to drag. This peak in step height occurred in water depths that were approximately the height of the ankle joint of the bird and were affected by stride velocity. In long-limbed birds, several joints can contribute to lifting a limb above the water. Our results show that while both the ankle and knee may exhibit altered kinematics in deeper water, most of the movement that correlated with changes in step height occurred in the ankle joint (Table 1; Table S1). Unfortunately, the feathers on our study animals obstructed the view of the hip, but future studies using methods such as XROMM (Andrada et al., 2015; Kambic et al., 2015; Kilbourne et al., 2016) could further test how the different limb joints contribute to changes in step height during wading.

Increasing the upward movement of the limb while in intermediate water depths may make it more challenging for flamingos to extend the limb forward when taking steps. Indeed, we found that when step height was at its highest, stride length was at its minimum in intermediate water depths. Conversely, longer strides were correlated with faster velocities in wading flamingos. In dogs, stride length increased and stride frequency decreased while wading in deep waters (Barnicoat and Wills, 2016). Taking fewer, longer strides rather than more frequent, shorter strides might be another mechanism for flamingos to reduce drag and energy expenditure while moving in deeper water by lowering the speed of limb motion through the water during wading steps. Although we were unable to collect metabolic data from flamingos, previous studies in wading horses found that respiratory frequency peaks at intermediate water depths around the height of the carpal joint, and that water depth has a greater influence on metabolic activity than speed of movements (Greco-Otto et al., 2017; Lindner et al., 2012).

Although the movements of the limbs were often correlated with either water depth or velocity, there were few statistically significant trends between the movements of the head and neck with water depth or velocity. The variability of head movements could reflect the use of the head for a large range of functions, including socialization, changes in gaze direction or feeding. However, the minimum distance between the head and body did show a weak trend towards increasing with increasing water depth and velocity, indicating that the neck was extended further anteriorly during deeper and faster steps. Pigeons also use neck extension when moving at higher velocities (Davies and Green, 1988). Future studies could provide further insight into these patterns by examining whether forward neck extension during faster strides is due to a passive or active mechanism.

Previous studies have suggested that the energetic costs of locomotion in semi-aquatic species may be higher in a given environment than the costs for an aquatic or terrestrial specialist, but that costs for a specialist are higher than those for a semi-aquatic species in the environments for which a specialist is not specialized (Fish and Baudinette, 1999). Semi-aquatic species may use a combination of morphological and kinematic adaptations to overcome the increased demands of their unique

environmental conditions (Blob et al., 2016; Provini et al., 2012, 2013). But while species that live at the interface between land and water must overcome a variety of physical challenges, they also have an opportunity to exploit the resources of a distinct environment (Blob et al., 2016; Kawano and Blob, 2013). The benefits of an increased niche, covering both water and land, may exceed the costs of moving through a challenging environment. Our results point toward various kinematic changes that can be employed to maintain performance in traits such as speed or stability while moving between different water depths. The capacity to sustain such performance, likely relating to the morphology of the limbs, could allow animals functional flexibility in shoreline environments in particular, where movements between land and water would be frequent (Abourachid and Höfling, 2012).

Long-legged birds, which possess a distinct limb morphology compared with that of other birds (Zeffer et al., 2003), have an increased need for stability. Interestingly, large wading birds often possess both long legs and a correlated long neck (Wilkinson and Ruxton, 2012), with flamingo taxa having the longest legs and neck relative to the body (del Hoyo et al., 1992). However, smaller waders such as yellowlegs or sandpipers have elongated legs correlated with deeper water habitats, but without extreme elongation of the neck (Baker, 1979), and semi-aquatic species such as swans exhibit long necks and shorter legs (Böhmer et al., 2019). Future studies could test how differences in anatomical proportions between smaller shorebirds and larger wading species, as well as ontogenetic changes in body proportions, might influence the diversity of wading kinematics.

Wading is a widespread locomotor behaviour that has received limited quantitative study. Our specific results for flamingos could have implications for the design of zoological enclosures that could help ensure that captive wading birds are kept in areas that limit negative locomotor difficulties while providing enough variety to stimulate the locomotor behaviours that are displayed in nature. For example, providing lagoons that gently grade through shallow to deep water would create opportunities for flamingos to continuously adjust their patterns of movement, rather than shift abruptly between patterns. More broadly, studies of wading locomotion could provide a useful point of comparison for understanding the reinvasion of aquatic habitats by terrestrial taxa (Fish, 1996; Young and Blob, 2015; Young et al., 2017), or provide a model for the design of bioinspired vehicles capable of stable and energy-efficient locomotion through a variety of habitats (McInroe et al., 2016). With broader comparisons across birds and other lineages, further studies of wading could build a framework for improving understanding of the diversity of mechanisms used in limbed locomotion.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: R.W.B.; Methodology: A.M.P., M.V.N., R.W.B.; Software: M.V.N.; Validation: A.M.P., M.V.N., R.W.B.; Formal analysis: A.M.P., M.V.N.; Investigation: A.M.P., R.W.B.; Resources: R.W.B.; Data curation: A.M.P., M.V.N.; Writing - original draft: A.M.P., R.W.B.; Writing - review & editing: A.M.P., M.V.N., R.W.B.;

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