HUMMINGBIRD MANEUVERING PERFORMANCE: AERODYNAMIC MECHANISMS AND PHYSIOLOGICAL CONSTRAINTS

by

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Abstract

The ability of a bird to maneuver in flight can determine its success at avoiding predators, catching prey, and other critical behaviors. Highly maneuverable animals, such as hummingbirds, are capable of diverse behaviors but it is unknown how their maneuvering is constrained by wing motion, wing morphology, and muscle capacity. The purpose of this dissertation was to determine: 1) if hummingbird wings create independent wakes; 2) if independent wingbeat kinematics are used to control maneuvers; and 3) how maneuverability is limited by intrinsic features, such as wing morphology, body mass, and physical properties of the air, versus facultative capacity, such as muscle power. The goal of chapter two was to determine if hummingbirds produce single or bilateral vortex wakes using flow visualization. The goal of chapter three was to determine if sustained maneuvers can be controlled by orienting the wings independently of the body. I tested this hypothesis by filming the three dimensional kinematics of a hummingbird feeding from a translating feeder. The goal of chapter four was to determine if the ability to perform voluntary maneuvers was associated with intrinsic or facultative features. I addressed this question using a tracking system to record a large data set of voluntary flight trajectories, with independent measurements of individual morphology and maximum muscle capacity. The goal of chapter five was to determine if maneuvering performance declines with increasing elevation and, if so, whether changes in oxygen availability or air density are most responsible. I addressed these questions by measuring maneuvering performance across elevation and in an airtight chamber with gas manipulations. Collectively, my results indicate that hummingbirds have wings that operate with a high degree of independence and that this feature influences their precision and control. Voluntary maneuvers at low elevation are primarily
influenced by facultative capacity, specifically burst power, and to a lesser extent by intrinsic limits, specifically wing aspect ratio. At higher elevations, maneuvering performance declines due to decreases in air density. This research demonstrates that the remarkable maneuverability of hummingbirds derives from their ability to control their wings independently and from high muscle power reserves for generating aerodynamic force.
Preface

This doctoral dissertation is a collection of four studies that explore different aspects of hummingbird maneuvering performance. I developed the central questions of these studies with the help and supervision of Dr. Doug Altshuler, and I took the lead in conducting the experiments, analyzing the data, and writing the manuscripts.

A version of chapter two was published in the journal *Experiments in Fluids* (Pournazeri *et al.*, 2012). The idea for this project was developed jointly by all four authors. S. Pournazeri and I developed the methods, collected and analyzed the data, and are co-first authors on the paper. D.L. Altshuler and I wrote the majority of the manuscript. The flow visualization data was supplemented with kinematic data extracted from unanalyzed videos of hovering hummingbirds taken by D.L. Altshuler between 2003-2006. I developed the computational framework that made the extraction of the kinematic measurements possible. This framework was additionally used in Altshuler *et al.* (2012), Read (2015), and chapter three of this dissertation.

I was the lead investigator for the study described in chapter three. A subset of the data came from videos collected by D.L. Altshuler with the intent of answering a different set of questions. I developed the central question in chapter three, re-purposed the videos, and developed the computational framework that made the kinematic analysis possible. D.L. Altshuler advised during the analysis and the writing. The statistical analysis was developed by K.M. Middleton in a previous study (Altshuler *et al.*, 2012).

I was the lead investigator for the studies in chapters four and five. I designed the experiments with the help of D.L. Altshuler, and conducted them myself. The experiments were based on videos filmed with an automated tracking system designed and programmed by A.D.
Straw for use with fruit flies (Straw et al., 2011). I built the hardware system and modified the software to make it suitable for use with hummingbirds. R.L. Dakin designed the statistical framework used, and T.L. Reed helped with the data collection and the techniques used for the gas substitution part of the study.

The procedures used for this experiment were approved by the UBC Animal Care Committee (certificate #a10-0223).
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* videos can be found at: http://hdl.handle.net/2429/54568; captions are provided in Appendix A
List of symbols and abbreviations

\( A \) .................................................................................................................................................... wing area
\( \text{AccHor}_{\text{max}} \) ................................................................................................................... maximum horizontal acceleration
\( \text{AccTot}_{\text{vel, max}} \) ....................................................................................................................
maximum velocity during an acceleration maneuver
\( \text{AccVD}_{\text{max}} \) .........................................................................................................................
maximum vertical downwards acceleration
\( \text{AccVU}_{\text{max}} \) ........................................................................................................................
maximum vertical upwards acceleration
\( A_{\text{disk}} \) ........................................................................................................................................
actuator disk area
\( \text{AICc} \) ........................................................................................................................................
Akaike information criterion
\( \text{Arc}_{\text{cent, max}} \) ...................................................................................................................
maximum centripetal acceleration during an arcing turn
\( \text{Arc}_{\text{rad}} \) .....................................................................................................................................
radius of an arcing turn
\( \text{Arc}_{\text{vel, avg}} \) ........................................................................................................................
average velocity during an arcing turn
\( \text{Bill \%} \) ........................................................................................................................................
bill insertion percentage
\( C_D \) ..................................................................................................................................................
coefficient of drag
\( C_L \) ..................................................................................................................................................
coefficient of lift
\( \text{CFD} \) ...........................................................................................................................................
computational fluid dynamics
\( \text{Cost}_{\text{ori}} \) .................................................................................................................................
cost associated with choosing orientation
\( \text{Cost}_{\text{ori}} \) .................................................................................................................................
cost associated with choosing reverse orientation
\( \text{DecHor}_{\text{max}} \) ............................................................................................................................
maximum horizontal deceleration
\( \text{DLT} \) ...............................................................................................................................................
direct linear transformation
\( \text{D\text{\'rag}} \) ...........................................................................................................................................
drag
\( \text{DS} \) ..................................................................................................................................................
downstroke
\( \text{EMG} \) ...............................................................................................................................................
electromyogram recording
\( \text{end} \) ................................................................................................................................................
endstroke
\( \text{\vec{F}}_{\text{aero}} \) ....................................................................................................................................
aerodynamic force
\( f \) ....................................................................................................................................................... wingbeat frequency
\( g \) ....................................................................................................................................................... gravitational constant
\( \text{hov} \) .................................................................................................................................................. hovering
\( J \) ......................................................................................................................................................... advance ratio
\( L \) ......................................................................................................................................................... wing length
\( \text{Lift} \) .............................................................................................................................................................. lift
\( \text{load} \) ............................................................................................................................................................. load-lifting
\( m \) ..................................................................................................................................................................... mass
\( \text{mid} \) ................................................................................................................................................................ midstroke
\( n \) .......................................................................................................................................................................... number
\( \text{O} \) \( \text{r} \) \( \text{b} \) ......................................................................................................................................................... body vector (calculated with tracking system)
\( \text{O} \) \( \text{r} \) \( \text{i} \) \( \text{n} \) \( \text{m} \) ....................................................................................................................................................... body vector calculated for the previous frame
\( \text{pFDR} \) ............................................................................................................................................................ positive false discovery rate
\( \text{PitchD}_{\text{vel,avg}} \) ................................................................................................................................................. maximum pitch-down velocity
\( \text{PitchU}_{\text{vel,avg}} \) ................................................................................................................................................. maximum pitch-up velocity
\( \text{PIV} \) ................................................................................................................................................................ particle imaging velocimetry
\( \text{PRT} \) ............................................................................................................................................................... pitch-roll-turn
\( \text{PRT}\% \) .......................................................................................................................................................... percentage of pitch-roll turns to total turns
\( \text{PRT}_{\text{deg}} \) ....................................................................................................................................................... degrees turned during a pitch-roll turn
\( \text{PRT}_{\text{time}} \) .................................................................................................................................................... time taken to perform a pitch-roll turn
\( Q_{\text{ori}} \) ................................................................................................................................. Kalman process covariance matrix for body orientation
\( Q_{\text{pos}} \) ................................................................................................................................. Kalman process covariance matrix for body position
\( R^2_{\text{GLMM(m)}} \) .............................................................................................................................. marginal coefficient of determination
\( R_{\text{ori}} \) ................................................................................................................................. Kalman observation covariance matrix for body orientation
\( R_{\text{pos}} \) ................................................................................................................................. Kalman observation covariance matrix for body position
\( \text{RWBA} \) ......................................................................................................................................................... relative wing bank angle
\( S \) ........................................................................................................................................................................ wing surface area
\( T \) ...................................................................................................................................................................... wingbeat period
\( T_D \) ........................................................................................................................................................... downstroke period
\( T_U \) ........................................................................................................................................................ upstroke period
\( U_p \) ............................................................................................................................................................ vertical direction vector
\( \text{US} \) ........................................................................................................................................................... upstroke
\( \bar{U}_{\text{tip}} \) ...................................................................................................................................................... average wingtip speed
\( \text{V}_{\text{body}} \) ................................................................................................................................................ body velocity
\( \text{V}_{\text{incident}} \) ........................................................................................................................................... incident velocity
\( \vec{V}_{\text{induced}} \) ..........................................................induced velocity

\( \vec{V}_{\text{tip}} \) ......................................................................wingtip velocity

\( V_{\text{el}} \) .............................................................velocity vector (calculated with tracking system)

\( V_{\text{el}}_{\text{mod}} \) ..................................................modified velocity vector tipped up 15° towards the vertical direction

\( w_{\text{b}} \) ..............................................................................body weight

\( WBA \) ...............................................................................wing bank angle

\( Yaw_{\text{vel, avg}} \) ..............................................................maximum yaw velocity

\( \alpha \) ..................................................................................geometric angle of attack

\( \dot{\alpha} \) ...........................................................................geometric angle of attack velocity

\( \alpha_{\text{aero}} \) .......................................................................aerodynamic angle of attack

\( \beta \) ..................................................................................stroke plane angle

\( \theta \) ..................................................................................elevation angle

\( \theta_{\text{GR}} \) ..........................................................................instantaneous elevation angle

\( \bar{\theta}_{\text{GR}} \) ..................................................................average elevation angle

\( \Theta_{\text{sp}} \) ..............................................................elevation amplitude (calculated in the stroke plane)

\( \rho \) ...................................................................................air density

\( \tau \) ..................................................................................downstroke ratio

\( \phi \) ..................................................................................position angle

\( \phi_{\text{GR}} \) ..........................................................................instantaneous wing position

\( \Phi_{\text{sp}} \) ..............................................................wingstroke amplitude (calculated in the stroke plane)

\( \chi_{\text{GR,XZ}} \) ............................................................lateral body angle

\( \chi_{\text{GR,YZ}} \) ............................................................frontal body angle

\( \Psi \) ...............................................................................travel angle

\( \omega \) ............................................................................wingtip angular velocity
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To Mandy. I couldn't have done it without you...
1. Introduction

Maneuverability is defined generally as the ability to change speed and direction (Dudley, 2002), and is critical to survival and reproduction. It plays an important role in competition, courtship, hunting and foraging, territory defense, escape from predation, and a variety of other behaviors (Hedenstrom and Rosen, 2001). However, because maneuverability is inherently difficult to measure, most studies of locomotion have focused on steady state behaviors that are made up of stereotypical, repetitive motions such as running (Biewener et al., 1983), gliding (Pennycuick, 1983), hovering (Ellington, 1984d), level flapping flight (Spedding, 1987a), swimming (Lauder, 2000), climbing (Isler and Thorpe, 2003), and hopping (Biewener and Baudinette, 1995). Although these studies are fundamental to understanding critical aspects of locomotor performance, it has been suggested that steady state behaviors are not the most relevant to an animal's survival on a day-to-day basis (Howland, 1974). The ability to accelerate (lizards: Huey and Hertz, 1984; birds: Warrick, 1998), takeoff (insects: Marden, 1987; hummingbirds: Tobalske et al., 2004), land (birds: Dial, 1992; bats: Riskin et al., 2009), fast-start (Domenici and Blake, 1997), and turn (Howland, 1974), are more likely to determine an animal's ability to escape a predator or catch prey (Walker et al., 2005).

Highly maneuverable animals, such as hummingbirds, are capable of diverse behaviors (Altshuler et al., 2012; Clark, 2009; Clark, 2011b; Clark et al., 2012; Felton et al., 2008; Feo and Clark, 2010; Read, 2015) but it is unknown how their maneuvering is constrained by wing motion, wing morphology, and muscle capacity. The purpose of this dissertation was to determine if hummingbird wings operate independently from each other with respect to aerodynamic wakes, if independent wingbeat kinematics are used to control maneuvers, and how
maneuverability is limited by intrinsic features, such as wing morphology, body mass, and physical properties of the air, and by facultative capacity, such as muscle power (Warrick, 1998). In this review I will describe the mechanical and aerodynamic underpinnings of aerial maneuvering performance, describe methods that have been used to study maneuvers, and introduce the questions that I will address in this dissertation and their relevance to understanding biomechanics, behavior, ecology, and evolution.

1.1 Aerodynamics

Flying animals use their wings to produce aerodynamic force, which can be decomposed into lift and drag component vectors. Lift is the vector component perpendicular to the oncoming air, whereas drag is the vector component parallel to the oncoming air. The equations for lift and drag are as follows:

\[ \vec{F}_{\text{aero}} = \vec{Lift} + \vec{Drag} \]

\[ \vec{Lift} = \frac{1}{2} C_L \rho A \vec{V}_{\text{incident}}^2 \]

\[ \vec{Drag} = \frac{1}{2} C_D \rho A \vec{V}_{\text{incident}}^2 \]

Aerodynamic force depends on several factors, some which are intrinsic properties of the wing and others that can be actively controlled by the animal. Lift and drag depend on air density ($\rho$), wing surface area ($A$), the coefficients of lift and drag ($C_L, C_D$), and incident velocity ($V$).
Aerodynamic force decreases with lower density and increases with greater surface areas. The coefficients of lift and drag are non-dimensional numbers that are affected by the shape of the wing and the angle that it encounters the oncoming air, known as the angle of attack ($\alpha_{aero}$). These coefficients are difficult to predict because they depend on many different factors (e.g. wing shape, material properties, camber) and therefore they are often measured empirically for a given angle of attack (e.g. Kruyt et al., 2014). Finally, the incident velocity is calculated with the following equation:

$$\vec{V}_{\text{incident}} = \vec{V}_{\text{tip}} + \vec{V}_{\text{induced}} + \vec{V}_{\text{body}}$$

Incident velocity is the vector sum of the velocity caused by the air speed ($\vec{V}_{\text{body}}$), the velocity that is produced by the motion of the wing ($\vec{V}_{\text{tip}}$), and the induced velocity ($\vec{V}_{\text{induced}}$) that results from the air being sucked into the wing in the same way that air is sucked into a fan. During level flapping flight, animals use wingbeat kinematics to generate enough aerodynamic force to overcome air resistance on the body and the wings, while still supporting their weight against gravity.

### 1.2 Measurements of aerodynamic forces

As interest in bioinspired engineering grows there have been several creative approaches to measuring the aerodynamic forces created by gliding and flapping animals. These include detailed force calculations based on the lift and drag equations (Ellington, 1984a; Norberg and Rayner, 1987; Pennycuick, 1968), direct measurements of the wake fields left by the wings (e.g. Kokshaysky, 1979; Spedding et al., 2003), scaled robotic models that recreate complex flapping
patterns (Birch and Dickinson, 2001), and pressure sensors that measure the circulation bound to wing surfaces (Usherwood et al., 2003).

Aerodynamic models of varying complexity can be used to estimate forces produced by the wings. For gliding animals, induced velocity and wingtip velocity are equal to zero, and the process is straightforward. The coefficients of lift and drag can be measured empirically using mounted preparations of the wings in a wind tunnel (Withers, 1981), or they can be inferred from the measured movements of the whole body (Pennycuick, 1983). Flapping wings are more complex and the aerodynamic forces are often calculated by dividing the wings into chordwise segments, calculating the forces created by each segment, and summing the force vectors over the entire wingstroke (Ellington, 1984b). This approach has its limitations: induced velocity is difficult to estimate (Sane, 2006) and is often calculated algebraically once all the other forces are balanced (Hedrick et al., 2002), and the model does not account for unsteady aerodynamic effects created by wing-wake interactions (Birch and Dickinson, 2003). As flapping wings encounter the vortices created during current and previous wingstrokes extra lift can be generated by mechanisms such as leading edge vortices, wake recapture, and long edge rotation of the wing. Unsteady effects can be difficult to calculate and the most common way of incorporating them into an aerodynamic model is through the use of Computational Fluid Dynamics (CFD), which is used to computationally simulate the flow of particles over an airfoil (Liu et al., 1998).

Direct measurement of the circulation in the wake structures created by gliding and flapping animals can replace complex estimates of aerodynamic forces and account for little understood unsteady aerodynamic mechanisms (Spedding et al., 2003). As an airfoil moves through the air it leaves behind a trail of vortices shed as a byproduct of lift generation. The
vortices are formed when air is accelerated downwards by the passing wing and according to Helmholtz's second theorem, because vortex filaments cannot end in the fluid they must connect to form closed rings. The circulation contained in the vortex rings is directly proportional to the amount of aerodynamic force generated and the shape of the vortex rings can be used to determine spatial and temporal differences in force production. Fixed wing aircraft and gliders leave a relatively simple vortex wake: the vortices form a single large ring that starts at takeoff and ends at landing (Henningsson and Hedenström, 2011). In contrast, flapping animals leave complex vortex patterns that are influenced by wing shape and wingbeat kinematics. Animals that use aerodynamically inactive upstrokes leave a wake that resembles a series of discrete rings. Animals that supinate their wings to create aerodynamically active upstrokes leave a ladder-like vortex structure of connected rings (Kokshaysky, 1979; Rayner, 1979; Spedding, 1987a; Spedding et al., 1984). It has been proposed that some flying animals can transition between inactive upstrokes at low flight speeds and active upstrokes at high flight speeds, and this represents the aerial equivalent of gaits (Hedrick et al., 2002; Tobalske, 2000). However, some studies have suggested that the transition is smooth and not discrete (Spedding et al., 2003). Animals that rely on high lift production from the upstroke such as hummingbirds (Warrick et al., 2005), swifts (Hubel et al., 2012) and hawkmoths (Willmott and Ellington, 1997) always use active upstrokes.

In addition to aerodynamic modeling and flow visualization a number of other techniques have been used to study aerodynamic force production. Model wings have been used to measure the effects of independently altering kinematic parameters and airfoil shape. These studies have shown that minor changes in wing rotation or angle of attack can influence the force production caused by unsteady state mechanisms (Bahlman et al., 2013b; Dickinson et al., 1999; Kruyt et
Finally, pressure transducers have been used to create maps of the airflow around the wings in free flying birds. This approach has demonstrated the differential roles played by different feathers, but to date has been limited to use with larger birds (Usherwood et al., 2003).

As methods of measuring aerodynamic forces become more sophisticated, there is increasing evidence for complex vortex structures left in the wake of flying animals. Studies have found direct evidence for leading edge vortices (Birch and Dickinson, 2001; Bomphrey et al., 2005; Muijres et al., 2008), wake recapture (Dickinson et al., 1999), cross-stream vortices (Spedding et al., 2003), and root vortices (Henningsson et al., 2011; Hubel et al., 2010a; Muijres et al., 2008). Additionally, studies have revealed important differences in the wake structure of different taxa. Birds can feather their wings during the upstroke to allow for aerodynamic inactivation in a way that bats and insects cannot (Muijres et al., 2008). While most bird wings create a single vortex ring per stroke, bat wings shed a root vortex that results in each wing generating its own vortex ring (Hubel et al., 2010a; Hubel et al., 2012; Muijres et al., 2008; Muijres et al., 2011). The ability of the wings to generate separate vortex rings and function independently may increase maneuvering performance, although this has not been tested.

1.3 Modulating aerodynamic force

Level flapping flight is a useful starting point for understanding aerodynamic performance. However, with the exception of long distance migrations, extended hovering bouts, or wind tunnel experiments, flying animals spend a majority of their airborne time stringing together sequences of maneuvers. A maneuver is any change in speed or direction and examples can range from simple (e.g. accelerations, decelerations, vertical climbs, descents, banked turns) to complex (e.g. crabbed turns, yaw turns, pitch-roll turns, skids, chandelle turns, barrel rolls). To
perform maneuvers animals increase aerodynamic force production beyond what is required for steady state flight, and use the excess force to change their momentum (Dudley, 2002).

Aerodynamic force is modulated by changing the wingstroke and the shape of the airfoil to affect the velocity, surface area, and lift coefficients found in the lift and drag equations. There are two functional categories of wing kinematics: variables that affect air velocity and variables that affect $C_l$, $C_d$ and area by dynamically altering wing shape and orientation.

The easiest and most effective way to modulate lift is by changing the velocity of the wing. Because the aerodynamic force is proportional to velocity squared, this has a disproportionate effect on lift production. The incident velocity of the wing can be increased by either increasing flight speed ($V_{\text{body}}$) or wing speed ($V_{\text{tip}}$). Induced velocity ($V_{\text{induced}}$) is also dependent on the wing speed, but it is usually small enough that it is swamped out by the other two velocity components, and therefore only slightly alters the effective angle of attack. The strategies used to increase incident velocity are highly dependent on physiological constraints and flight modes. Gliding animals take advantage of gravity to increase body velocity, as they have little capacity to increase wingtip velocity (Bahlman et al., 2013a). Likewise, for large fliers whose flapping velocity is constrained by the length of their wings (Pennycuick, 1975), increasing body velocity remains the most energy efficient method of increasing incident velocity. For this reason, large birds often rely on flight strategies such as dynamic soaring or riding thermals to maximize air speed while minimizing flapping. On the other end of the spectrum, for hovering animals wingtip velocity is the only way to increase air velocity. This requires extremely high wingtip speeds, which in turn imposes limits to wing length, aerodynamic force production, and body size.

Wingtip velocity can be altered by modulating wingbeat frequency, wingbeat amplitude,
or the proportion of time spent in downstroke. Although amplitude and frequency affect aerodynamic force in similar ways, there is some evidence that individual birds prefer to increase amplitude over frequency. When hummingbirds are challenged to fly at low air densities they compensate for the reduced aerodynamic force production by increasing wingbeat amplitude substantially and wingbeat frequency modestly (Altshuler and Dudley, 2003; Chai and Dudley, 1995; Chai and Dudley, 1996). Additionally, when hummingbirds are challenged to increase aerodynamic force production in response to incrementally added weights they do so by increasing amplitude (Mahalingam and Welch, 2013). However, when the weight is increased to obtain maximal transient lifting performance, hummingbirds respond by increasing both frequency and amplitude (Altshuler et al., 2004b; Altshuler et al., 2010a; Chai et al., 1997). The preference for increasing amplitude over frequency may reflect intrinsic properties of the flight muscles.

A second way that animals can increase aerodynamic force is by altering angle of attack and wing shape to change area and lift and drag coefficients. For a given wing shape there is a narrow angle of attack range that maximizes lift and minimizes drag (Kruyt et al., 2014). By dynamically morphing wing area, camber, and aspect ratio, flying animals may increase lift to drag ratios. Under the simplest flapping models with no dynamic wing shape control, the upstroke produces downward forces that are counterproductive to staying aloft. Flying animals have developed two strategies for overcoming this challenge, folding or supinating the wing during upstroke. Folding the wing during upstroke results in a smaller surface area that produces less negative lift. Supinating, or twisting the wing, changes the angle of attack so that the upstroke produces positive lift (Tobalske, 2000). Although there is evidence that many flying animals actively morph their wings to modulate aerodynamic force during different flight modes,
1.4 Redirecting aerodynamic forces

To perform maneuvers, flying animals increase aerodynamic force production and then redirect the excess force to effect changes in momentum (Warrick, 1998; Warrick and Dial, 1998; Warrick et al., 1998). For linear accelerations, climbs, and banked turns the wings are tilted forward, upwards or laterally, often by reorienting the body. The magnitude of the aerodynamic force determines how much of the force can be redirected while still supporting the bodyweight against gravity. Body axis rotations may represent less costly methods of changing direction (Altshuler et al., 2012; Hedrick et al., 2009), although geometric and anatomical restrictions of the wing shape, body shape, and shoulder excursion may limit the ability to roll, pitch, and yaw.

1.5 Implementation of specific maneuvers

Recently, several studies have focused on the initiation and maintenance of aerial maneuvers. Maneuvers are by nature difficult to study because they are transient and there are multiple sources of variation. Individuals may have preferences for how they execute a change in momentum based on morphology, muscle power capacity, efficiency, or motivation. One common method for obtaining repeatable behaviors is by constraining maneuvers through the use of obstacle courses. Tunnels (Warrick, 1998) and towers (Berg and Biewener, 2008; Jackson and Dial, 2011; Tobalske and Dial, 2000) have been used to study simple linear maneuvers such as horizontal and vertical accelerations, and it has been proposed that maximum acceleration can be used as a proxy for quantifying more complex maneuvering performance (Warrick, 1998).
Likewise, right-angle corridors have been used to elicit banked turns (performed by rolling the body axis: Hedrick and Biewener, 2007; Iriarte-Diaz and Swartz, 2008; Ros et al., 2011) and crabbed turns (performed by reorienting the wings: Iriarte-Diaz and Swartz, 2008; Ros et al., 2014), although in some cases the same individual can choose to perform either one (Iriarte-Diaz and Swartz, 2008; Ros et al., 2015). For hovering animals like hummingbirds and hawkmoths, motorized feeder tracking has been used to measure energetics of linear accelerations (hawkmoths: Sprayberry and Daniel, 2007) and detailed wing kinematics of turns (yaw turns in hummingbirds: Altshuler et al., 2012; banked turns in hummingbirds: Read, 2015). Enriched flight chambers have demonstrated how animals maneuver to avoid obstacles (Warrick et al., 1998; Williams and Biewener, 2015), turn in confined spaces (Hedrick et al., 2009), and perform takeoffs and landings (Jackson and Dial, 2011; Riskin et al., 2009). Several studies have measured stereotyped escape responses, although these maneuvers are not always repeatable. Visual stimulation produces highly repeatable banked turns in flies (Muijres et al., 2014), but startled hummingbirds perform a range of escape maneuvers (Clark, 2011b). Finally, certain behavioral stimuli have been used to elicit repeatable maneuvers. Hummingbirds performing territorial and mating displays perform highly choreographed and spectacularly maneuverable display dives (Clark, 2009), shuttles (Feo and Clark, 2010), and long axis rotations (Felton et al., 2008). Constrained maneuvers are an excellent way to measure the kinematic basis of specific maneuvers and to compare similar performances across individuals, however, they may not reflect actual performance in a natural setting (Irschick, 2003).

### 1.6 Free-flight maneuvering performance

To understand the effects of maneuvering performance on behavior, ecology, and
evolution, it is important to measure the breadth of an animal's maneuvering capabilities in a natural or semi-natural setting. However, voluntary maneuvering behavior presents a problem for experimental biologists: how do you compare repeated maneuvers when an animal is free to vary its motion across translational and rotational axes, and across time and space? There have been two approaches used to overcome these problems and to quantify self-selected, functional maneuvering performance. The first is to creatively design experimental treatments that compare assays of maneuvering performance. An example of this is measuring the time it takes to navigate an obstacle course (Aldridge, 1986) or the time it takes for an animal to be captured by a predator in an arena (Walker et al., 2005). These types of experiments quantify maneuvering performance, but allow for animals to self-select and perform different trajectories.

The second method used to compare free-flight maneuvering performance is to use a computational approach to categorize and compare similar trajectories. With a sufficiently large sample size it is possible to identify similar trajectories or similar features from different types of trajectories. Wagner (1986) compared velocities, accelerations, and yaw turns in chasing houseflies. Egelhaaf and collaborators used a mathematical clustering approach to discover maneuvering primitives in blowflies (Braun et al., 2010) and hoverflies (Geurten et al., 2010). They identified complex maneuvers by searching for repeated sequences of primitives in a large data set. Shelton et al. (2014) measured maximum mechanical power generation and banked turns performed during cliff swallow chases. Both mechanical power and instantaneous turning radius are measurements that can be compared across very different trajectories. The study of self-selected maneuvering performance is a relatively new field with the potential to unite the study of locomotion with questions of behavior, ecology, and evolution, in a way that was not previously possible. As of yet, there have been no studies that have measured the morphological
and physiological determinants of individual maneuvering performance, the effects of different environmental conditions on individual maneuvering performance, or the effects of ecological role on interspecific maneuvering performance.

1.7 Hummingbirds as a model for studying maneuvering performance

Hummingbirds are a good model organism for studying maneuverability because of their obvious agility, ease of training for complex behavioral experiments, and well-described flight ability. Hummingbirds possess the ability to perform maneuvers using all six ranges of translational and rotational motion, including the ability to hover (Greenewalt, 1960), fly backwards (Sapir and Dudley, 2012), and even briefly fly upside down. During display dives, Anna's hummingbirds can reach speeds of over 60 mph, attaining the highest body length per second speed known for any animal (385 length/sec) and experiencing acceleration forces of up to nine times the gravitational constant (Clark, 2009). In addition, hummingbirds have a wide range of complex behaviors that can be exploited to incite maneuvering performance in a controlled setting. Hummingbirds in captivity will set up and defend territories (Tiebout, 1993), feed from moving feeders (Altshuler et al., 2012; Read, 2015), and even perform territorial or mating displays (Clark et al., 2013). Hummingbirds also have several features which make them ideal for structuring natural experiments (Feinsinger and Chaplin, 1975): they have an extensive radiation with a well known phylogeny (McGuire et al., 2008), they inhabit a variety of habitats and ecological niches (Feinsinger, 1976), and they have a large range of wing and body morphology (Altshuler et al., 2004a).
1.8 Conclusions

The goal of my dissertation research was to determine if hummingbird wings operate independently from each other with respect to aerodynamic wakes, if independent wingbeat kinematics are used to control maneuvers, and how maneuverability is limited by intrinsic features, such as wing morphology, body mass, and physical properties of the air, and by facultative capacity, such as muscle power.

In the second chapter I present evidence for a previously hypothesized but undocumented vortex structure found in the wake of hovering hummingbirds. Using a novel method of low-tech flow visualization I test the hypothesis that hovering hummingbirds use bilateral vortex jets. Previously, two vortex flow patterns had been proposed for the wake of hovering hummingbirds: 1) the two wings form a single, merged vortex ring during each stroke (Rayner, 1979); and 2) the two wings form bilateral vortex loops during each stroke (Altshuler et al., 2009). The structure of the vortex loop and the ability of the wings to operate with a degree of independence may influence the high level of maneuverability that hummingbirds possess.

The goal of the third chapter was to determine if sustained maneuvers can be controlled by orienting the two wings independently of the body. I tested this hypothesis by presenting the three dimensional wing kinematics and the resultant quasi-steady aerodynamic model for a hummingbird performing a controlled lateral flight maneuver while feeding from a translating feeder.

In the fourth chapter I asked the question: what are the biomechanical determinants of maneuverability? It has been proposed that maneuverability is determined by both intrinsic constraints, such as body mass, wing size and shape, and by facultative capacity, such as muscle power output (Warrick, 1998). However, how morphological and physiological factors affect
individual maneuvering performance is unknown. I addressed this question using an automated, high-speed tracking system to record a large data set of voluntary flight trajectories, with independent measurements of individual morphology and maximum muscle capacity. In this chapter I developed a computational framework that can be used to compare hummingbird maneuvering performance across individuals, experimental treatments, and species.

The goal of the fifth chapter was to determine if maneuvering performance declines with increasing elevation and, if so, whether changes in oxygen availability or air density are most responsible. I addressed the first question by using the tracking system developed in the fourth chapter to test maneuvering performance of individual hummingbirds translocated between high and low elevations. To address the second question I measured maneuvering performance of hummingbirds flying in variable density and oxygen gas mixtures.
2. Hummingbirds generate bilateral vortex loops during hovering: evidence from flow visualization

2.1 Introduction

Research in animal aerodynamics has demonstrated that the wake patterns, which can be visualized with increasing detail, differ among species and by flight mode. Comparisons across forward flight speeds in bats, birds, and insects have revealed both differences and some examples of convergence. The general pattern described for birds has been that the wake produced by the wings is connected over the body, generating a single vortex loop at slow speeds and changing circulation at higher speeds (Kokshaysky, 1979; Spedding, 1987a; Spedding et al., 1984; Spedding et al., 2003). Bats, in contrast, have been shown to generate bilateral vortex wakes—one per wing—at slow speeds and more continuous shedding at moderate speeds (Hedenström et al., 2007; Muijres et al., 2008). Both patterns have been seen in insects, with hawkmoths producing one vortex wake (Bomphrey et al., 2005) and bumblebees producing bilateral wakes (Bomphrey et al., 2009). Particle image velocimetry (PIV) methods have improved in temporal and spatial resolution over the last few years as have the coverage of species of different sizes and flight modes. One of the significant additions to the emerging model of the wakes of flying animals is that vortices can be shed from the wing roots in both birds and bats over a wide range of sizes and flight speeds, although the strength and persistence

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1 A version of this chapter was published as (Pournazeri et al., 2012). The idea for this project was developed jointly by all four authors. S. Pournazeri and I developed the methods, collected and analyzed the data, and are co-first authors on the paper. D.L. Altshuler and I wrote the majority of the manuscript. The flow visualization data was supplemented with kinematic data extracted from unanalyzed videos of hovering hummingbirds taken by D.L. Altshuler between 2003-2006. I developed the computational framework that made the extraction of the kinematic measurements possible.
of these root vortices varies by species and speed (Hedenström et al., 2009; Henningsson et al., 2011; Hubel et al., 2010b; Hubel et al., 2012; Johansson and Hedenström, 2009; Muijres et al., 2011; Muijres et al., 2012). The presence of root vortices indicates that if and when the body is included in producing vertical force, this requires some time to develop. Other aerodynamic consequences of root vortices are still to be demonstrated, but the Kutta-Joukowski and Kelvin-Helmholtz theorems indicate that these shedding patterns will lead to differences in lift-generating mechanisms and the energetic cost of flight (Hedenström et al., 2007; Rayner and Gordon, 1998).

Hovering flight is useful for examining the relationships between animal performance and its aerodynamic wake because in the absence of forward flight the sources of the wake are restricted to the wings and the wake interactions with the body. Several features of the flow around hummingbird wings have been well described (Warrick et al., 2005; Warrick et al., 2009), but a thorough description of the wake topology is not available. It had been proposed that a hovering hummingbird generates one vortex ring per stroke (Ellington, 1984d; Pennycuick, 1988; Rayner, 1979; Rayner and Gordon, 1998), which would match the vortex shedding pattern proposed for larger birds during slow flight (Spedding et al., 1984; Spedding et al., 2003).

Altshuler et al. (2008) made PIV measurements in a horizontal plane beneath the hummingbirds close to the tail. These measurements revealed source flows induced by vortices, which appeared on each side of the animal at an interval of double the wingbeat frequency. They proposed that each wing of a hovering hummingbird generates its own vortex loop during each downstroke and upstroke. However, they did not have images of the vortices. One prediction of the proposed wake topology is that there should be bilateral jets, one per wing.

Hovering presents at least two challenges for PIV recordings. In the absence of forward
speed, the wake does not trail behind but instead piles up beneath the animal, which can cause the vortices to occur very close to and possibly be occluded by the body at low wake stream velocities. Also, because the wings are active during both up- and down-stroke, each stroke can disrupt previous stroke’s wake. One solution to these problems is to compile a time-resolved history of the flow field (Bomphrey et al., 2006). However, for hovering animals, sequential measurement of the vortex progression over the course of a single stroke requires a specialized fast response PIV system.

Previous PIV studies with hovering hummingbirds used 2D approaches that included descriptions of flows around cross-sections of the wing and thin slices from the lateral, rear, and underneath perspectives (Altshuler et al., 2009; Warrick et al., 2005; Warrick et al., 2009). Proof of the bilateral vortex loop model requires a perspective of the 3D flow, which can be obtained by visualization of the flow field. Here, I present the results from films of hovering hummingbird in a white plume emitted by the heating of dry ice. Additional high-speed videography from multiple perspectives was used to describe the position, velocity, and phase relationships of the wing tips and wing roots in normal air. These kinematic data were used to refine four different vortex topologies for comparison with the visualized structures. The topologies differ with respect to the number of jets (i.e., one vs. two) per stroke and the connections of the wakes (i.e., via the wingtip or wing root).

2.2 Methods

2.2.1 Animals

Six male Anna’s hummingbirds (*Calypte anna*) were captured on the campus of the
University of California, Riverside (UCR), and used for the flow visualization measurements. Another four male *C. anna* were captured on the campus of the California Institute of Technology (Caltech) for the wingbeat kinematic measurements. The hummingbirds were housed in campus vivarium, trained to feed from artificial feeders, and fed a diet of sugar water and specially formulated hummingbird nectar (Nektar-Plus, Nekton GmbH). At the conclusion of the experiments, all of the birds were banded and released at the original site of capture. All procedures were approved by the UCR and Caltech Institutional Animal Care and Use Committees.

### 2.2.2 Flow visualization

The flow visualization measurements were made between January and March 2011 at UCR. The flight chamber (0.5 m × 0.6 m × 0.6 m) was constructed with two clear acrylic sides for filming, two black cardboard sides to increase contrast with the background, and a mesh floor and ceiling to allow air circulation. I trained the hummingbirds to hover at a feeder in the center of the chamber surrounded by a white plume, which allowed for visualization of the wake patterns. The plumes were formed by adding dry ice to hot water above the cage. The water temperature accelerated the sublimation process and resulted in a plume that entered the cage through the mesh top, enveloped the bird, and exited through the bottom of the cage. The plume extended only ~5-15 cm around the feeder so that the bird could enter and exit it at will. I filmed the region in front of the feeder from the side and rear perspectives using two synchronized high-speed digital cameras (Fastec Imaging Troubleshooter, Vision Research Miro 4) recording at 500 frames per second. Both cameras used the tip of the feeder as the main point of focus. I recorded eight trials for each bird, and each trial’s videos contained ~150 wingbeat cycles. The
visibility of the vortices associated with the wake structures depended upon their position and orientation relative to the camera. However, each of the six birds approached the feeder from a slightly different angle, so every video essentially provided a unique view of the wake structures.

### 2.2.3 Wingbeat kinematics

The wingbeat kinematic measurements were made between December 2003 and April 2006 at Caltech. The acrylic flight chamber (1 m × 1 m × 1 m) had three white sides and three clear sides for filming with three high-speed cameras (Photron APX). The hummingbirds were trained to hover feed from a small artificial feeder located in the center of the chamber. The three cameras recorded at 1,000 frames per second. Points at the wingtips, shoulder, and the wing root were digitized frame by frame using DLTdv software (Hedrick, 2008) running in the Matlab platform (MathWorks Inc., Natick, MA). A multi-point calibration object was filmed immediately before and after flight trials. I analyzed one hovering sequence for each bird.

The wing stroke angles were calculated from Cartesian coordinates of the digitized anatomical landmarks in 3D space. The position angle $\phi$ is defined as the angle between the projection of the wingtip or wing root into the horizontal plane and a line defined by the midpoint between the wingtips at pronation and the head. The values span from $0^\circ$, directly behind the bird, to $180^\circ$, directly in front of the bird. The elevation angle $\theta$ is defined as the angle between the wingtip or wing root, the shoulder, and the projection of the wingtip or wing root into the horizontal plane. The values span from $-90^\circ$, directly below the bird, to $+90^\circ$, directly above the bird, with $0^\circ$ at the horizontal position. The downstroke period $T_D$ is defined as the time from the rearward most excursion of the position angle to the forward most excursion of the position angle in a given wingbeat. The upstroke period $T_U$ is the time from the forward to the
rearward most excursion within each wingbeat. The downstroke ratio $\tau$ is defined as $T_D / T$, where $T$ is the wingbeat period.

The 3D positions of the body and wing points were imported to a 3D modeling program (sketchup.google.com) to generate four simplified versions of the single and bilateral vortex topologies. The most likely topology was oriented to match with the body orientation and wing positions of the hummingbird in the plume to assist in the identification of flow structures.

### 2.2.4 Vortex terminology

I present terminology to describe the observations from the flow visualization experiment. It is now recognized that vortex wakes are complex, interconnected structures (Spedding et al., 2003), so labeling specific segments as discrete vortices is an oversimplification that is useful for comparison in the absence of a completely resolved flow field. When possible, I have used existing terminology, but I have also had to modify some terms to match the observed structures. Three distinct families of vortices are defined: (1) structures shed from the wing tip, (2) from the wing root, and (3) during stroke reversal.

**Wingtip** vortices are formed by the pressure differences above and below the wing. This pressure difference induces a flow around the wing (Green, 1995), which forms into a vortex that is shed off the wingtip during the progression of the stroke. Following the Prandtl lifting-line theory (Anderson, 1991), these vortices can also be explained through the shed of the rolled-up streamwise vorticity that is generated due to the spanwise variation of bound circulation (i.e., lift), which is a result of the finite spanwise length of the wing (three-dimensional effects).

**Wing root** vortices are observed near the wing root. Wing root vortices are generated through a similar mechanism as wingtip vortices, where streamwise vorticity of opposite spin
rolls up into a distinct vortex. These vortices have been observed in insects, birds, and bats with varying strength and persistence (Bomphrey et al., 2009; Hedenström et al., 2009; Hedrick et al., 2007; Henningsson et al., 2011; Hubel et al., 2010b; Hubel et al., 2012; Johansson and Hedenström, 2009; Muijres et al., 2008; Muijres et al., 2011). The cause and function of these vortices has not been fully established.

At the termination of each stroke, the wing sheds a vortex that can be caused by several sources. Due to a rapid change in translational velocity (deceleration), and assuming that the change in the descending velocity along the wing chord is negligible, a region with a significantly high velocity gradient (i.e., high vorticity) is developed at the leading edge of the wing. As the vorticity rolls up in the wake, it forms a stopping vortex with the same sign as the bound vortex that is shed as the wing tip reverses (Anderson, 1991; Hedenström et al., 2007; Warrick et al., 2005; Warrick et al., 2009). Additional sources can include changes in the angle of attack and camber, which lead to variation in bound circulation, thereby causing vortex shedding. During the initiation of the next stroke when the wing undergoes an acceleration in the opposite direction, a starting vortex is formed, which is rotating in the opposite direction to the new bound vortex of the wing but in the same direction as the stopping vortex from the previous stroke. Because I did not observe separate stopping and starting vortices, I use the term reversal vortex to refer to what may be the combination of the two structures. Reversal vortices occur at the beginning and ending of each stroke, so I use the terms pronating and supinating reversal vortices to distinguish the vortices that occur behind and in front of the bird, respectively.
2.3 Results

2.3.1 Flow visualization

The videos of hummingbirds hovering within the white plume demonstrate clear development of flow structures. I first describe a sequence of plume entrainment on the left side of a hummingbird (Bird 6, trial 5, video 2.1). Seven successive frames (59-65) from this video depict the first half of a downstroke (Fig. 2.1). The panels on the left are raw images, whereas the panels on the right are the exact same images but with arrows illustrating the location and direction of prominent flow structures.

After the wing pronates at the end of the upstroke and start of the downstroke (frame 60), a counterclockwise pronating reversal vortex is shed. As the wing continues its stroke, a wingtip vortex is shed, first visible in frame 63. This vortex is formed as a result of the roll-up of the streamwise vorticity that is shed from the wing. The wingtip vortex occurs throughout the entire course of the downstroke. However, in the video, it only becomes clearly visible as a vortex when its cross-section is perpendicular to the camera (frame 63), and it continues to show up until the vortex vector becomes almost in line with the image plane. The pronating reversal and wingtip vortices form a continuous horizontal loop that induces a strong vertical jet-shaped like an hourglass, converging above and diverging below the wing.

I next take advantage of multiple perspectives of the birds’ positions with respect to the plume to describe additional flow features (Fig. 2.2). The beginning of the third trial of bird 6 provides a clear perspective of a train of vortex loops on the right side of the animal (video 2.2). Several structures are visible in frame 101 (Fig. 2.2a, b). This frontal perspective of the bird is shown immediately after wing pronation. The wingtip vortex created by the previous upstroke is
visible (b1), as well as both the supinating reversal (b2) and wingtip (b3) vortices created by the previous downstroke. The large wingtip vortex created by two downstrokes earlier is also visible (b6), but the smaller supinating reversal (b5) and wingtip (b4) vortices from the previous upstroke have already dissipated. The straight arrows represent the extrapolated location of the dissipated vortices.

Later in the same video, the plume has expanded and the bilateral jets and associated vortex loops shed from the wings are visible (Fig. 2.2d, e). This image is also a frontal view of the bird immediately after wing pronation. The wingtip vortices from the previous downstroke (e1 and e2) and the right supinating reversal vortex (e3) are visible. The supinating reversal vortex for the left wing is not visible, although its hypothesized location is indicated (e4). The left and right wings have generated individual jet streams (e5 and e6).

A wider field of view of the rear perspective of a hummingbird hovering in the plume is available for the second trial of bird 5 (video 2.3). Frame 58 (Fig. 2.2g, h) occurred at the start of the downstroke and contains a view of both the pronating reversal (h1) and wingtip (h2) vortices created by the previous downstroke. It also provides a clear view of the shape of the vortex tube (h3) connecting the vortices.

The lateral perspective of the wake structure from a hovering hummingbird has been described in previous PIV studies (Warrick et al., 2005; Warrick et al., 2009), and I provide that perspective here (video 2.4) for comparison. At frame 37, the reversal vortices are parallel or nearly so with respect to the camera plane and, therefore, not visible. The wingtip vortices from both the upstroke and the downstroke are visible. Two sets of vortices can be observed at stroke transition: k1 is the wingtip vortex shed at the beginning of the current upstroke, and k2 is the wingtip vortex shed at the end of the previous downstroke (Fig. 2.2j, k). At the previous
upstroke-downstroke transition, there is a single vortex (k3), which is likely to contain the wingtip vortices that have either merged by this point or are too close to be resolved. At the previous downstroke-upstroke transition, the wingtip vortex from the downstroke is still prominent (k5), but the wingtip vortex from the upstroke has just disappeared. The red arrow indicates its hypothesized position (k4). The previous upstroke-downstroke vortex (k6) has now dissipated.

### 2.3.2 Kinematics experiment

The wing stroke amplitude ($\Phi$), wingbeat frequency ($f$), and downstroke ratio ($\tau$) of the hummingbirds from both experiments are presented in Table 1. The hummingbirds in the kinematics experiment exhibited values for these variables that are similar to what has previously been reported for male *C. anna* during hovering (Altshuler et al., 2010b). On average, the hummingbirds in the flow visualization experiment used lower stroke amplitudes and higher wingbeat frequencies, but the individual birds were also more variable. Bird 4, for example, used a stroke amplitude/wingbeat frequency combination that was nearly identical to the average values for the kinematics experiment. An image sequence of flow development on the left side of the bird is presented in Fig. 2.3 and in video 2.5. The vortex dipole in frame 351 consists of a wingtip vortex on the left and either a reversal or wing root vortex on the right. From this perspective of the bird, both are possible because the position at which pronation occurred was nearly perpendicular to the image plane of the camera.

The average instantaneous position ($\phi$) and elevation ($\theta$) angles of the left and right wing tips and wing roots are depicted in Fig. 2.4. The position angles of the wingtips and wing roots have similar amplitudes, but with the wingtips leading at all stages of the stroke. The elevation
traces exhibit a double harmonic pattern, but again with the wing tips leading at all phases. Thus, the wing root reverses in both position and elevation after the wing tip. The left and right wings followed nearly identical patterns.

2.3.3 Wake pattern

The wingbeat kinematics and flow visualizations were used to visualize four potential topologies for hovering hummingbirds. These new but still simplified models are based upon previous hypotheses of the single (Rayner, 1979) and bilateral (Altshuler et al., 2009) vortex loops and other possible explanations for the flow patterns observed here (Fig. 2.5). The wing stroke is assumed to impart the same downward momentum to the vortices in all four models. The downward velocity was estimated by tracing wingtip vortices over sequences of images in the flow visualization experiment. The images were calibrated using a ruler that was placed vertically next to the feeder and filmed immediately after the experiment. The calibration was then applied to the flow visualization images and analyzed using ImageJ. The average descent velocity based on 60 wingbeats from six individuals was 2.6 m/s (±0.2 m/s SD). This velocity is a coarse approximation and was not used to calculate aerodynamic force and efficiency.

The outer paths of the models follow the paths of the wing tips over the stroke amplitudes recorded during hovering flight in the kinematics measurements. For the merged vortex ring model (Fig. 2.5a), the wingtip vortex path follows an extrapolated wingtip trajectory to merge the left and right sides. The indented merged vortex loop model is a version of the single loop model that includes reversal vortices. It assumes that the connection between the left and right sides occurs near the body, which produces an hourglass-shaped loop (Fig. 2.5b). The bilateral vortex loops model accounts for the reversal vortices by assuming that these are connected by root
vortices (Fig. 2.5c). Because I did not observe separate starting and stopping vortices, these are assumed to either merge or appear very close together in this and the indented merged vortex loop model. The bilateral vortex loops model was oriented to match each of the bird positions in Fig. 2.2, where it is presented in the right column. The concentric vortex rings model is presented for the scenario in which the stroke amplitude is close to 180°, and the wingtips and wing roots each form closed loops unifying the left and right sides (Fig. 2.5d). Three-dimensional animations of the four topologies are available in the videos 2.6-2.9.

2.4 Discussion

2.4.1 Flow visualization

Previous PIV measurement of the horizontal plane underneath hovering Anna’s hummingbirds, C. anna, provided vector topologies that implicated the presence of bilateral vortex loops in the wake (Altshuler et al., 2009). However, only the source flows caused by the jets could be distinguished and not the associated vortices. Here, I made high-speed image sequences of the wake structures formed by C. anna hovering in white plumes. Videos recorded from rear (Figs. 2.1, 2.2g) and frontal (Fig. 2.2a, d) perspectives revealed the presence of bilateral jets and associated vortices, which disproves the merged vortex ring model (Fig. 2.5a), at least for this species of hummingbird. There are two potential topologies that can account for observations by the previous PIV study (Altshuler et al., 2009) and the current flow visualization: an indented merged vortex loop (Fig. 2.5b) and the bilateral vortex loop models (Fig. 2.5c).

The difference between the models that include bilateral jets is the presence or absence of
root vortices. Vortex dipoles surrounding the bilateral jets were shed during both up- and down-strokes. However, because of the proximity and alignment of the root and reversal vortices at the start and end of the stroke, it is not certain which structure was visualized in each frame. Assuming that there are no root vortices, the most common wake structure expected would be the topology suggested by (Spedding et al., 2003) where the body is included in the lift generation. This can be valid in forward flight. However, the distinct feature of hovering flight compared to other flight modes is the absence of forward flight speed, which essentially excludes the body from the lift generation. The flow speed across the wing will increase with distance from the body but should be zero at the wing-body junction. This decrease in flow speed, which is also a decrease in circulation toward the body, should be accompanied by a vortex shed close to the root. The total excursion of the wing root during normal hovering is considerably less than the length of the body, making it highly implausible that the vortices shed from the left and right wing roots connect to each other. Rather, this root vortex should connect the supinating and pronating vortices from the same wing. This will form a closed loop and lead to a bilateral vortex wake structure.

The two potential topologies with bilateral jets (Fig. 2.5b,c) include the assumption that the vortices produced by each stroke form connected streams rather than separate structures. I did not discern separate stopping and starting vortices, which is consistent with the flow patterns generated at the downstroke-upstroke transition (supination), but not of the upstroke-downstroke transition (pronation) reported for rufous hummingbirds, Selasphorus rufus (Warrick et al., 2009) (see Warrick et al., 2009 supplementary material, Fig. 6). Because the transition at both supination and pronation occurs very fast relative to the descent rate of the flow passing the bird, these vortices are spatially very close and, it appears, merge to form a single vortex. The wake
created by a hovering *C. anna* should, therefore, consist of a vertically connected vortex ladder rather than separated vortex loops. It is unknown whether the wing-wake interactions at stroke transition include strong components of wing rotation and wake capture (Dickinson et al., 1999).

The vortex structure influences the lift generated by the wings. The wake patterns of Blackcaps (*Sylvia atricapilla*, Johansson and Hedenström, 2009) include wing root vortices that could be either opposite or same sign relative to the wingtip vortices. The upwash and downwash induced by these vortices can reduce or enhance the lift, respectively (Wang and Wu, 2010). In the present study, I observed vortex dipoles under each wing with the outer (wingtip) pole always of opposite sign to the inner (reversal or wing root) pole. This suggests that a wing root vortex generates upwash directly beneath the hummingbird. This structure can be seen in image sequences presented here (e.g., Fig. 2.2d, e), and its signature also exists in PIV results presented in Altshuler et al. (2009) as a sink flow behind the bird body (e.g., their Fig. 8). Although the upwash between the bilateral loops is relatively weak, it can slightly reduce the total lift by generating a local negative lift. Thus, when all else is equal, the single vortex loop will produce higher aerodynamic force due to the larger downwash area, absence of the upwash, and inclusion of the body in lift generation. For a hovering animal, the vertical force must by definition balance the body weight regardless of wake topology, but the single vortex ring wake will be more aerodynamically efficient (Muijres et al., 2012; Norberg et al., 1993).

Animals attempting to maximize lift should expand the area of downwash, whereas animals aiming to minimize power expenditure would benefit from producing the wake that meets the aerodynamic requirements for the smallest muscle output. A potential advantage of each wing producing a vortex loop is that differences in the size and orientation of the wake could confer enhanced ability to produce left-right asymmetries in aerodynamic force.
This level of control would be advantageous for maneuverability (Henningsson et al., 2011), potentially at the expense of stability.

The original vortex model for hovering in hummingbirds (Fig. 2.5a) was based on early measurements from a tropical hummingbird (*Florisuga fuscus*) that used a relatively high stroke amplitude and close to horizontal stroke plane (Stolpe and Zimmer, 1939). Although necessarily simplistic, this model of a merged vortex ring (Ellington, 1999; Pennycuick, 1988; Rayner, 1979; Rayner and Gordon, 1998), such as that produced by helicopters, allowed for consideration of how aerodynamics could influence the metabolic costs of hovering flight (Epting and Casey, 1973) and related ecological and biogeographical constraints (Feinsinger and Chaplin, 1975; Feinsinger and Colwell, 1978; Feinsinger et al., 1979). An exciting prospect of the new topology of the hovering wake is that this may also contribute to new understanding of hummingbird physiological ecology.

### 2.4.2 Wingbeat kinematics

When hovering in normal air, hummingbirds used an average wing stroke amplitude of 153.6°, but the average stroke amplitude of the birds studied in the white plumes was only 129.8°. It has been demonstrated that hummingbirds will modulate stroke amplitude and wingbeat frequency under a variety of conditions (Ortega-Jimenez and Dudley, 2012) and can match the lift requirements for different environmental and experimental conditions. Across taxa, high-elevation hummingbirds use higher stroke amplitudes during hovering than low-elevation taxa (Altshuler and Dudley, 2003; Altshuler et al., 2010a). Measurements of the same taxa at different elevations reveal that hovering stroke amplitudes can increase by as much as 24° over a 1,000-m gain in elevation (Buermann et al., 2011). During experimental reduction of air density,
hovering Ruby-throated hummingbirds *Archilochus colubris* increase stroke amplitude from ~155° to a geometric limit near 180° (Chai and Dudley, 1995). Hummingbirds challenged to hover when loaded with weights increase their stroke amplitudes up to the ~180° limit from hovering values that are between 22° and 40° lower depending on the species (Altshuler and Dudley, 2003; Chai and Millard, 1997; Chai et al., 1997). Although it is reasonable to assume that hummingbirds will have bilateral vortex loops when using stroke amplitudes typical of hovering in normal air, this flow pattern could change as the wing stroke amplitude approaches 180°. The vortices shed from the two wings will approach each other as the stroke amplitude increases and may cancel each other out when the parallel reversal vortices collide. Under such circumstance, I hypothesized that the wake topology could consist of a root vortex ring, positioned within a wing tip vortex ring (Fig. 2.5d).

When hovering in the plume, the hummingbirds used lower stroke amplitudes but higher wingbeat frequencies on average. What could cause such an obvious change in wingbeat kinematics? I tested one hypothesis that the plume could be influencing the air density by comparing the recorded frequency of a Galton whistle (after Dudley 1995) in air and within the plume, as well as within larger clouds of dry ice, and within an airtight chamber. I did not detect any changes in air density greater than 1%. It is also possible that a higher concentration of CO₂ could influence wingbeat kinematics by compromising oxygen metabolism. However, the higher wingbeat frequencies within the plume must derive from an increase in the contractile frequencies of the oxygen-demanding power muscles, which does not support this hypothesis. Instead, reductions in oxygen availability cause decrease in wingbeat frequency without a consistent effect on wing stroke amplitude during hovering flight in hummingbirds (Altshuler and Dudley, 2003). Thus, the cause of the lower stroke amplitudes is presently unknown, but I
propose that the higher wingbeat frequency may be a behavioral adjustment to allow for a rapid escape from an environment with reduced visibility and that maintaining vertical position with an elevated wingbeat frequency will necessarily require lower stroke amplitude.
Figure 2.1. Flow visualization of a hovering hummingbird from the rear-left perspective, demonstrating sequential development of a vortex loop on the left side during a downstroke. The time interval between frames is 2 ms and sequence runs from top to bottom. The panels on the left are unmanipulated images. The panels on the right are the same images but with the bird outlined in white and key features of the flows indicated by color: blue (downstroke wing tip vortex), purple (reversal vortex), green (air jet). This image sequence comes from frames 59–65 (labeled in the lower right of each panel) of the video (2.1) of trial #5 from bird 6.
Figure 2.2. Visualization of the vortex wake from multiple perspectives. Unmanipulated images are presented in the left panels. The central panels contain the same images with the bird outlined in white and key features of the flows indicated by color: blue (downstroke wingtip vortex), red (upstroke wingtip vortex), purple (reversal vortex), green (air jet). Straight arrows indicate the hypothesized locations or structures that are no longer present. The numbers build from most recent (1) to oldest (highest number) shedding events. The accompanying video names, bird numbers, and trial are provided underneath the left panels. The bilateral vortex model has been placed in similar orientations for comparison in the right panels. The frontal perspective is presented after a supinating rotation (A, B) and then again at the beginning of downstroke (D, E). An off-axis rear perspective with a wide field of view is presented for a wing at the initiation of a downstroke (G–I). A lateral view is presented at mid-downstroke with the wingtip paths during the down- and up-strokes indicated in blue and red, respectively (G–L).
Figure 2.3. Image sequence of a hummingbird hovering in the plume with wingbeat frequency and wing stroke amplitude values that fall within the range of birds hovering in normal air. The development and progress of a jet including shed wing tip and either reversal or wing root vortices during the downstroke is visible on the left side of the animal. The vortices are indicated by blue arrows in frame 351. The amplitude of this specific downstroke was 146°.
Figure 2.4. The average instantaneous wing position ($\phi$) and elevation ($\theta$) angles of the wing tips and wing roots from free-flight measurements of four male Anna’s hummingbirds (Calypte anna) during hovering flight. The downstroke phase is indicated in light blue and the upstroke is in light red. The mean stroke kinematic pattern is presented twice in succession for viewing the stroke transitions.
Figure 2.5. Schematic representations of the simplified vortex topologies. For all four topologies, the downstrokes are indicated in blue and upstrokes are indicated in red. The merged vortex ring model (A) is similar to the structures shed from a helicopter. The indented, merged vortex loop model (B) can account for wingtip and reversal vortices (purple), but has a junction at the proximal region of the latter in place of root vortices. The bilateral vortex loop model (C) accounts for wingtip, wing root, and reversal vortices. The concentric vortices model (D) may occur at wing stroke amplitudes approaching 180°.
Table 2.1. Wingbeat kinematics of hummingbirds used in the flow visualization and kinematic experiments. The sample sizes ($n$) refer to the number of wingbeats analyzed to determine the wing stroke amplitude ($\Phi$, in degrees), wingbeat frequency ($f$, in hertz), and downstroke ratio ($\tau$, in %). For the flow visualization measurements, the sample size of wingbeats analyzed was lower for the stroke amplitude than for the other variables. The experiment average by type of measurement is indicated by average with standard errors in parentheses. Rows with bird numbers contain individual means with standard errors in parentheses.

| Flow Visualization | |
|-------------------|-----------------|-----------------|-----------------|-----------------|
| bird #            | $n$             | $\Phi$ (°)      | $n_f$           | $f$ (Hz)        | $\tau$ (%)     |
| 3                 | 10              | 116.32 (1.03)   | 33              | 41.56 (0.37)    | 44.98 (0.63)   |
| 4                 | 10              | 154.08 (0.98)   | 26              | 38.51 (0.29)    | 46.45 (0.37)   |
| 5                 | 10              | 121.55 (0.86)   | 21              | 44.73 (0.33)    | 45.92 (0.41)   |
| 6                 | 10              | 127.05 (1.09)   | 21              | 39.55 (0.39)    | 50.37 (0.64)   |
| average           |                 | 41.09 (1.37)    |                 | 46.93 (1.18)    | 129.75 (8.40)  |

| Kinematics | |
|------------|-----------------|-----------------|-----------------|
| bird #     | $n$             | $\Phi$ (°)      | $f$ (Hz)        | $\tau$ (%)     |
| 7          | 15              | 148.94 (1.17)   | 39.08 (0.20)    | 50.00 (0.34)   |
| 8          | 11              | 164.38 (0.91)   | 38.62 (0.31)    | 46.29 (0.43)   |
| 9          | 14              | 149.36 (0.96)   | 38.05 (0.18)    | 49.72 (0.48)   |
| 10         | 14              | 151.65 (0.54)   | 38.27 (0.26)    | 47.26 (0.33)   |
| average    |                 | 153.58 (3.65)   | 38.51 (0.22)    | 48.32 (0.91)   |
3. Banking right to fly left: controlled lateral flight of hummingbirds

3.1 Introduction

Hummingbirds and many insects are capable of sustained hovering flight, as well as a suite of related steady-state maneuvers such as yaw turns (Altshuler et al., 2012), vertical ascents (Vance et al., 2014), backwards flight (Sapir and Dudley, 2012), and lateral flight. These maneuvers can be incorporated in complex behaviors such as feeding and sexual displays. For example, hummingbirds are capable of docking at flowers and artificial feeders moving back and forth in the wind (personal observation), and both lateral flights and yaw turns have been described during displays to females (Felton et al., 2008; Hurly et al., 2001). Lateral flight has also been observed for brief periods in freely flying fruit flies (Ristroph et al., 2009; van Breugel and Dickinson, 2014), hoverflies (Collett and Land, 1975), blowflies (Nachtigall, 1979), and houseflies (Wagner, 1986a), but the wing kinematics and aerodynamic mechanisms that enable the maneuver are not fully understood. Lateral flight is of particular biomechanical interest because it is a translational maneuver that in principle only requires a force asymmetry between the left and right wings for initiation and steady-state control (Zhang and Sun, 2011).

Previous studies that documented lateral flight raised the question of what is the kinematic source for force in the lateral direction. Two hypothesis have been proposed, which are aerodynamically similar but kinematically distinct. The key assumption underlying both

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2 A subset of the data used for this chapter came from videos collected by D.L. Altshuler with the intent of answering a different set of questions. I developed the central question, re-purposed the videos, and developed the computational framework that made the kinematic analysis possible. D.L. Altshuler advised during the analysis and the writing. The statistical analysis was developed by K.M. Middleton in a previous study.
hypotheses is that flapping wings function like the actuator disc of a helicopter, meaning that the time-averaged output force is normal to the stroke plane (Weis-Fogh, 1972). The first hypothesis is that the actuator disc (stroke plane) is essentially fixed to the body, and it is necessary to reorient the body to direct force into the direction of travel (force vectoring). This hypothesis is derived from studies with insects including tethered fruit flies (Götz, 1968; Vogel, 1966), and free-flying fruit flies (David, 1978) and house flies (Wagner, 1986b). A second hypothesis is that the actuator disc, and therefore the output force, can be reoriented off the body axis. Thus, the animal tilts its stroke plane in the direction of motion while maintaining a constant body posture with respect to the ground. This hypothesis was proposed as a possible explanation for the lateral flights observed in blowflies (Blondeau, 1981; Nachtigall, 1979). Wagner (1986b) argued against this second hypothesis and proposed that force vectoring can explain the strong lateral movements observed in hoverflies (Collett and Land, 1975) and blowflies (Nachtigall, 1979), assuming that the animals roll about their longitudinal body axis during the maneuver. These studies did not include detailed measurements of wingbeat kinematics due to limited availability of multi-camera, high-speed videography.

Recent studies with detailed wingbeat kinematics of rapid maneuvers performed by freely flying insects (Fry et al., 2003; Muijres et al., 2014) and birds (Hedrick et al., 2007; Ros et al., 2011) support the force vectoring hypothesis: time-averaged output force is orthogonal to the stroke plane, and the stroke plane is oriented by redirecting the body axis. Asymmetries in stroke amplitude, angle of attack and wing deviation were observed for at least some wingbeats during these brief turning maneuvers. However, one study with fruit flies indicates that asymmetries in wingbeat kinematics can lead to an orientation of aerodynamic forces that contradicts the assumption of the actuator disc (Ristroph et al., 2009). During two maneuvers, the tilt of the
stroke plane did not fully account for the magnitude of the lateral acceleration. The authors proposed that the lateral force was further supplemented by a drag-based mechanism caused by differences in the time of wing rotation (flip) between the left and right wings.

The original aim of the present study was to examine the neuromuscular and biomechanical control of sustained lateral flight as hummingbirds fed continuously from a translating feeder. Initial examination of the digitized data revealed a surprising result: hummingbirds performed controlled lateral flight by banking the stroke plane off the body axis, but opposite to the direction of travel. This indicates that 1) hummingbirds can control lateral flight using a mechanism other than force vectoring, and 2) that controlled lateral flight performed at a feeder involves forces not predicted by the tilt of the stroke plane. Here I present this result and focus my analysis on explaining these two unexpected results.

3.2 Methods

3.2.1 Animals

Six adult male Anna's hummingbirds (*Calypte anna*) were captured near the campus of the California Institute of Technology and two additional male Anna's hummingbirds were captured near the campus of the University of British Columbia using drop-door traps (Russell and Russell, 2001). The hummingbirds were housed in individual cages and fed *ad libitum* with a solution of artificial nectar (Nektar-Plus, Nekton, Pforzheim, Germany) and sugar water. Before the experiments, the birds were allowed to acclimate to the flight chamber. The procedures were approved by the Institutional Animal Care and Use Committee of the California Institute of Technology and the Animal Care Committee at the University of British Columbia.
3.2.2 Experimental setup and training

Experiments were performed in an acrylic cube (0.9 m³) with three clear sides and three white sides to provide backgrounds for filming (Fig. 3.1a). The birds were trained to feed from an artificial feeder made out of a 25 ml syringe mounted on a 45cm linear slide table powered by a stepper motor (X18 and MD2b, Arrick Robotics, Tyler TX). The feeder was oriented horizontally and 90° to the axis of table motion so that the hummingbird could perform pure lateral flight while feeding during feeder movement. The birds were trained to feed on command once every 20 minutes (Altshuler et al., 2012). Six birds were filmed during hovering and while moving laterally at 0.15 m/s in both the left and right directions.

The trials were recorded using three internally synchronized high-speed cameras (Photron APX, San Diego CA; fps: 1000 frames/sec, shutter: 1/6000sec). The cameras were placed dorsally, laterally and above the bird. Calibration was performed using direct linear transformation (DLT) with a 27-point calibration object in DLTdv5 software (Hedrick, 2008). Eight points were digitized on each hummingbird: right shoulder, left shoulder, right wing tip, left wing tip, right 5th primary, left 5th primary, top of the head, and the tip of the middle tail feather. The 2D points were transformed to 3D coordinates that were filtered with a zero-phase, fourth-order low-pass Butterworth filter. The filter cut-off frequencies were six times the wingbeat frequency for the shoulders and tail, eight times the wingbeat frequency for the wingtip and 5th primary, and twice the wingbeat frequency for the head. To compare wingbeat kinematics with electromyography data, the filtered kinematic data were upsampled from 1000 samples/sec to 10,000 samples/sec using a cubic spline (interpolate package, Scientific Tools for Python). I defined the pronation time for each wing as the time of minimum excursion in the stroke plane and the supination time as the time of the maximum excursion.
The flow visualization trials were performed with two birds using a different stepper motor and linear actuator (M-drive 23 Motion Control, Schneider Electric, Marlborough CT). The birds were filmed performing controlled lateral flight at 15cm/s and 30cm/s while flying through a plume of CO$_2$ created by dropping dry ice cubes into hot water. This method is described in detail in Pournazeri et al. (2012). The trials were filmed from the dorsal perspective using a single camera (M120, Vision Research, Wayne NJ, USA; fps: 1000 frames/sec, shutter: 1/1110 sec).

3.2.3 Frame of reference and kinematic variables

To compare the kinematics across wingbeats, I used two frames of reference defined by the position of the wings at the start and end of downstroke and upstroke: one gravitational and one stroke-centered. The frames of reference and most of the kinematic variables are described in detail elsewhere (Altshuler et al., 2012). From these two frames of reference I calculated 14 kinematic variables for each downstroke and upstroke. Seven variables were calculated in the gravitational frame of reference: average wingtip speed ($\bar{U}_{\text{tip}}$), lateral body angle ($\chi_{\text{GR,XZ}}$), frontal body angle ($\chi_{\text{GR,YZ}}$), instantaneous position angle ($\phi_{\text{GR}}$), instantaneous elevation angle ($\theta_{\text{GR}}$), average elevation angle ($\bar{\theta}_{\text{GR}}$), and the stroke plane angle ($\beta$). The stroke plane angle is calculated solely from the X and Z coordinates of the wingtips, and therefore only measures the angle relative to the horizontal plane from the lateral perspective. It does not describe other features of the stroke plane. In the stroke-centered frame of reference I calculated the wingstroke amplitude ($\Phi_{\text{SP}}$) and elevation amplitude ($\Theta_{\text{SP}}$).

I calculated several new measurements compared to Altshuler et al. (2012) to assess lateral flight performance. The wing bank angle ($WBA$) is defined as the difference in the average
elevation angle between the left and right wings, divided by 2. By convention, when the left wing is elevated and the right wing is depressed the \( WBA \) is positive, and when the right wing is elevated and the left wing is depressed the \( WBA \) is negative. The relative wing bank angle (\( RWBA \)) is a measure of the wing bank relative to the frontal body axis and is calculated as the absolute value of the sum of the \( WBA \) and \( \chi_{GR,YZ} \). When the wings are perpendicular to the body axis the \( RWBA \) is zero. The geometric angle of attack (\( \alpha \)) is calculated as the angle between the plane of the wing and the horizontal for a given wing elevation (Fig. 3.1b). By this convention, a geometric angle of 0° signifies that the wing is oriented parallel to horizontal, and at 90° the wing is oriented vertically. The plane of the wing was defined by three points: the shoulder, the wingtip, and the tip of the 5th primary feather. This measure of the hummingbird geometric angle of attack is calculated in the gravitational frame of reference and therefore differs from analogous measures in the body-centered frame of reference (Kruyt et al., 2014; Tobalske et al., 2007). The wing rotation velocity (\( \dot{\alpha} \)) is calculated as the derivative of the geometric angle of attack and the wingtip angular velocity (\( \omega \)) is calculated with the spherical law of cosines for the wing position and deviation angles. The travel angle (\( \Psi \)) represents how the bird is oriented with respect to the feeder (Fig. 3.1c,d) and is defined as the angle between the feeder, which defines the x-axis, and the wingtip path dividing line. The latter is defined as a line connecting the midpoints between the wingtips at the upstroke/downstroke transition and downstroke/upstroke transition (Altshuler et al., 2012). The travel angle is positive when the bird is facing the direction of motion, as shown in figure 3.1d, and negative when the bird is facing away from the direction of motion. The bill insertion (Bill %) is the percentage of the exposed culmen inside the artificial feeder, calculated using ImageJ software. The frame of reference transformations and the calculations of the kinematic variables were made using custom software written in Python.
I used a Quasi-steady state aerodynamic analysis based on the blade-element model developed by Weis-Fogh (1973) and employed by others (e.g., Fry et al., 2005; Kruyt et al., 2014). This approach integrates the instantaneous lift and drag forces occurring along chord-wise sections (blades) of the wing over the time course of the wingstroke. By definition, quasi-steady state aerodynamic models do not include rotational lift generating mechanisms that are known to act on reciprocating wings (Altshuler et al., 2005). The instantaneous, quasi-steady lift and drag acting on each flapping wing are calculated as:

\[
\vec{Lift} = \frac{1}{2} C_L \rho S R_2 \vec{V}_{\text{incident}}^2
\]

\[
\vec{Drag} = \frac{1}{2} C_D \rho S R_3 \vec{V}_{\text{incident}}^2
\]

where \( \rho \) is the air density (1.225 kg m\(^{-3}\)), \( S \) is the surface area of the wing (6.78E\(^{-4}\) m\(^2\)) and \( R_2 \) and \( R_3 \) are the second and third moments of the wing (.499 and .552 respectively). The wing and body mass measurements used in the model are species-averaged values for \( C.\ anna \) that were reported in a previous study (Kruyt et al., 2014). \( C_L \) and \( C_D \) are the coefficients of lift and drag, respectively, which were calculated using the aerodynamic angle of attack (\( \alpha_{\text{aero}} \), °), defined as the angle of attack of the wing relative to the incident velocity (\( V_{\text{incident}} \)):

\[
C_{L,\ down} = 0.0031 + 1.5842 \times \cos(0.0301 \alpha_{\text{aero}} + 4.7124)
\]
\[ C_{D, \text{down}} = 8.3171 + 8.1909 \times \cos(0.0073 \alpha_{\text{aero}} + 3.1416) \]

\[ C_{L, \text{up}} = 0.0028 + 1.1251 \times \cos(0.0332 (\alpha_{\text{aero}} - 180) + 4.6963) \]

\[ C_{D, \text{up}} = 1.1993 + 1.0938 \times \cos(0.0281 (\alpha_{\text{aero}} - 180) + 3.1277) \]

These equations were empirically derived for \( C. \text{anna} \) wings and presented in Kruyt et al. (2014), and differ here only in the sign convention of \( \alpha_{\text{aero}} \) with respect to the up- and down- strokes.

The incident velocity was calculated as:

\[ \vec{V}_{\text{incident}} = \vec{V}_{\text{tip}} + \vec{V}_{\text{induced}} + \vec{V}_{\text{body}} \]

where \( \vec{V}_{\text{tip}} \) is the velocity of the wingtip calculated from the motion of the wing, \( \vec{V}_{\text{body}} \) is the velocity of the bird (0 for hovering, 0.15 ms\(^{-1}\) for lateral flight), and \( \vec{V}_{\text{induced}} \) is the velocity of the air induced by the motion of the wings. The induced velocity was estimated using the Rankine-Froude model which assumes a flat stroke plane and a downward directed velocity. Pennycuick (1968) derives two equations for induced velocity, one for hovering and one for forward flight at speeds greater than 1 ms\(^{-1}\). Because the speed of the controlled lateral flight trials was much less than 1 ms\(^{-1}\) I use the hovering equation for all of my calculations:

\[ \vec{V}_{\text{induced}} = \sqrt{\frac{mg}{2 \rho A_{\text{disk}}}} \]
where \( m \) is the mass (0.0468 kg), \( g \) is the gravitational constant, and \( A_{\text{disk}} \) is the area swept out by the actuator disk, estimated by the equation:

\[
A_{\text{disk}} = \left( \frac{1}{180} \right) \Phi_{\text{sp}} \pi L^2
\]

where \( \Phi_{\text{sp}} \) is the calculated stroke amplitude, and \( L \) is the wing length (0.0525m). To calculate \( V_{\text{tip}} \) and \( \alpha_{\text{aero}} \) I used the average wingbeat kinematics, assuming a 39 Hz wingbeat frequency for hovering and a 41 Hz wingbeat frequency for lateral flight, as well as a downstroke:upstroke ratio of 48:52 for both hovering and lateral flight.

The lift acts in the direction of the vector obtained by taking the cross product of the leading edge of the wing and \( \vec{V}_{\text{incident}} \), in the positive vertical direction, whereas the drag acts in the direction of \( \vec{V}_{\text{incident}} \). The instantaneous forces were calculated for 200 equally spaced time points during the course of the wingbeat and then the average forces in the global vertical, forward, and lateral directions were calculated.

### 3.2.5 Electromyography

The procedures for making electromyographic recordings (EMGs) in hummingbirds are described in detail elsewhere (Altshuler et al., 2010b; Altshuler et al., 2012). Briefly, up to four pairs of double-bonded 99.99% silver wires separately insulated with heavy polyimide (HML, bifilar, California Fine Wire, Grover Beach, CA, USA) were inserted in the pectoralis major and pronator superficialis muscles. An attempt was made to insert one wire pair into each muscle on the left and right sides, but not all insertions were successful. During surgeries, birds were
anesthetized with isoflourane and wires were inserted by forming a hook with a hypodermic needle and then using the needle to insert the wire into the muscle. A forceps was used to hold the wire in place while removing the needle and then moving the wire slightly to secure it to a bundle of fibers. A fifth insertion was made for the ground electrode, which consisted of a single silver wire with HML insulation. All wires were attached first to the skin and then to the intervertebral fascia using suture. The signals from the EMG wires were amplified 1000X (model 1700, A-M Systems, Sequim, WA, USA) and digitally acquired (Digidata 1440, Molecular Devices, Sunnyvale, CA, USA) along with the camera trigger signal for synchronization with the wingbeat kinematics. The online filters were set wide open at 0.1 Hz (high-pass) and 10 kHz (low-pass), and subsequent filtering and analyses were conducted offline using custom scripts written in Matlab (Mathworks Inc., Natick, MA, USA). All EMG signals were processed using a fourth-order Butterworth filter at between 3 and 12 times the wingbeat frequency. A timing and an intensity measure for each activation burst were analyzed. Recordings from the *pectoralis major* had one burst per cycle whereas recordings from the *pronator superficialis* has two bursts per cycle.

### 3.2.6 Statistical analysis

To quantify the changes in kinematic and muscle activation features I used a mixed-model ANOVA with wing position (leading wing, hovering wing, trailing wing) as the fixed effect and bird as random effect. The kinematic and electromyographic measurements for the leading wings were averaged for both left and right lateral flights and likewise for the trailing wings. The hovering wingbeat kinematics are an average of the left and right wings. The downstroke and upstroke parameters were analyzed separately. For models with significant
Overall ANOVAs ($\alpha = 0.05$), a post hoc analysis was used to test for significant differences between leading wing and hovering wing, trailing wing and hovering wing, and leading wing and trailing wing using general linear hypothesis tests corrected for multiple comparisons (Hothorn et al., 2008). Similarly, for the body kinematics I used a mixed-model ANOVA with direction of travel (right lateral, hover, left lateral) as the fixed effect and bird as random effect, and when the results were significant ($\alpha = 0.05$) I performed the three post hoc comparisons to test for significant differences between right lateral flight and hovering, left lateral flight and hovering, and right and left lateral flight.

Analysis of the cross-correlations between kinematic and electromyographic measurements largely follow Altshuler et al. (2012). Briefly, I considered pairwise cross-correlations among kinematic measurements and between kinematic and electromyographic measurements each for a series of 16 consecutive wingbeats. I included lag values of -2, -1, 0, 1, and 2, corresponding to wingbeat lags of -1, -1/2, 0, 1/2, and 1. Significance of cross-correlations was determined analytically using 10,000 randomizations for each variable pair, because randomly permuted data can show significant apparent cross-correlation (Altshuler et al., 2012). To aid in visualization of cross-correlation analysis result, I colorized pivot-tables of P-values according to significance level.

Because I carried out many statistical tests on closely related data (80 hypothesis tests in Table 3.1 and Table 3.2, combined with 2,340 in the cross-correlation analysis), correction for multiple comparisons was necessary to prevent inflation of family-wise error rate overall (Curran-Everett, 2000). I performed a positive false discovery rate (pFDR) analysis, with the goal of controlling familywise error rate at 0.05 (i.e., no more than 5% “false positives”; Storey, 2002). The “smoother” option in the R (v. 3.1.0; R Foundation for Statistical Computing)
package qvalue (v. 1.38.0) was used to determine an adjusted α level for the statistical tests. The results of this analysis, which uses the empirical distribution of P-values to estimate the rate of true null hypotheses indicates that at $\alpha = 0.017$, family-wise error rate is controlled at 5%. Therefore, I use this modified alpha level for inferences (Table 3.1 and Table 3.2).

### 3.3 Results

#### 3.3.1 Wingbeat kinematics of controlled lateral flight

Six male Anna's hummingbirds ($Calype anna$) were filmed during both hovering and controlled lateral flight at 15 cm/s while feeding from a translating, artificial feeder. This speed was the maximum that could be steadily achieved by the stepper motor and linear slide. I later filmed two additional $C. anna$ males for flow visualization using a different motor and slide, which could achieve smooth motion at faster speeds. These two birds were able to easily feed from a feeder moving at 30 cm/s. Feeder movements began with a brief acceleration phase and ended with a brief deceleration phase, but only sequences with constant velocity were analyzed.

Ignoring deformations, the instantaneous wing motion of a hummingbird can be described using three angles in the gravitational frame of reference (Fig. 3.2). Wing position angles ($\phi_{GR}$) were sinusoidal and highly repeatable for both wings across all three treatments. Elevation angles ($\theta_{GR}$) followed a similar time course for the left and right wing during hovering but differed during lateral flight. The leading wing was elevated relative to the trailing wing during most of the wingbeat but was lower than the trailing wing during supination. This pattern was observed in every bird in almost all wingbeats and is readily apparent in plots of the average wingstrokes overlaid onto bird silhouettes (Fig. 3.3).
During lateral flights, the two wings also differed in the time course of the geometric angle of attack ($\alpha$) for most of the wingbeat (Fig. 3.2). On average, the change in the angle of attack (wing rotation) was delayed in the leading wing and advanced in the trailing wing during the transition from downstroke to upstroke (supination). The two wings were more synchronized during the transition from upstroke to downstroke (pronation). However, the time course of the angle of attack was more variable than the other wing angles within and between birds. The derivative of the angle of attack, the rotational velocity ($\dot{\alpha}$), also differed between the two wings during lateral flight (Fig. 3.4). The leading wing rotated slower during the last half of the downstroke and faster during the first half of the upstroke, relative to the trailing wing. The wingtip angular velocity ($\omega$) was similar between the leading and trailing wings. The two online supplementary videos of leftward lateral flights from the dorsal perspective demonstrate the differences in elevation angle and angle of attack between leading and trailing wings. Individual frames from video 3.1 of pronation, mid-downstroke, supination, and mid-upstroke from a single wingbeat are shown in figure 3.5.

To analyze the differences in the wingstroke patterns observed during hovering and controlled lateral flight, I defined stroke-specific kinematic parameters that are comparable to other studies of animal flight (Table 3.1). Generally, during lateral flight the stroke plane angle ($\beta$) of the leading wing increased, whereas the stroke plane angle of the trailing wing decreased relative to hovering. I was unable to statistically analyze the stroke plane angle using a linear mixed model because this variable had a bimodal distribution. During lateral flight the stroke amplitude ($\Phi_{SP}$) was lower than hovering flight, however there was no significant difference between leading and trailing wings. The average wingtip speed ($U_{tip}$) was faster during the downstroke compared to the upstroke, but there was no significant difference between the
leading and trailing wings during lateral flight. I present two measures to describe the deviation of the wing from the body and the stroke plane, the average elevation angle ($\bar{\theta}_{GR}$) and the elevation amplitude ($\Theta_{sp}$). During lateral flight the leading wing was elevated and had a low elevation amplitude, and the trailing wing was lowered and had a high elevation amplitude, compared to hovering. The geometric angle of attack was analyzed statistically at four key positions: the middle ($\alpha_{mid}$) and the end ($\alpha_{end}$) of the downstroke and the upstroke. The leading wing angle of attack was similar to hovering wings during the downstroke but was significantly shallower compared to hovering wings during the middle of the upstroke. The trailing wing had a steeper angle of attack during the downstroke, but was similar to hovering during the upstroke. The angles of attack at the end of the upstroke were similar for all flight modes.

Whole body kinematic measurements are presented in Table 3.2. The percentage of the bill inserted into the feeder (Bill %) did not differ significantly among treatments. The downstroke was significantly faster during lateral flight than hovering. Birds tended to face the direction of travel during lateral flight, but I did not analyze this with a mixed model because travel angle ($\Psi$) has no meaning during hovering flight. The body angle from the lateral perspective ($\chi_{GR,XZ}$) did not differ significantly across treatments. From the frontal perspective ($\chi_{GR,YZ}$), the hummingbirds' bodies were significantly tilted to the right during flight to left but were close to vertical (90°) during hovering and flight to the right. During controlled lateral flight the bird used a wing bank angle ($WBA$) that was tilted opposite to the direction of motion, and the bird did not maintain a perpendicular wing bank angle ($RWBA$) between wings and the dorsal body axis. These patterns held for both downstroke and upstroke.

The hummingbirds did not orient their stroke plane into the direction of travel during sustained lateral flight. To discard the possibility that these counterintuitive wingbeat patterns
were a result of the bird being dragged by the moving feeder, I measured the percentage of the bill inserted into the feeder. This number had a broad range: the maximum value was 54\% and the minimum value was only 2\% (Fig. 3.3a,d-e). Bill insertion percentage did not vary consistently with flight mode (Fig. 3.6b). Bill insertion percentage was unrelated to the wing bank angle (Fig. 3.6c), and individuals that maintained a low insertion also tilted their wings opposite to the direction of travel. Additionally, when the feeder was modified so that there was no possibility of tethering, the wings remained banked opposite to the direction of travel (Fig. 3.6f). Collectively, these results do not suggest that the bird is being pulled along by the feeder.

3.3.2 Quasi-steady State analysis

The observed kinematic patterns during lateral flight indicate that hummingbirds generate a net force that is not normal to the wing stroke plane. This observation is inconsistent with the actuator disc model proposed by Weis-Fogh (1972). Broadly speaking, there are two possible explanations for this pattern. The first is that a more complex, blade-element model, incorporating left-right differences in wing deviation and angle of attack, can lead to a net force that is not normal to the average stroke plane. This hypothesis can be evaluated using a quasi-steady aerodynamic analysis. An alternative hypothesis is that unsteady mechanisms are generating the force components to propel hummingbirds in the direction of travel.

The quasi-steady forces in the vertical, thrust, and lateral components are presented for the average wingbeat pattern during left lateral, hovering, and right lateral flight (Fig. 3.7a-c). These force components are expressed in a bird centered frame of reference. The quasi-steady analysis for hovering flight largely supports observations from other studies such as left-right symmetry for the vertical and thrust components, asymmetry in force output between the
downstroke and upstroke, and 66% weight support (0.66\(w_b\)) (Fry et al., 2003; Kruyt et al., 2014; Warrick et al., 2005). There was an unexpected asymmetry between backwards and forwards thrust for all three flight modes. During left lateral flight, the net thrust was 0.09\(w_b\) in the forward direction. Net thrust was 0.11\(w_b\) of body weight in the forward direction during hovering and right lateral flight. This result was probably due to wing twist. The proximal section of the wing had a very high angle of attack and the distal section had a much lower angle of attack, but the measure of \(\alpha\) was based on a flat plane that incorporated both sections. This led to a high estimate for drag and forward thrust during the upstroke.

The left and right wings produce opposite lateral forces, which essentially sum to zero over the course of the full hovering wingbeat (0.07\(w_b\) towards the left). During lateral flight, the net vertical and thrust components are similar to hovering flight, but the lateral components do not sum to zero. However, the net lateral forces are oriented opposite to the direction of travel. During lateral flight to the left, the average lateral force is 0.07\(w_b\) towards the right, and during lateral flight to the right, the average lateral force is 0.06\(w_b\) to the left. Because the birds had non-zero travel angles (21.9° during left lateral flights and 10.3° during right lateral flights), I also calculated the quasi-steady force in the direction of travel, which was 0.03\(w_b\) to the right during left lateral flight and 0.04\(w_b\) to the left during right lateral flight. It is important to note that if the forward thrust component during the upstroke is inflated (see above), then the quasi-steady forces opposite to the direction of travel will be even stronger. This analysis therefore indicates that although asymmetries in wingbeat kinematics are sufficient to generate lateral forces, the observed kinematic patterns are not consistent with a quasi-steady mechanism for controlling lateral flight in hummingbirds.
I next used flow visualization via sublimation of dry ice to examine the orientation of the jets caused by the wings. This visualization approach works best for the impulse jets caused by the flapping of the wings and is therefore another method to describe the feasibility of quasi-steady mechanisms. Controlled lateral flight through the smoke revealed the presence of two jets, one under each of the leading and trailing wings (Fig. 3.8). The primary flows generated during mid-strokes were oriented in the direction of travel and the resulting momentum impulse is opposite to the direction of travel. This result is in agreement with the quasi-steady analysis.

3.3.3 Motor activation

Muscle activation patterns derived from electromyogram recordings of pectoral and wing muscles were analyzed in terms of timing and intensity. As was the case for a previous study of yaw turns in Anna's hummingbirds (Altshuler et al., 2012), I found no statistically significant relationships between muscles activations and flight treatments. Because feeder tracking involves a sequence of wingbeats, I further analyzed the wing- and body-specific variables and EMG variables using cross-correlation analysis. Most of the kinematic variables showed significant associations with other kinematic variables, and most of these associations persisted over multiple time lags (+1 wingbeat, Fig. 3.9). However, several kinematic variables including frontal travel angle, bill percent, body angle, and stroke amplitude had few, if any, associations with other variables. There were no significant associations between EMG and kinematic variables.
3.4 Discussion

Hummingbirds performing controlled lateral flight while feeding from a translating feeder produced asymmetrical wingbeats with substantial differences in the time course of the instantaneous elevation angle and geometric angle of attack (Fig 3.2). Analyses on stroke-averaged kinematics revealed a number of significant differences in wing motion between the leading and trailing wings. The average elevation angle of the leading wing was higher, and of the trailing wing was lower, relative to the hovering wingbeat pattern (Fig. 3.3, 3.5). The leading wing also had a lower elevation amplitude and steeper stroke plane angle relative to the trailing wing (Table 3.1). Again, the hovering wingbeat had intermediate values. The overall result of this experiment is that the wings of Anna's hummingbirds are banked away from the direction of travel during sustained lateral flight, although the birds maintained a vertical body orientation as in hovering. Thus, the tilt of the stroke plane during steady lateral motion was not generated by a reorientation of the body frame, but rather a change in kinematics relative to the body. This pattern was unaffected by the extent of insertion of the bill in the feeder (Fig. 3.6). Collectively, these results suggest that controlled lateral flight performed at a feeder involves forces not predicted by the tilt of the stroke plane.

There are at least four possible sources for the lateral force in the direction of travel, including: 1) docking to the moving feeder, 2) bilateral asymmetries during the translational phases of the stroke, 3) bilateral differences in wing-wake interaction during stroke reversal, and 4) intermittent wingbeats banked towards the direction of travel. The analysis of feeder docking does not suggest that the feeder itself is the lateral force mechanism but it may be that slight contact between the bill and feeder, even as little as 2%, is indeed sufficient to pull the bird. If true, this represents rather remarkable behavior that could explain how hummingbirds are able to
precisely feed from flowers moving in the wind.

I did observe consistent differences in wing elevation, angle of attack and rotation velocity between the leading and trailing wings. Aerodynamic mechanisms operating during the translational phases of the stroke may be accurately approximated from a quasi-steady analysis, which indicates that the wing asymmetries do generate significant lateral force (Fig. 3.7). Indeed, the estimated lateral forces were oriented opposite to the direction of travel. Flow visualization showed that the induced flow through the vortex structures created by each of the wings is normal to the stroke plane and slightly inclined in the direction of lateral motion (Fig. 3.8). If this coarse flow structure is interpreted as representing the momentum imparted to the fluid during the translational phases of the stroke, then these results are consistent with the quasi-steady analysis.

Another possible explanation for the direction of lateral motion is the force provided by unsteady aerodynamic mechanisms that are not captured by either the quasi-steady model or the flow visualizations. Because the advance ratio is so low ($J = 0.014$ and $0.030$ for the kinematic analysis and smoke visualization, respectively), one potential source of force results from the interaction of the wing with the wake of the previous stroke (Altshuler et al., 2005; Birch, 2003; Dickinson et al., 1999). Although it has not been formally evaluated, the force due to wing-wake interactions may have large lateral components because the surface of each wing, which is a coarse predictor for the direction of any net pressure force, is oriented perpendicular to the direction of travel. I also observed a bilateral asymmetry in the progression of the angle of attack between the leading and trailing wings, which led to differences in the wing rotation velocity and in the timing of wing supination. These differences may cause unsteady aerodynamic effects that are sufficient to oppose and overcome the lateral forces generated by the banked wings to drive
the lateral flight in the direction observed. Although I cannot directly test this hypothesis with my data (or likely any data collected from live animals), this hypothesis could be evaluated using a dynamically-scaled robot or an accurate CFD model.

Finally, it is possible that intermittent wingbeats banked towards the direction of travel are sufficient to provide lateral force but do not have a strong influence on the average wingstroke pattern. If this is true, then precise lateral velocity is modulated with a sequence of alternating wingbeats used to accelerate and break, as opposed to a single, stereotyped wingstroke. This intriguing possibility could be tested with a timeseries analysis of long stretches of controlled lateral flight.

Another unexpected finding of this study is that a specific combination of kinematic features used by hummingbirds performing controlled lateral flight are also observed during asymmetric maneuvers described in other studies. The elevated wing has a lower elevation amplitude and delayed rotation during supination, relative to hovering. The lowered wing has a higher elevation amplitude and advanced rotation during supination, relative to hovering. The same combination was observed in the "sashay" and "dodge" lateral flight maneuvers of fruit flies (Ristroph et al., 2009), although they used a different approach for describing the kinematic patterns. However, to perform these maneuvers, flies elevated the trailing wing, in contrast to laterally flying hummingbirds which elevated the leading wing. A previous study examined the wingbeat kinematics that Anna’s hummingbirds employed to make yaw turns, which is another maneuver involving asymmetry between the left and right wings (Altshuler et al., 2012). In that study, the geometric angle of attack was not measured, but the association between wing elevation and elevation amplitude was observed during yaw turns where the outside wing was elevated. Given that the association among wing elevation, wing elevation amplitude, and wing
rotation timing has now been observed for two taxa during four asymmetrical maneuvers, an
obvious question for future research is whether this association is driven by morphological
constraints, timing constraints, or aerodynamic efficiency?

Controlled lateral flight is slow and steady, requiring precision in lateral acceleration and
balancing of all moments. In contrast, the free-flight maneuvers that have been studied in detail
for insects and birds (Fry et al., 2003; Hedrick et al., 2007; Iriarte-Díaz and Swartz, 2008;
Muijres et al., 2014) were fast and transient, and it may be more difficult to control accelerations
and balance moments when using force vectoring. How often is controlled lateral found in
nature? Hummingbirds have been known to use lateral flight to perform a variety of different
tasks including escape behaviors (Clark, 2011b), competitive interactions, aerial insect capture
(Yanega and Rubega, 2004), feeding from moving flowers or feeders, and a wide variety of
shuttle displays (Clark, 2011a; Clark et al., 2012; Hurly et al., 2001). The wing kinematics of
these behaviors have not been studied in-depth, but it is likely that many of them are
significantly different than those used for controlled lateral flight. Escape behaviors and
competitive interactions require high levels of agility but a lower level of precision compared to
feeder tracking. When the primary goal is to escape or hit an opponent, this can be accomplished
with a series of impulsive darts and dodges. Likewise, aerial insect capture requires high levels
of agility, but using an extreme bill gape helps reduce the levels of control and precision needed
(Yanega and Rubega, 2004). For all three of these behaviors it is expected that hummingbirds
would primarily use force vectoring kinematics. The precision to feed from flowers and feeders
moving in windy conditions may lead birds to use a strategy more similar to controlled lateral
flight, although at high speeds they may change to a form of force vectoring. Hummingbird
courtship displays are highly varied and species-specific and may be performed with either
method depending on the flight features that are being put on display. A display that showcases accelerations and changes in direction is likely performed by force vectoring whereas a display that involves stability, control, and precision may use mechanisms similar to those used in controlled lateral flight. The increased portability of high-speed cameras should allow for more extensive documentation of how lateral flight is performed in natural settings.
Figure 3.1. Methods used to study and quantify controlled lateral flight. (A) Hummingbird feeder tracking was studied in an acrylic flight chamber. The feeder was moved left and right using a stepper motor and linear slide. Three high-speed cameras were placed orthogonally and filmed the bird during hovering and lateral flight. Trailing EMG electrodes were connected to an extracellular amplifier just outside of the chamber. (B) The kinematic variables are presented in Altshuler et al. (2012), and one new variable, the geometric angle of attack $\alpha$, is introduced here. $\alpha$ is calculated relative to a plane defined by the shoulder to wingtip vector and the horizontal. (C) A top view of a bird moving laterally to the left. Six digitized anatomical features are presented in this panel: left wingtip (red), right wingtip (blue), left shoulder (green), right shoulder (orange), head (magenta), and tail (cyan). (D) The frame of reference was transformed by aligning every frame to the head, and every wing stroke to the midpoints between the stroke transitions. The travel angle $\Psi$ is the angle between the wingtip path dividing line and the orientation of the feeder, which is aligned with the x-axis. $\Psi$ is positive when the bird is facing the direction of motion and negative when it is facing away. The bill % is the percentage of the exposed culmen inserted into the feeder.
Representative Wingbeat Traces

Average Wingbeat Traces

θ GR (°)

ϕ GR (°)

α (°)

25 milliseconds 1 wingbeat cycle

left wing right wing

1 wingbeat cycle
Figure 3.2. Time course of wing angles illustrate left-right differences in wing deviation and angle of attack during controlled lateral flight. Representative traces from one individual in each of three treatments (right lateral flight, hovering, and left lateral flight) are presented on the left. The position angle $\phi$, deviation angle $\theta$, and geometric angle of attack $\alpha$ are presented for each treatment. The scale bar of 25 ms is valid for all representative traces. The average wingbeat traces, calculated across all individuals, are presented twice in succession on the left side of the figure. Lines represent average values and transparent bands indicate the standard errors of the mean calculated across birds. The timing for all average traces is normalized by down- and up-stroke, with each representing 50% of the wingbeat. Downstrokes are represented in gray and upstroke are represented in white. The left wing is indicated in red and the right wing is indicated in blue.
Figure 3.3. The average wingbeat kinematics from three perspectives indicate strong difference in wing elevation and kinematic angle of attack between controlled lateral flight and hovering. Left controlled lateral flight is given in the left column with rows 1-3 depicting the front, side, and top views, respectively. The fourth row depicts the kinematic angle of attack. These same perspectives for hovering and right controlled lateral flight are given in the middle and right columns, respectively. The right wingtip path is given in blue and the right shoulder is given in orange. The left wingtip paths is given in red and the left shoulder is given in green. The scale bar of 1 cm is valid for all panels. Black arrows represent direction of flight. Stroke direction is indicated by small blue and red arrows for columns 1-3. The downstroke is shaded in gray for row 4.
Figure 3.4. The leading wing rotates slower during the mid-downstroke and faster during the mid-upstroke relative to the trailing wing during controlled lateral flight. The wing rotation velocity $\dot{\alpha}$ is calculated as the derivative of the geometric angle of attack, and is presented in the top three traces for the three treatments. The wingtip angular velocity $\omega$ is calculated with the spherical law of cosines for the wing position and deviation angles, and is presented in the bottom three traces. The average traces across all birds are presented twice to allow for visualization of the transitions between strokes. Other features of the plots are the same as in figure 2.
Figure 3.5. A dorsal perspective on controlled lateral flight illustrates that the leading wing is elevated, rotates slower during the downstroke, and faster during the upstroke. A time lapse of images from the same wingbeat during controlled lateral flight to the left are spread out for clarity. The downstroke begins at 788 ms (white text) and the mid-downstroke occurs at 795 ms. The upstroke begins at 799 ms and the mid-upstroke occurs at 804 ms. The distance between each pair of images is equivalent to the distance the bird and feeder would move over 20 wingbeats. The feeder was moving at 15 cm/second. The entire movie from which this sequence is obtained is available in the online supplementary materials (Movie 1).
right lateral flight
hovering flight
left lateral flight

specific WBA DS (°)

bill insertion %

wingbeat #

bill insertion %

specific WBA DS (°)

wingbeat #

bill insertion %
Figure 3.6. The wing bank angle is unaffected by the extent to which the bill is inserted into the feeder. (A) The bill insertion percentages are plotted for all trials. Hovering trials are indicated in green, and controlled lateral flight to the left and right are indicated in red and blue, respectively. (B) The direction specific wing bank angle (WBA_{DS}) is plotted across all trials. By this convention a positive value signifies the leading wing is elevated and the trailing wing is depressed. In the hovering trials a positive value signifies the left wing is higher than the right wing. Hovering trial averages are calculated as left - right. The leading wing is consistently higher than the trailing wing across all trials with a moving feeder. (C) The wing bank angle was not related to bill insertion. The 95% confidence intervals for the slope included a slope of 0 in all cases. In this panels, each point represents the mean of the entire trial and the error bars represent standard error of the mean along both axes. Hovering trial averages are calculated as left-right and are not included in the model fit. (D) A frame shot from the camera filming from the top is presented for bird #1 moving to the left. For this wingbeat, the bird has a low travel angle (19°), 51% of its bill inserted in the feeder, and a difference between the average elevation angles of the leading and trailing wings of 8°. (E) An equivalent frame shot is presented for bird #6, also moving to the left. In this wingbeat, the bird had a high travel angle (44°), 2% of its bill inserted in the feeder, and a difference between the average elevation angles of the leading and trailing wings of 7°. (F) Frame shots from a video of a bird moving at 30 cm/second to the left while feeding from a feeder with a large window designed so that the bird cannot be dragged along. The images are taken at two mid-downstrokes (162 ms, 497 ms) and the subsequent mid-upstrokes (497 ms, 508 ms). In each of the four images the wings are banked opposite to the direction of travel.
Figure 3.7. The quasi-steady aerodynamic analysis reveals that wing translation generates net lateral forces opposite to the direction of travel during lateral flight. The instantaneous vertical (upper panels), forward (middle panels), and lateral (lower panels) forces were calculated using a blade-element analysis. The columns depict the forces based on the average wingbeat kinematics (Fig. 3) during left lateral, hovering, and right lateral flight. Instantaneous forces generated by the left (red) and right (blue) wings, and the net force (black) are expressed relative to body weight. The net force over the whole wingbeat and along each axes is depicted by the solid yellow line. The downstroke is depicted in gray and the upstroke is depicted in white.
Figure 3.8. Flow visualization during controlled lateral flight illustrates that each wing produces jets that are orthogonal to the stroke plane. Bird #7 was filmed from the dorsal perspective during controlled lateral flight to the left in the presence of a CO2 plume created by the sublimation of dry ice. The complete movie is available in the online supplementary materials (Movie 2). The frame at 89 ms provides a view of the jet generated by the left (leading) wing immediately after mid-downstroke. This frame is provided twice, once as an unmanipulated image (A) and again with the outline of the bird (white) and the jet (red) included (B). The frame at 288 ms from the same video provides a view of the jet generated by the right (trailing) wing immediately after mid-downstroke. Unmanipulated (C) and outlined (D) images are provided. The feeder was moving at 30 cm/second.
| \( \alpha_{\text{end}} \) | \( \alpha_{\text{mid}} \) | \( \theta_{\text{sp}} \) | \( \phi_{\text{sp}} \) | \( \theta_{GR} \) | \( \theta_{GR, YZ} \) | \( \chi \) | \( \Psi \) | \( T \) | \( WBA \) | \( \alpha_{\text{end}} \) | \( \alpha_{\text{mid}} \) | \( \theta_{\text{sp}} \) | \( \phi_{\text{sp}} \) | \( \theta_{GR} \) | \( \theta_{GR, YZ} \) | \( \chi \) | \( \Psi \) | \( T \) | \( WBA \) |
| 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.01 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.02 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.03 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.04 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.05 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.06 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.07 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.08 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.09 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.10 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.11 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.12 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.13 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.14 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.15 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.16 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| 0.17 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |

**Left wing**

- \( \alpha_{\text{end}} \)
- \( \alpha_{\text{mid}} \)
- \( \theta_{\text{sp}} \)
- \( \phi_{\text{sp}} \)
- \( \theta_{GR} \)
- \( \theta_{GR, YZ} \)
- \( \chi \)
- \( \Psi \)
- \( T \)
- \( WBA \)

**Right wing**

- \( \alpha_{\text{end}} \)
- \( \alpha_{\text{mid}} \)
- \( \theta_{\text{sp}} \)
- \( \phi_{\text{sp}} \)
- \( \theta_{GR} \)
- \( \theta_{GR, YZ} \)
- \( \chi \)
- \( \Psi \)
- \( T \)
- \( WBA \)
**Figure 3.9. A matrix of pairwise cross correlations among kinematic variables during controlled lateral flight and hovering.** Values shows are analytical P-values based on the median number of times that observed cross-correlations were significant relative to randomized data (see Altshuler et al., 2012 for additional details). The lags of 1 and -1 represent the preceding and subsequent whole wingbeats while the lags of -1/2 and 1/2 represent wingstrokes. P-values are shaded for ease of comparison. Cells shaded red are significant at $\alpha = 0.017$, the alpha level adjusted for a positive false discovery rate of 5% (see Methods). The blue shading represents the strength of $P$-values that were not significant. Cross-correlations among kinematic variables on the left side of the hummingbirds are shown in the left column and cross-correlations among kinematics on the right side are shown in the right columns. A full list of variables and their abbreviations is provided in Table 3.1 and Table 3.2.
Table 3.1. Mixed model ANOVA (mma) of wing kinematics: stroke plane angle (β), average elevation angle (θGR), stroke amplitude (ΦSP), elevation amplitude (ΘSP), average wingtip speed (Utip), and angle of attack at mid-stroke (αmid) and end-stroke (αend) for both downstroke (DS) and upstroke (US) by wing flight mode (leading wing, hovering wing, trailing wing). Bird was included as a random effect within the model. The degrees of freedom for each ANOVA are 2, 10. For models with significant ANOVAs three post-hoc comparisons were made: leading vs hovering (L-H), trailing vs hovering (T-H), and leading vs trailing wings (L-T). The means are presented along with the P values for the ANOVA and the three post-hoc comparisons. The P values for βDS and βUS are not presented because the distribution of these variables is bimodal due to the frame of reference chosen and thus violate the assumptions of the ANOVA.

<table>
<thead>
<tr>
<th></th>
<th>Leading</th>
<th>Hovering</th>
<th>Trailing</th>
<th>mma</th>
<th>L-H</th>
<th>T-H</th>
<th>L-T</th>
</tr>
</thead>
<tbody>
<tr>
<td>βDS (º)</td>
<td>14.2</td>
<td>10.0</td>
<td>5.6</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>βUS (º)</td>
<td>12.5</td>
<td>7.2</td>
<td>-0.5</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>θGR,DS (º)</td>
<td>13.0</td>
<td>9.1</td>
<td>5.2</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>θGR,US (º)</td>
<td>9.5</td>
<td>5.6</td>
<td>1.2</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ΦSP,DS (º)</td>
<td>146.2</td>
<td>150.9</td>
<td>136.3</td>
<td>0.020</td>
<td>0.519</td>
<td>0.002</td>
<td>0.057</td>
</tr>
<tr>
<td>ΦSP,US (º)</td>
<td>146.7</td>
<td>151.2</td>
<td>135.7</td>
<td>0.012</td>
<td>0.540</td>
<td>&lt;0.001</td>
<td>0.026</td>
</tr>
<tr>
<td>ΘSP,DS (º)</td>
<td>6.2</td>
<td>8.8</td>
<td>14.6</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ΘSP,US (º)</td>
<td>9.8</td>
<td>15.8</td>
<td>22.7</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Utip,DS (m/s)</td>
<td>11.0</td>
<td>11.2</td>
<td>10.8</td>
<td>0.137</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Utip,US (m/s)</td>
<td>10.2</td>
<td>10.4</td>
<td>9.9</td>
<td>0.007</td>
<td>0.177</td>
<td>&lt;0.001</td>
<td>0.050</td>
</tr>
<tr>
<td>αDS,mid (º)</td>
<td>30.3</td>
<td>34.1</td>
<td>39.1</td>
<td>0.003</td>
<td>0.103</td>
<td>0.018</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>αDS,end (º)</td>
<td>74.7</td>
<td>80.8</td>
<td>93.4</td>
<td>0.017</td>
<td>0.500</td>
<td>0.049</td>
<td>0.001</td>
</tr>
<tr>
<td>αUS,mid (º)</td>
<td>116.7</td>
<td>127.1</td>
<td>130.9</td>
<td>0.002</td>
<td>&lt;0.001</td>
<td>0.369</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>αUS,end (º)</td>
<td>74.5</td>
<td>81.4</td>
<td>80.1</td>
<td>0.327</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3.2. Mixed model ANOVA (mma) of whole body kinematics: bill percentage inserted in feeder (Bill %), wingstroke period (T), travel angle (Ψ), lateral body angle (χ_{GR,XZ}), frontal body angle (χ_{GR,YZ}), wing bank angle (WBA), and relative wing bank angle (RWBA) by maneuver (left lateral flight, hovering, right lateral flight). Bird was included as a random effect within the model. The degrees of freedom for the Bill % ANOVA is 2, 5, and for the other parameters is 2, 8. For models with significant ANOVAs three post-hoc comparisons were made: left vs hovering (L-H), right vs hovering (R-H), and left vs right (L-R). The means are presented along with the P values for the ANOVA and the three post-hoc comparisons. The P values for Ψ are not reported because travel angle is not a meaningful measurement during hovering.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Left</td>
<td>Hovering</td>
<td>Right</td>
<td>mma</td>
<td>L-H</td>
</tr>
<tr>
<td>Bill %</td>
<td>24.8</td>
<td>19.8</td>
<td>27.7</td>
<td>0.683</td>
<td>-</td>
</tr>
<tr>
<td>T_{DS} (ms)</td>
<td>11.8</td>
<td>12.3</td>
<td>11.8</td>
<td>0.029</td>
<td>0.005</td>
</tr>
<tr>
<td>T_{US} (ms)</td>
<td>12.8</td>
<td>13.3</td>
<td>12.7</td>
<td>0.049</td>
<td>0.029</td>
</tr>
<tr>
<td>Ψ (º)</td>
<td>21.9</td>
<td>-</td>
<td>-10.3</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>χ_{GR,XZ,DS} (º)</td>
<td>59.1</td>
<td>66.8</td>
<td>56.5</td>
<td>0.064</td>
<td>-</td>
</tr>
<tr>
<td>χ_{GR,XZ,US} (º)</td>
<td>57.6</td>
<td>65.4</td>
<td>54.5</td>
<td>0.047</td>
<td>0.052</td>
</tr>
<tr>
<td>χ_{GR,YZ,DS} (º)</td>
<td>3.5</td>
<td>0.2</td>
<td>-1.8</td>
<td>0.009</td>
<td>0.007</td>
</tr>
<tr>
<td>χ_{GR,YZ,US} (º)</td>
<td>2.6</td>
<td>0.2</td>
<td>-1.3</td>
<td>0.041</td>
<td>0.068</td>
</tr>
<tr>
<td>WBA_{DS}</td>
<td>4.0</td>
<td>0.0</td>
<td>-2.6</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>WBA_{US}</td>
<td>7.9</td>
<td>-0.6</td>
<td>-7.4</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RWBA_{DS}</td>
<td>7.5</td>
<td>0.1</td>
<td>4.4</td>
<td>0.001</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RWBA_{US}</td>
<td>10.5</td>
<td>-0.4</td>
<td>8.7</td>
<td>&lt;0.001</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>
4. Burst muscle performance predicts the speed, acceleration, and turning performance of hummingbirds

4.1 Introduction

The ability of an animal to maneuver can determine its success at avoiding predators, obtaining food, and performing other fundamental behaviors that define the margin between life and death (Hedenstrom and Rosen, 2001; Walker et al., 2005; Webb, 1976). Most biomechanical research on birds has focused on either brief (e.g. take off) or steady-state movements (e.g. forward flight) that can be studied most readily in the laboratory. Maneuverability is therefore one of the most important but least understood aspects of animal locomotion. Warrick and coworkers (Warrick and Dial, 1998; Warrick et al., 1998) proposed that there are both intrinsic and facultative influences on maneuvering performance. For animals that perform powered flight, intrinsic maneuverability is defined by the physical limitations imposed by wing size and shape (e.g., Norberg and Rayner, 1987), but muscle power reserves should allow them to facultatively overcome the costs of suboptimal morphology, achieving higher levels of performance by sacrificing efficiency. Although compelling, this hypothesis has never been tested explicitly.

Wing morphology is defined using measures of size (e.g. area or length) and non-dimensional measures of shape (e.g., aspect ratio). Wing area and aspect ratio have significant and well known effects on the aerodynamics of flight in animals (Pennycuick, 1975), and should affect maneuvering performance. Wing morphology influences flight efficiency
(Feinsinger and Chaplin, 1975), and is correlated with ecological roles (Feinsinger, 1976; Feinsinger and Colwell, 1978; Feinsinger et al., 1979; Warrick, 1998) and competitive ability (Altshuler, 2006; Feinsinger and Chaplin, 1975; Feinsinger and Colwell, 1978; Feinsinger et al., 1979). Because these previous studies concerned species and gender comparisons, less is known about how individual variation in wing morphology influences performance, especially with respect to maneuverability. One complication is that different wing sizes and shapes can be favored depending on the specific maneuver performed, e.g., yaw versus banked turns. Given the diversity of flight behaviors, it is unclear if the requirements for maneuvering exert strong selection on wing morphology.

Muscle power reserves in flying animals are defined as the difference between the minimum aerodynamic power required to fly and the maximum aerodynamic power generated during burst performance (Chai and Dudley, 1999). Power reserves indicate how much additional force is available for other flight behaviors, such as accelerating while maintaining or increasing altitude, or directing force laterally to perform a level turn. Power reserves could also be used to compensate for suboptimal wing positions and angles used during body rotations or when wing movements are anatomically or spatially constrained (Warrick, 1998). Altshuler and coworkers (Altshuler, 2006; Altshuler et al., 2004a) demonstrated that variations in ecological role across species are more strongly associated with burst muscle capacity than morphological parameters such as wing loading. Burst muscle capacity is also associated with species- and gender-specific competitive ability at different elevations. Altshuler (2006) suggested that the relationship between burst muscle capacity and competitive ability may be mediated through maneuvering performance.

Voluntary maneuvers are difficult to constrain experimentally and are not expected to be
as stereotyped as simpler flight behaviors, as demonstrated by studies on escape reactions (Clark, 2011b; Muijres et al., 2014) and obstacle avoidance (Iriarte-Díaz and Swartz, 2008; Williams and Biewener, 2015). Even when maneuvering can be constrained within a laboratory setup, such as when hummingbirds track a revolving feeder, there is considerable variation within and among individuals in wingbeat kinematics (Altshuler et al., 2012). Unconstrained and voluntary maneuvers have been recorded from hoverflies (Geurten et al., 2010), blowflies (Braun et al., 2010), and cliff swallows (Shelton et al., 2014), but without the ability to track individuals with sufficiently high throughput to address animal-to-animal variability. A major goal of this study was to obtain a large data set on voluntary maneuvering performance to determine if flight maneuvers were repeatable within individuals. It was hypothesized that intrinsic performance should be repeatable between measurements of the same individual. Facultative performance could be repeatable, but would also depend on motivation. If measurements of maneuvering performance were not repeatable, then the intrinsic contributions due to fixed morphological or anatomical traits would be negligible.

I studied the free-flight maneuvering performance of Anna's hummingbirds (Calypte anna) in a large flight cage both alone and in the presence of an interspecific competitor. I used a high-throughput computational approach to obtain a large number of flight sequences. These trajectories were parsed into a set of performance metrics based on body position and orientation. I also measured individual morphology and maximum load-lifting performance. This analysis addressed three questions: 1) Is maneuvering performance repeatable across trials?, 2) What are the relative contributions of morphology, and burst muscle capacity to maneuvering performance?, and 3) Does motivation state induced by the presence of a competitor alter maneuvering performance?
4.2 Methods

4.2.1 Animals and experimental trials

I captured and filmed 20 adult male Anna's hummingbirds (*Calypte anna*) at the University of California, Riverside (n = 8 birds in July-October 2009; n = 4 birds in January-March 2010) and the University of British Columbia (n = 8 birds in December 2013-April 2014). The hummingbirds were housed in individual cages and fed *ad libitum* with a solution of artificial nectar (Nektar-Plus, Nekton, Pforzheim, Germany) and sucrose. The flight arenas were large rectangular cages (3 x 1.5 x 1.5 m) built with an aluminum frame and had either garden mesh (California) or clear acrylic (British Columbia) side panels. The cages contained multiple perches and a single feeder hung from the roof of the cage.

Before the first trial, each bird was allowed to acclimate to the flight arena and learn where the perches and the feeder were located. The trials began once the birds were actively exploring the cage and consistently visiting the feeder and multiple perches. At this point, I recorded a two-hour solo trial for each bird. Following solo flight trials (between 0-23 days later), birds were paired and filmed for another two hours in competition trials. One bird in each pair was marked with a small square of retro-reflective tape placed between the shoulder blades for identification. The birds filmed in British Columbia had one competition trial and the birds in California had two competition trials. In the latter case, the second trial consisted of previously unknown opponents that were chosen randomly from the remaining pool. The competition trials involved chases, displacements, and aerial displays but very little contact. Regardless, I monitored the competition trials to ensure that no birds were harmed or excluded from the feeder.

Following each round of solo and competition trials, I performed asymptotic load-lifting
experiments using the techniques described in Chai et al. (1997), and subsequently used in other studies estimating maximum burst power output (Altshuler, 2006; Altshuler et al., 2004b; Altshuler et al., 2010b; Chai and Millard, 1997). Here, I use the mass of maximum number of beads lifted by each individual as a measure of burst muscle capacity. Immediately following load-lifting, I weighed the birds and photographed both wings in an outstretched position against white paper with a reference scale (Chai and Dudley, 1995). Measurements of wing length, wing area, aspect ratio, and the non-dimensional moments of wing area (Ellington, 1984c) were calculated using custom analysis software in MATLAB (The MathWorks, Natick, MA, USA).

All procedures were conducted under approval of the Institutional Animal Care and Use Committee at the University of California, Riverside and the Animal Care Committee at the University of British Columbia.

### 4.2.2 Tracking system

A customized automated tracking system was used to measure both body position and orientation of flying birds in three dimensions. A general explanation of the tracking algorithm and hardware components is described in Straw et al. (2011). Body orientation was estimated using the algorithm described in Appendix B, and this required fitting orientations to the body axis in each 2D image. For this, I implemented software to align cropped images containing just the bird such that the center of intensity mass remained stationary in the sequence of cropped images. A series of 5 images was stacked and a maximum intensity projection was used to segment the time-varying flapping wings from constantly dark body pixels. From such maximum intensity projections, the 2D orientation of the body axis was estimated when the eccentricity of an ellipse fit to the binarized body pixels exceeded a threshold. I adapted this system for
recording hummingbird solo and competitive flight trajectories with four or five digital cameras (GE680, Allied Vision Technologies, Burnaby, Canada). The cameras were mounted on the ceiling and recorded at 640 x 480 pixels resolution at 200 frames per second (figure 4.1a). The filming volume was calibrated by moving a single light-emitting diode throughout the arena, and using an automated self calibration algorithm (Svoboda et al., 2005). The scale, rotation, and translation of the calibration was matched with a 3D model of the flight arena reconstructed from the 2D images using Direct Linear Transformation (Abdel-Azziz and Karara, 1971).

To minimize the effect of errors in the 3D tracking, I used a forward/reverse non-causal Kalman filter (Rauch–Tung–Striebel smoother). One set of smoothing parameters was used for estimating translational position of birds, and was chosen so that 7 traces of a tracked, falling object averaged a peak acceleration of 9.8 m/s$^2$. A second set of smoothing parameters was used for orientation estimation, and was chosen by comparing re-projections on the original video. Examples of maneuvers with different smoothing parameters, including the chosen values, are given in figure 4.1c and 4.1d. Other details of the specific approach for tracking multiple hummingbirds are provided in appendix B.

4.2.3 Maneuvering performance metrics

The first stage of analysis was estimating instantaneous velocities, accelerations, and headings from the raw tracking data. Translational velocity and acceleration were calculated from body position data and separated into vertical and horizontal components. Two rotational velocities, azimuthal and pitch, were calculated from the body orientation vector, which was represented in spherical coordinates as azimuth and pitch angles. Heading was calculated as the instantaneous direction of the horizontal translation velocity, and the heading velocity was
calculated as the derivative of heading.

The second stage of analysis used the velocity, acceleration, and orientation data to search for a series of ten stereotyped maneuvers that were independent of time and distance scales (figure 4.1b). These include 1) total acceleration, 2) horizontal acceleration, 3) horizontal deceleration, 4) vertical upward acceleration, 5) vertical downward acceleration, 6) pitch-up rotation, 7) pitch-down rotation, 8) yaw turn, 9) arcing turn, and 10) pitch-roll turn. These ten maneuvers are not meant to be mutually exclusive, exhaustive, or to divide the entire filming session into a set of discrete behaviors, but instead are intended to extract simple measurements that can be used as an assay for maneuvering performance. Because I assumed that a new maneuver must involve a change in velocity, the first search parameter was to find sequences bounded by velocity maxima and minima, or vice versa. I next describe the additional search parameters and the performance metrics used to quantify each maneuver.

The five translational maneuvers were defined using translational velocity maxima and minimal, and only sequences with at least 25 cm of travel were analyzed. The total acceleration maneuver \((AccTot)\) was bounded by total velocity minima and maxima, and had one performance metric, the maximum translational velocity \((AccTot_{vel, max})\). The horizontal acceleration maneuver \((AccHor)\) was bounded by horizontal velocity minima and maxima, and was constrained to no more than 10 cm of vertical distance traveled. The performance metric for each maneuver was the maximum horizontal acceleration \((AccHor_{max})\). The horizontal deceleration maneuver \((DecHor)\) and its performance metric, maximum horizontal deceleration \((DecHor_{max})\), were similar except bounded by horizontal velocity maxima and minima. The vertical upward acceleration \((AccVU)\) and vertical downward acceleration \((AccVD)\) maneuvers were bounded by vertical velocity minima and maxima. The performance metrics were maximum upward
(Acc\textsubscript{VU}\textsuperscript{max}) and maximum downward (Acc\textsubscript{VD}\textsuperscript{max}) accelerations. All translational accelerations and decelerations are expressed as positive values, so that higher values represent a higher level of performance.

The three rotational maneuvers were pitch-up (Pitch\textsubscript{U}), pitch-down (Pitch\textsubscript{D}), and yaw turns (Yaw). These sequences were bounded by the roots of the azimuthal and pitch velocities. In contrast to translational maneuvers, which were defined by the maxima and the minima of the velocities, the rotational maneuvers begin and end with changes in rotational velocity direction. Thus, the performance metrics are average rotational velocities over the whole maneuver instead of maximum accelerations or decelerations. An additional constraint common to all three rotational maneuvers is that the linear distance traveled was less than 10 cm.

The pitch-up and pitch-down maneuvers were defined as having continuous pitch velocity in the upward or downward direction, respectively. Only maneuvers with a total pitch rotation greater than 45° were analyzed. From these maneuvers I calculated either the average pitch-up (Pitch\textsubscript{U}\textsubscript{vel, avg}) or pitch-down (Pitch\textsubscript{D}\textsubscript{vel, avg}) velocity as the performance metrics. Defining yaw turns is challenging because unlike most birds and insects, hummingbirds fly with an upright body posture. When the body posture is near vertical, azimuthal rotation is implemented by rolling the body axis, but when the body posture is near horizontal, azimuthal rotation is implemented by yawing the body axis. I therefore define yaw turns as azimuthal changes in direction when the body pitch angle is below 75°. An additional constraint specific to yaw turns was a requirement for at least 90° change in azimuth. From these trajectories I measured the average yaw velocity (Yaw\textsubscript{vel, avg}) as the performance metric.

In addition to five translational and three rotational maneuvers I also considered two maneuvers that are turns with translational components. Arcing turn maneuvers (Arc) were
defined as sequences with a heading velocity > 90°/sec, a minimum total translational velocity > 0.5 m/s, a total distance traveled > 25 cm, and a vertical distance traveled < 10 cm. These search parameters reliably extract arcing turns that occur in the horizontal plane. To compare arcing turns of different shapes and scales I clipped the trajectories to a length of 25 cm centered at the sharpest point of the turn. From the clipped trajectory I analyzed three performance metrics, average velocity \((Arc_{\text{vel, avg}})\), radius \((Arc_{\text{rad}})\) and the maximum centripetal acceleration \((Arc_{\text{cent, max}})\).

The latter two were calculated using the following equations:

\[
Arc_{\text{rad}} = \frac{Arc_{\text{distance traveled}}}{\Delta \text{Heading}_{\text{radians}}}
\]

\[
Arc_{\text{cent, max}} = \frac{Arc_{\text{vel, avg}}^2}{Arc_{\text{rad}}}
\]

Pitch-roll turn maneuvers (PRT) have been described in hummingbirds and are characterized by the following sequence: a) deceleration, b) increase in pitch to near vertical, c) azimuthal rotation by rolling the body, and d) acceleration in a new direction (Clark, 2011). These maneuvers were identified by searching for sequences of deceleration followed by acceleration with a maximum pitch > 75°. These sequences were clipped to a linear distance of 25 cm centered on the point of the lowest translational velocity. Only clipped sequences in which the total vertical displacement was less than 10 cm were analyzed. The performance metrics for pitch-roll turns were the time taken \((PRT_{\text{time}})\) and the degrees turned \((PRT_{\text{deg}})\).

Arcing turns and pitch-roll turns are two different mechanisms for generating a change in heading, with no overlap in the data set. I analyzed how morphology, burst capacity, and
competitor presence influenced the relative use of these two turns. The pitch-roll percent (PRT%) is defined as the number of pitch-roll turns divided by the total number of arcing and pitch-roll turns extracted from each trial. The tracking system and data processing were performed using custom programs written in Python (Python Software Foundation, 2012).

4.2.4 Statistical analysis

The automated digitization produced a small number of extreme tracking errors, which I did not want to unduly influence the statistical analysis. I accordingly removed values >5 SDs more extreme than the mean for each performance metric. The trimmed values comprised only 0-0.31% of the original pooled sample size for each metric. I next calculated the mean of each performance metric for each bird-trial combination (n= 52 means; 20 birds in 20 solo trials and 16 paired competition trials). All statistical analyses were performed on the bird-trial means using R 3.1.1 (R Development Core Team, 2014).

To quantify the repeatability of the performance metrics (question 1), I estimated how much of the variation is attributable to differences among individual birds (Nakagawa and Schielzeth, 2010). For each performance metric, I fit an intercept-only mixed effects model that included estimates of the intercept and the random effect of individual. Such a model has two variance components, one for the random effect of individual and a residual variance. I calculated repeatability as the individual variance divided by the total variance (individual plus residual). I used parametric bootstrapping with 5000 iterations to obtain confidence intervals for these repeatability estimates via the bootMer function in the lme4 (v1.1.7) package.

Because the second question involved evaluating several possible scenarios for the influence of morphology and burst performance on maneuverability, I used an
information-theoretic approach to multi-model inference (Burnham and Anderson, 2002). Unlike dichotomous null hypothesis testing, this approach quantifies support for multiple hypotheses, and it avoids the problem of eliminating potentially important predictors when two or more alternative models are equally well supported. The output for interpretation includes the effect size and relative importance of each predictor, and there are no null hypotheses or P values associated with this approach.

I considered eight candidate mixed-effects models that could plausibly explain variation in each maneuvering performance metric (table 4.1). All candidate models included bird identity as a random intercept and were fit using the nlme (v 3.1-117) package. The intercept-only model included an estimate of the intercept but no fixed effects. Other candidate models are listed in table 4.1; in addition to fixed effects of morphology and burst muscle capacity, these models include fixed effects of competitor presence, body mass, and experiment. Experiment had three levels, one for each round of trials (California 2009, 2010, British Columbia 2014) to account for differences such as location, time of year, and filming conditions.

Two issues arose in the preliminary examination of data. The first is a nuisance variable: six of the performance metrics were significantly influenced by the number of days a bird had been in captivity. I therefore included an additional fixed effect of the number of days since capture when analyzing these six metrics (see table 4.1). The second issue was that one of the metrics, the heading change in pitch-roll turns ($PRT_{deg}$), had significant outliers (Grubb’s test, all $G > 3.09$, all $p < 0.03$; figure 4.2). I verified that these outliers were correct and not the result of errors in the tracking system. However for proper statistical analysis, I omitted these outliers from the analysis of heading change in pitch-roll turns. This ensured that all fitted Gaussian models for all performance metrics met the assumptions of normality and homoscedasticity of
residuals. The best-fit model for heading change in pitch-roll was the same regardless of whether the outliers are included.

To quantify the variance explained by the fixed effects of interest in each model, I calculated the marginal $R^2_{\text{GLMM}(m)}$ using the r.squaredGLMM function in the MuMIn (v1.10.5) package (Nakagawa and Schielzeth, 2013). This measure does not have all the properties of a traditional coefficient of determination, but like $R^2$ it ranges from 0 to 1, and it is an appropriate estimate of the variance explained by the fixed effects in a mixed model. I removed the effect of experiment and the number of days post-capture when calculating $R^2_{\text{GLMM}(m)}$, because these were not effects of interest.

I evaluated the support for different models using the Akaike information criterion (AICc) adjusted for small sample sizes. This was calculated using the MuMIn (v 1.10.5) package with maximum likelihood estimation. I defined the group of supported models as those with a difference in AICc < 2 from the best-fit model for each performance metric. If no other models came within 2 AICc units of the best-fit model, I present effect size measures, their confidence intervals, and $R^2_{\text{GLMM}(m)}$ for only that model. Otherwise, I present averages of all supported models. Details of all candidate models are provided in the online supplement.

The third question concerned the influence of competitor presence on the performance metrics. If the confidence interval for the coefficient estimate of competitor presence excluded zero, I examined the magnitude and direction of that effect. Positive coefficient estimates indicate that performance was higher during competitive flights, whereas negative coefficients indicate that performance was lower during competitive trials.
4.3 Results

4.3.1 Repeatability of performance

A large sample of values was obtained for each performance metric. Sample sizes and descriptive statistics are provided in table 4.2. All performance metrics based on total and horizontal linear accelerations and complex turns were highly repeatable, with >80% of the variation in these metrics attributable to differences among individuals (figure 4.3). The rotational performance metrics and the percent of turns that were pitch-roll turns were moderately repeatable, with 40-70% of the variation in these metrics attributable to among-individual differences. The vertical accelerations $AccVU_{\text{max}}$ and $AccVD_{\text{max}}$ were not repeatable, as the 95% confidence intervals for repeatability of these metrics overlapped zero.

4.3.2 Performance in relation to burst muscle capacity

The best-supported models for each performance metric are given in table 4.3. Burst muscle capacity was an important predictor for most of the maneuvering performance metrics. Birds that lifted more weight (accounting for their body mass) tended to accelerate and decelerate faster, and they tended to perform maneuvers with higher velocity (figure 4.4). However, weight lifted was not an important determinant of vertical acceleration and deceleration, as candidate models including that predictor were not supported. Birds that lifted more weight also executed pitch-up and pitch-down maneuvers with higher rotational velocities. Burst capacity was not a strong determinant of yaw performance. Although yaw velocity was somewhat positively related to mass lifted (figure 4.4), candidate models of yaw velocity that included mass lifted as a predictor were not well-supported.
Burst capacity was also associated with some, but not all performance metrics related to complex turns. Birds that lifted more weight for their body mass tended to execute faster, larger radius arcing turns (figure 4.4). However, the centripetal acceleration of arcing turns was not associated with load-lifting. Hummingbirds with higher load-lifting capacity executed pitch-roll turns in less time. Burst capacity was not a strong determinant of heading change during pitch-roll turns. Lastly, birds with higher muscle capacity used pitch-roll turns for proportionately more of their heading changes.

4.3.3 Performance in relation to morphology

Wing morphology, specifically the aspect ratio, was an important predictor for three performance metrics: velocity, centripetal acceleration, and the percent of direction changes that were pitch-roll turns (figure 4.5). Hummingbirds with long, narrow wings tended to perform maneuvers with higher velocity, and arcing turns with higher centripetal accelerations, relative to birds with short, wide wings. Birds with higher aspect ratio wings also used proportionately more arcing turns than birds with low aspect ratio wings.

4.3.4 Effect of competitor on performance

I did not detect a substantial effect of competitor presence on many of the performance metrics (table 4.3). Two metrics, horizontal acceleration and deceleration were affected, but in the direction opposite to what I predicted. Specifically, birds exposed to competitors performed maneuvers with lower acceleration (−0.45 m/s² difference on average) and lower deceleration (−0.46 m/s²) relative to solo flight (figure 4.6a, b). One metric, pitch-down velocity, did increase during competitive trials (figure 4.6c) as predicted (0.06 rev/s difference on average). I had no
prediction for how competition would influence the relative use of pitch-roll and arcing turns, but found that birds used proportionately more arcing turns in the presence of competitors (figure 4.6d). During competitive trials, 32% of direction changes were arcing turns (and 68% pitch-roll) on average, whereas in solo flight, only 18% of direction changes were arcing turns (and 82% pitch-roll).

4.4 Discussion

The goal of this study was to determine the relative contributions of wing morphology and muscle capacity to maneuverability in flight. I found that hummingbirds maneuvered with highly repeatable performance while flying in a large enclosure (figure 4.3). Maximum weight lifted during load-lifting trials predicted most of the performance metrics that I measured, such that birds with higher burst capacity flew faster, had higher horizontal accelerations, faster rotations, and higher performance during complex turns (figure 4.4). Additionally, birds with higher aspect ratio wings performed higher velocity maneuvers and turns with higher centripetal acceleration (figure 4.5). When flying in the presence of a competitor, hummingbirds used faster pitch velocities, although they used slower horizontal accelerations and decelerations. During competition trials birds increased the proportion of arcing turns used (figure 4.6). Collectively, these results suggest that burst muscle capacity is the most important predictor of maneuverability, that wing morphology underlies some elements of maneuvering performance, and that body angular velocity and arcing turns are associated with the presence of competitors.

Almost all of the performance metrics were highly repeatable, which indicates a potential role for intrinsic influences of wing morphology in determining maneuverability. However, aspect ratio was the only morphological parameter that predicted performance, and only for a
limited set of maneuvers. Aspect ratio is a key determinant in wing efficiency for fixed wings, such as during gliding (Pennycuick, 1983), and it has recently been demonstrated that higher aspect ratio wings correspond to higher power factors in the revolving wings of hummingbirds (Kruyt et al., 2014). I found that aspect ratio had a strong effect on the few performance metrics that it predicted, but did not affect most features of maneuvering performance. This suggests a limited role for efficiency in many features of maneuvering.

The limited role for intrinsic maneuverability stands in contrast to the pronounced role for facultative maneuverability. Load-lifting is measured as a transient escape maneuver that is likely anaerobic and performed inefficiently. All hummingbirds reach maximum load-lifting performance at a geometric limit set by the amplitude of the wings: wing stroke amplitude cannot extend much past 180° without the two wings interfering with each other physically and aerodynamically (Chai and Dudley, 1995; Chai and Millard, 1997; Chai et al., 1997). Maximum load-lifting also elicits a substantial increase in wingbeat frequency but as a constant fraction of baseline wingbeat frequency (Altshuler and Dudley, 2003). Thus, maximum load-lifting performance involves brief increases in muscle strain and muscle velocity to physically imposed limits. Although the relationship between burst capacity and maneuvering performance metrics is evidence for the importance of facultative maneuverability, it is important to note that burst capacity is itself limited intrinsically by muscle anatomy and physiology (Altshuler et al., 2010b). The capacity to increase muscle strain and velocity has previously been shown to influence foraging behavior and competitive ability (Altshuler, 2006) and the results of the current study demonstrate that it also underlies multiple features of maneuvering performance.

The two performance metrics that were not repeatable are vertical accelerations and decelerations, which were expected to be important based on previous observations of
hummingbird competitive interactions (Altshuler, 2006) and mating displays (Clark, 2009). Moreover, vertical performance was not well predicted by morphology, burst capacity, or competitor presence. The dimensions of the experimental chamber likely influenced the observations of vertical performance. Hummingbirds in captivity tend to fly near the top of their cages, and the vertical dimension of the chamber (1.5 m) may have limited vertical movement.

Male hummingbirds are extremely aggressive towards conspecifics (Carpenter et al., 1983; Kodric-Brown and Brown, 1978) and other species of hummingbirds (Stiles and Wolf, 1970; Wolf et al., 1976). The most territorial species will vigorously defend territories (Carpenter et al., 1983) and lekking sites (Rico-guevara and Araya-Salas, 2015). In staged competition studies, paired hummingbirds will also establish and defend territories (Tiebout, 1993). I originally intended to use competition to elicit high levels of flight activity and maneuvering performance in territorial male Anna's hummingbirds (Stiles, 1982). However, I found that competitor presence affected only a small number of the maneuvering performance metrics that I measured. Pitch-down velocity increased with competition whereas horizontal acceleration and deceleration actually decreased. I do not know why these three metrics (in addition to PRT% -see below) were strongly affected by competition and why they were affected in the directions observed. However, there are several possible causes for why competitor presence did not affect the other metrics: 1) I was unable to elicit a high level of competition or territoriality, 2) the birds may have worked out dominance without the aggressive interactions normally seen outdoors, and 3) the interactions required to establish dominance may have been very brief such that they comprised only a minuscule sample of the maneuvers I analyzed (Maynard Smith, 1974). This experiment was not designed to study the effects of maneuvering performance on competitive success, although this represents an important topic for future investigation. Laboratory
performance tests do not always reflect field behavior (Irschick, 2003) and outdoor studies of maneuvering performance will be important for understanding the role of maneuverability in competitive interactions. Recent advances in video tracking have made three-dimensional studies of maneuvering in a natural setting possible (Shelton et al., 2014; Theriault et al., 2014).

The most substantial result of competitor presence was the increase in the use of arcing over pitch-roll turns. These two types of turns represent different strategies for changing direction that differ in duration and amount of heading change. Arcing turns require less time but are used for smaller heading changes, whereas pitch-roll turns are longer but can be used to change heading by 180° (figure 4.2). Given that hummingbird agonistic interactions can involve direct contact and stabbing with bills (Clark and Russell; Rico-guevara and Araya-Salas, 2015; Tiebout, 1993), slow turns in place could make a bird more vulnerable during competitive interactions.

The relative use of arcing and pitch-roll turns was the only metric in this study that was influenced by all of morphology, burst capacity, and competitor presence. The minimum radius of an arcing turn is limited by the maximum centripetal acceleration that a bird can generate while maintaining lift. The speed of a pitch-roll turn is limited by the ability to decelerate and then accelerate. Birds with higher wing aspect ratio may have preferred arcing turns because they were able to generate higher centripetal accelerations. Birds with higher burst power may have favored pitch-roll turns because they had higher accelerating and decelerating performance. These observations suggest the hypothesis that high aspect ratio and high burst capacity enhance maneuverability. This hypothesis could be evaluated by comparing hummingbird species that differ in wing shape, foraging strategy and burst capacity (Altshuler, 2006; Altshuler et al., 2004a; Altshuler et al., 2010a; Kruyt et al., 2014).
Figure 4.1. A multi-camera, automated tracking system extracted hummingbird body position and orientation from solo and competitive flights. The trajectory shown for one bird (A) is also presented in supplementary video 4.1. Stereotyped maneuvers were classified in each trajectory (B) and between one and five performance metrics were calculated from each maneuver. Maneuvers within a trajectory may be overlapping (e.g. #4,5,6). The trajectory presented in B is a 2D view of the trajectory shown in A. Body position and orientation were smoothed with an extended Kalman filter (C, D). The effects of four different sets of smoothing parameters are presented for an arcing turn (maneuver #9 in B) and an upward acceleration (maneuver #1 in B). Level of smoothing had little effect on the performance metrics measured from the maneuvers. Shown here are the unsmoothed position and orientation (black trace and text), the chosen levels of smoothing (blue; $R_{pos}$ and $R_{ori}$ matrices presented in appendix B), a lower level of smoothing (green; 0.1 x $R_{pos}$; 0.1 x $R_{ori}$), and a higher level of smoothing (red; 10 x $R_{pos}$; 10 x $R_{ori}$).
Figure 4.2. Degrees turned and elapsed time for pitch-roll (PRT) and arcing (Arc) turns. Data points are bird-trial means (n = 52), with grand means indicated with lines. On the right are histograms for the pooled dataset. The three outliers for degrees turned in pitch-roll turns were included when calculating the grand means but not in the model analyses.
Figure 4.3. Most maneuvering performance metrics are highly repeatable. Values > 70% are considered to have high repeatability, 40-70% moderate repeatability, and < 40% low repeatability. A metric is considered not repeatable if its 95% confidence intervals overlap zero.
Figure 4.4. Burst capacity was associated with 9 of 14 maneuvering performance metrics. Each panel shows partial residual performance (y-axis) in relation to the mass of weights lifted (x-axis) for the most supported candidate model with burst performance as a predictor. Partial residual values (y-axis) account for the other fixed effects in that model. Lines show model predictions assuming the median value of continuous predictors, and averaging across experiments and levels of competitor presence. Prediction lines are dashed for metrics where burst performance was not identified as an important predictor. Color is used to denote data points from the same bird.
Figure 4.5. Aspect ratio was associated with three maneuvering performance metrics. Each panel shows partial residual performance (y-axis) in relation to wing aspect ratio (x-axis) from a best-fit model that identified aspect ratio as an important predictor. All other features as in figure 4.
Figure 4.6. Competitor presence was associated with four maneuvering performance metrics. Each panel shows residual performance (y-axis) in relation to competitor presence from a best-fit model where competitor presence had a detected effect. All other features as in figure 4.
Figure 4.7 Most maneuvering performance metrics are positively related to one another. The color of each cell in this matrix indicates the strength of the Pearson’s correlations between two metrics, with darker blue indicating a stronger positive relationship, and darker red indicating a stronger negative relationship. Metrics are ordered by relationship strength. Note that a lower duration of pitch-roll turns ($PRT_{\text{time}}$) indicates higher performance; thus, the strong negative relations between $PRT_{\text{time}}$ and most other metrics indicate that birds with higher speeds, accelerations and rotational velocities executed higher performance pitch-roll turns.
Table 4.1. Candidate models of maneuvering performance. All models include an intercept as well as a random effect of bird identity to account for repeated measures of individuals.

<table>
<thead>
<tr>
<th>model</th>
<th>fixed effects</th>
<th>description</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>solo/comp + experiment + body mass + wing length</td>
<td>wing size</td>
</tr>
<tr>
<td>2</td>
<td>solo/comp + experiment + body mass + wing aspect ratio</td>
<td>wing shape</td>
</tr>
<tr>
<td>3</td>
<td>solo/comp + experiment + body mass + wing length + wing aspect ratio</td>
<td>wing size &amp; shape</td>
</tr>
<tr>
<td>4</td>
<td>solo/comp + experiment + body mass + weight lifted</td>
<td>burst power</td>
</tr>
<tr>
<td>5</td>
<td>solo/comp + experiment + body mass + weight lifted + wing length</td>
<td>burst power &amp; wing size</td>
</tr>
<tr>
<td>6</td>
<td>solo/comp + experiment + body mass + weight lifted + wing aspect ratio</td>
<td>burst power &amp; wing shape</td>
</tr>
<tr>
<td>7</td>
<td>solo/comp + experiment + body mass + weight lifted + wing length + wing aspect ratio</td>
<td>burst power, wing size &amp; shape</td>
</tr>
<tr>
<td>8</td>
<td>intercept-only</td>
<td></td>
</tr>
</tbody>
</table>

* Candidate models 1-7 also include a fixed effect of days post-capture for the following metrics: $AccTot_{pow, max}$, $AccTot_{vel, max}$, $AccHor_{max}$, $DecHor_{max}$, $Arc_{vel, avg}$, and $Arc_{cent, max}$.
Table 4.2. Descriptive statistics and sample sizes for maneuvering performance. Grand mean values were calculated by first taking the mean of each bird’s trial average, and then taking the mean of means across birds ($n = 20$ birds in 20 solo trials and 16 paired competition trials).

<table>
<thead>
<tr>
<th>performance metric</th>
<th># trajectories</th>
<th>grand mean</th>
<th>[range of bird means]</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>linear accelerations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Acc_{Tot, max}$</td>
<td>71,007</td>
<td>2.22 m/s</td>
<td>[1.20, 2.94]</td>
</tr>
<tr>
<td>$Acc_{Hor, max}$</td>
<td>47,287</td>
<td>6.30 m/s²</td>
<td>[2.96, 8.83]</td>
</tr>
<tr>
<td>$Dec_{Hor, max}$</td>
<td>51,245</td>
<td>6.67 m/s²</td>
<td>[9.03, 3.45]</td>
</tr>
<tr>
<td>$Acc_{VU, max}$</td>
<td>6,935</td>
<td>3.78 m/s²</td>
<td>[2.98, 4.67]</td>
</tr>
<tr>
<td>$Acc_{VD, max}$</td>
<td>9,284</td>
<td>3.58 m/s²</td>
<td>[4.69, 2.68]</td>
</tr>
<tr>
<td><strong>rotational velocities</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Pitch_{U, avg}$</td>
<td>6,085</td>
<td>1.13 rev/s</td>
<td>[0.91, 1.34]</td>
</tr>
<tr>
<td>$Pitch_{D, avg}$</td>
<td>14,807</td>
<td>1.00 rev/s</td>
<td>[1.19, 0.78]</td>
</tr>
<tr>
<td>$Yaw_{avg}$</td>
<td>12,660</td>
<td>1.52 rev/s</td>
<td>[1.32, 1.75]</td>
</tr>
<tr>
<td><strong>pitch-roll</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$PRT_{deg}$</td>
<td>17,133</td>
<td>133.3 °</td>
<td>[34.9, 162.7]</td>
</tr>
<tr>
<td>$PRT_{time}$</td>
<td>17,133</td>
<td>0.47 s</td>
<td>[0.38, 0.60]</td>
</tr>
<tr>
<td><strong>arcing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$Arc_{rad}$</td>
<td>6,945</td>
<td>0.48 m</td>
<td>[0.14, 0.70]</td>
</tr>
<tr>
<td>$Arc_{vel, avg}$</td>
<td>6,945</td>
<td>1.57 m/s</td>
<td>[0.80, 2.26]</td>
</tr>
<tr>
<td>$Arc_{cent, max}$</td>
<td>6,945</td>
<td>6.59 m/s²</td>
<td>[3.42, 10.80]</td>
</tr>
<tr>
<td><strong>use of turns</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$PRT%$</td>
<td>24,078</td>
<td>0.69</td>
<td>[0.39, 0.87]</td>
</tr>
</tbody>
</table>
Table 4.3. Maneuvering performance in relation to burst power, wing morphology, and competitor presence (n = 20 birds in 20 solo trials and 16 paired competition trials).

Standardized beta coefficients and $R^2_{\text{GLMM(m)}}$ are reported for either the best-fit model, or, if there was support for more than one model, the average of supported models. The standardized beta coefficient is a measure of effect size that can be compared among predictors in the same model. Relative importance is a measure of the weight of evidence in favor of a predictor subject to model selection, on a scale from 0-1, and is reported for burst capacity and wing morphology variables as these alone were subject to model selection. Marginal $R^2_{\text{GLMM(m)}}$ provides a measure of the combined explanatory power of fixed effects of interest (competitor presence, burst capacity, and wing morphology effects combined). Detail of all candidate models are provided in Appendix C.

<table>
<thead>
<tr>
<th>model set</th>
<th>support for</th>
<th>fixed effects</th>
<th>std beta coef [95% CI]</th>
<th>relative importance</th>
<th>$R^2_{\text{GLMM(m)}}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$AccTot_{\text{vel, max}}$</td>
<td>wing shape + burst</td>
<td>competitor presence mass</td>
<td>$-0.04 [-0.18, 0.11]$</td>
<td>--</td>
<td>0.66</td>
</tr>
<tr>
<td></td>
<td></td>
<td>weight lifted</td>
<td>$0.09 [-0.03, 0.20]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>wing length</td>
<td>$0.12 [-0.002, 0.24]$</td>
<td>1.00</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>wing aspect ratio</td>
<td>$-0.08 [-0.21, 0.06]$</td>
<td>0.25</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>experiment (CA1)</td>
<td>$0.18 [-0.02, 0.38]$</td>
<td>0.77</td>
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<tr>
<td></td>
<td></td>
<td>experiment (CA2)</td>
<td>$0.18 [0.38, 1.41]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>days post-capture</td>
<td>$0.79 [0.30, 1.28]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$-0.003 [-0.01, 0.002]$</td>
<td>--</td>
<td></td>
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<tr>
<td>$AccHor_{\text{max}}$</td>
<td>burst + competition</td>
<td>competitor presence mass</td>
<td>$-0.45 [-0.85, -0.06]$</td>
<td>--</td>
<td>0.37</td>
</tr>
<tr>
<td></td>
<td></td>
<td>weight lifted</td>
<td>$0.15 [-0.34, 0.66]$</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>wing aspect ratio</td>
<td>$0.42 [-0.09, 0.93]$</td>
<td>1.00</td>
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<tr>
<td></td>
<td></td>
<td>experiment (CA1)</td>
<td>$0.48 [-0.35, 1.30]$</td>
<td>0.34</td>
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<tr>
<td></td>
<td></td>
<td>experiment (CA2)</td>
<td>$3.86 [2.06, 5.67]$</td>
<td>--</td>
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<tr>
<td></td>
<td></td>
<td>days post-capture</td>
<td>$3.80 [2.04, 5.55]$</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$-0.01 [-0.03, 0.01]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>$DecHor_{\text{max}}$</td>
<td>burst + competition</td>
<td>competitor presence mass</td>
<td>$-0.46 [-0.80, -0.12]$</td>
<td>--</td>
<td>0.40</td>
</tr>
<tr>
<td></td>
<td></td>
<td>weight lifted</td>
<td>$0.27 [-0.17, 0.72]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>wing aspect ratio</td>
<td>$0.47 [0.02, 0.93]$</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>experiment (CA1)</td>
<td>$0.38 [-0.37, 1.14]$</td>
<td>0.30</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>experiment (CA2)</td>
<td>$3.67 [2.06, 5.29]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>days post-capture</td>
<td>$3.93 [2.43, 5.42]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$-0.01 [-0.03, 0.01]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>$AccVU_{\text{max}}$</td>
<td>intercept-only</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0 (NA)</td>
</tr>
<tr>
<td>$AccVD_{\text{max}}$</td>
<td>intercept-only</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0 (NA)</td>
</tr>
<tr>
<td>$PitchU_{\text{vel, avg}}$</td>
<td>burst</td>
<td>competitor presence mass</td>
<td>$0.02 [-0.02, 0.06]$</td>
<td>--</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td></td>
<td>weight lifted</td>
<td>$0.0001 [-0.04, 0.04]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>experiment (CA1)</td>
<td>$0.04 [-0.01, 0.08]$</td>
<td>1.00</td>
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<tr>
<td></td>
<td></td>
<td>experiment (CA2)</td>
<td>$0.13 [0.04, 0.22]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$0.17 [0.06, 0.29]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>$PitchD_{\text{vel, avg}}$</td>
<td>competition + burst</td>
<td>competitor presence mass</td>
<td>$0.06 [0.01, 0.10]$</td>
<td>--</td>
<td>0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>weight lifted</td>
<td>$0.01 [-0.04, 0.05]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>wing length</td>
<td>$0.05 [-0.01, 0.10]$</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>experiment (CA1)</td>
<td>$0.04 [-0.03, 0.11]$</td>
<td>0.33</td>
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<tr>
<td></td>
<td></td>
<td>experiment (CA2)</td>
<td>$0.15 [0.01, 0.3]$</td>
<td>--</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>$0.25 [0.11, 0.38]$</td>
<td>--</td>
<td></td>
</tr>
<tr>
<td>$Yaw_{\text{vel, avg}}$</td>
<td>intercept-only</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0 (NA)</td>
</tr>
<tr>
<td>model set</td>
<td>support for</td>
<td>fixed effects</td>
<td>std beta coef [95% CI]</td>
<td>relative importance</td>
<td>$R^2_{\text{GLMM(oo)}}$</td>
</tr>
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<td>-----------</td>
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<td>------------------------</td>
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</tr>
<tr>
<td>$PRT_{\text{log}}$</td>
<td>intercept-only</td>
<td>NA</td>
<td>NA</td>
<td>NA</td>
<td>0 (NA)</td>
</tr>
<tr>
<td>$PRT_{\text{size}}$</td>
<td>burst</td>
<td>competitor presence</td>
<td>-0.001 [-0.01, 0.01]</td>
<td>–</td>
<td>0.16</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mass</td>
<td>-0.01 [-0.03, 0.003]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>weight lifted</td>
<td>-0.02 [-0.04, -0.004]</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
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<td>wing length</td>
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</tr>
<tr>
<td></td>
<td></td>
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<td>–</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>experiment(CA2)</td>
<td>-0.12 [-0.17, -0.08]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>$Arc_{\text{rad}}$</td>
<td>burst</td>
<td>competitor presence</td>
<td>-0.02 [-0.07, 0.02]</td>
<td>–</td>
<td>0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mass</td>
<td>0.01 [-0.03, 0.06]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>weight lifted</td>
<td>0.08 [0.03, 0.13]</td>
<td>1.00</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>experiment(CA1)</td>
<td>0.20 [0.10, 0.30]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>experiment(CA2)</td>
<td>0.31 [0.19, 0.44]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>$Arc_{\text{vel, avg}}$</td>
<td>burst</td>
<td>competitor presence</td>
<td>-0.01 [-0.10, 0.09]</td>
<td>–</td>
<td>0.38</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mass</td>
<td>0.03 [-0.06, 0.13]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>weight lifted</td>
<td>0.14 [0.04, 0.24]</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>wing aspect ratio</td>
<td>0.13 [-0.02, 0.29]</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>experiment(CA1)</td>
<td>0.77 [0.39, 1.15]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>experiment(CA2)</td>
<td>0.72 [0.31, 1.15]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>days post-capture</td>
<td>-0.001 [-0.005, 0.002]</td>
<td>–</td>
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<tr>
<td>$Acc_{\text{cent, max}}$</td>
<td>wing shape</td>
<td>competitor presence</td>
<td>0.29 [-0.37, 0.94]</td>
<td>–</td>
<td>0.40</td>
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<tr>
<td></td>
<td></td>
<td>mass</td>
<td>-0.20 [-0.74, 0.34]</td>
<td>–</td>
<td></td>
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<tr>
<td></td>
<td></td>
<td>wing aspect ratio</td>
<td>1.09 [0.19, 1.99]</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>experiment(CA1)</td>
<td>5.93 [4.02, 7.84]</td>
<td>–</td>
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<tr>
<td></td>
<td></td>
<td>experiment(CA2)</td>
<td>0.85 [-1.59, 3.28]</td>
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<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>days post-capture</td>
<td>-0.04 [-0.06, -0.02]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td>$PRT%$</td>
<td>competition + wing shape + burst + wing size</td>
<td>competitor presence</td>
<td>-0.14 [-0.19, -0.09]</td>
<td>–</td>
<td>0.31</td>
</tr>
<tr>
<td></td>
<td></td>
<td>mass</td>
<td>-0.001 [-0.04, 0.05]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>weight lifted</td>
<td>0.06 [0.002, 0.12]</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>wing aspect ratio</td>
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<tr>
<td></td>
<td></td>
<td>experiment(CA1)</td>
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<td>1.00</td>
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</tr>
<tr>
<td></td>
<td></td>
<td>experiment(CA2)</td>
<td>0.10 [-0.11, 0.31]</td>
<td>–</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>days post-capture</td>
<td>0.39 [0.14, 0.65]</td>
<td>–</td>
<td></td>
</tr>
</tbody>
</table>
5. Mechanical constraints on burst power reserves decrease maneuvering performance in hummingbirds

5.1 Introduction

Animals flying at high elevation face the dual challenges of reduced oxygen availability, which constrains metabolic input, and decreased air density, which constrains mechanical power output. Hummingbirds have one of the most expensive forms of flight but are nonetheless common in high elevation habitats throughout the western hemisphere (Brown and Kodric-Brown, 1979; Carpenter et al., 1983; Feinsinger et al., 1979; Stiles, 1980). Research with hummingbirds has demonstrated that the metabolic and mechanical costs of hovering increase at higher elevations (Altshuler and Dudley, 2003; Chai and Dudley, 1996; Welch, and Suarez, 2008). Additionally, experiments with variable gas mixtures have revealed that reduction in oxygen availability leads to decreases in wingbeat frequency, while reduction in air density leads to increases in wingbeat amplitude (Altshuler and Dudley, 2003; Chai and Dudley, 1995). It is expected that other features of flight performance should also be influenced by environmental changes along elevational gradients (Altshuler and Dudley, 2006; Altshuler et al., 2004b). In particular, hummingbirds rely on their high level of maneuverability to perform complex mating displays (Clark, 2009; Clark et al., 2012; Felton et al., 2008; Feo and Clark, 2010), capture insects (Stiles, 1995; Yanega and Rubega, 2004), and aggressively defend territories (Altshuler, 2006; Carpenter et al., 1983). However, how maneuvering performance is affected by the reduced oxygen and decreased air density found at high elevations remains largely unknown.

Maneuvering flight requires the ability to generate and reorient excess aerodynamic force...
(Dudley, 2002). The capacity for hummingbirds to generate additional force for brief periods of time can be measured using the technique of asymptotic load-lifting, and is often referred to as the burst power reserves (Chai and Millard, 1997; Chai et al., 1997). I recently tested the hypothesis that performance during voluntary maneuvers was associated with an individual hummingbird's power reserves (Chapter 4). Hummingbirds with higher power reserves accelerated, decelerated and turned faster than birds with lower power reserves. Moreover, the magnitude of the power reserves was the most common predictor of different features of maneuvering performance, much more than wing morphology or even the presence of a competitor.

At high elevations, hummingbird power reserves are reduced. Hummingbirds caught at high elevation sites demonstrated significantly lower power reserves compared to individuals of the same species caught at lower elevation sites (Altshuler, 2006). Because load-lifting experiments have not been combined with gas mixture trials, it is unknown how muscle capacity at high elevation is influenced by oxygen availability and air density. If power reserves directly affect maneuvering performance, then birds flying at high elevation should also experience a decrease in maneuverability.

Given the association between power reserves and maneuvering performance (Segre et al.), I sought to determine if hummingbird maneuvering performance declines at higher elevation (figure 5.1c: H1). I measured maneuvering behavior of Anna's hummingbirds (*Calypte anna*) using a high-speed video tracking system. The same birds were tested during solitary and competitive flights in a large flight chamber at both a low elevation (326m) and at a high elevation (3800m) site. The high elevation site was chosen because it is slightly higher than their natural range and high enough to impose a challenge to power reserves without detrimentally
restricting flight capabilities (Altshuler and Dudley, 2003). This experiment revealed significant declines in all of the maneuvering performance metrics that I considered. I designed an additional experiment to determine if the declines in maneuvering performance were caused by changes in oxygen availability (figure 5.1c: H2) or air density (figure 5.1c: H3). This experiment tested the maneuvering performance of hummingbirds in an airtight flight chamber infused with either nitrogen, to lower oxygen availability, or heliox, to lower air density (Altshuler and Dudley, 2003; Chai and Dudley, 1996). Collectively, these experiments address the question: what are the challenges to hummingbird maneuvering performance imposed by high elevations?

5.2 Materials and methods

I captured and filmed 16 adult male Anna's hummingbirds near the University of California, Riverside (n = 8 birds in June-October 2009) for the translocation experiment and near the University of British Columbia (n = 8 birds in December 2013-April 2014) for the gas substitution experiment. The hummingbirds were captured with drop-door traps, housed in individual cages, and fed ad libidum with a solution of artificial nectar (Nektar-Plus, Nekton, Pforzheim, Germany) and sucrose. All of the procedures were conducted under approval of the Institutional Animal Care and Use Committee at the University of California, Riverside and the Animal Care committee at the University of British Columbia.

Birds were filmed during free-flight to describe their maneuvering performance. The maneuverability trials were conducted in a large flight cage (3 x 1.5 x 1.5 meters), built with a metal frame and garden mesh (translocation) or plexiglass (gas substitution) side panels, and containing several perches and a feeder. The birds were allowed time to acclimate to the cage and were then filmed flying individually for two hours. Each bird was also filmed flying in the
presence of a randomized and previously unknown competitor for a separate two-hour trial. During the competition trials one bird was marked with a piece of retro-reflective tape. After data processing was complete the digitized maneuvers were re-projected onto the videos, and a team of digitizers attributed each maneuver to the marked or unmarked individual. To determine the burst power capacity of each bird, body mass was recorded and load-lifting trials were conducted using the same methods described in previous studies (Altshuler, 2006; Chai et al., 1997).

5.2.1 Tracking system

During the maneuverability trials the birds were filmed using a multi-camera tracking system that automatically measures body position and orientation. A detailed description of the tracking system and performance metrics is available in Chapter 4. Briefly, I extracted a series of stereotyped maneuvers: total accelerations, horizontal accelerations, horizontal decelerations, arcing turns, hovering pitch-up rotations, hovering pitch-down rotations, and hovering yaw turns. For each type of maneuver I calculated one or two performance metrics. I focused on performance metrics that are repeatable and reliably surveyed by this experimental setup (Chapter 4). For total accelerations maneuvers I measured the maximum velocity ($Acc_{Tot\text{vel}, \text{max}}$). For horizontal accelerations and decelerations I measured the maximum accelerations ($Acc_{Hor\text{max}}$) and decelerations ($Dec_{Hor\text{max}}$) respectively. From the arcing turns I measured the maximum centripetal acceleration ($Arc_{cent, \text{max}}$). From the hovering pitch-up, pitch-down, and yaw maneuvers I measured average rotational velocity ($Pitch_{U\text{vel}, \text{avg}}$, $Pitch_{D\text{vel}, \text{avg}}$, $Yaw_{\text{vel}, \text{avg}}$).

5.2.2 Translocation experiment

Hummingbirds were captured at low elevations and transported to a high elevation site at
White Mountain Research Center's Barcroft Laboratory (elevation 3800 m, $\rho = 0.79 \text{ kg/m}^3$, oxygen partial pressure = 102 mm Hg) in two groups. The first capture group included three birds that were given four weeks to acclimate to the high altitude site, and the second capture group included five birds that were given two weeks to acclimate. Once the filming was completed the birds were transported back to the low elevation site at the University of California Riverside (elevation 326 m, $\rho = 1.18 \text{ kg/m}^3$, oxygen partial pressure = 153 mm Hg) and allowed to acclimate for four weeks before being filmed. Birds were kept in cloth bags during transport and were monitored for signs of stress and hand-fed every thirty minutes. All trials were conducted at 22ºC, and every bird was filmed once in solo flight and twice in competitive flight.

5.2.3 Gas substitution experiment

Hummingbirds were filmed at the University of British Columbia (elevation 91 m, $\rho = 1.13 \text{ kg/m}^3$, oxygen partial pressure = 159 mm Hg, average lab temperature = 18.5ºC) in three gas treatments designed to match the air density and oxygen concentrations in the translocation study. The first treatment was in air, the second treatment added nitrogen to create normodense and hypoxic conditions, and the third treatment added helium and oxygen (heliox) to create hypodense and normoxic conditions. The oxygen content and air density within the sealed acrylic flight chamber were controlled with a gas mixer (MFC-4, Sable Systems, Las Vegas NV, USA) that regulated mass flow valves (MC-20 & MCP-100, Alicat Scientific, Tuscon AZ, USA).

During gas mixing, a relief port in the side of the chamber was left open to allow oxygen to escape and a small electric fan circulated the contents of the chamber. Oxygen concentrations and air density were monitored continuously during gas mixing and every thirty minutes during
filming. Oxygen was measured with an oxygen analyzer (OM-11, Model 242B, Beckman Instruments, Pasadena CA, USA) and air density was calculated similar to previous studies (Altshuler and Dudley, 2003; Chai and Dudley, 1996) where the frequency of a Galton whistle inside the flight chamber was recorded (Raven Lite 1.0, Cornell Lab of Ornithology, Ithaca NY, USA) and calculated with a custom Matlab script (Matlab, Mathworks, Natick MA, USA). This frequency, as well as the chamber’s temperature, humidity, and barometric pressure was used to calculate the initial air density within the chamber and subsequent changes in whistle frequency with the addition of heliox were used to determine changes in air density. Additional nitrogen or heliox was added to the flight chamber as needed to maintain the air density and oxygen concentration of hypodense treatments (average air density = 0.79 kg/m$^3$, average oxygen partial pressure = 157 mm Hg) and hypoxic treatments (average air density = 1.10 kg/m$^3$, average oxygen partial pressure = 101 mm Hg) as closely as possible to those at the White Mountain Research Center (elevation 3800 m, air density = 0.79 kg/m$^3$, oxygen partial pressure = 102 mm Hg). For each gas mixture treatment, every bird was filmed once in solitary flight and once in competition against a previously unknown opponent.

5.2.4 Statistical analysis

The data sets from California and British Columbia were analyzed separately because they resulted from two different experiments. However, it is important to note that the data sets also differed in time of year, air temperature, and visual environment of the flight chamber between California and British Columbia experiments.

Quality checking of the video tracking revealed few errors, but the system occasionally produced unrealistic changes in position and orientation. Because I did not want tracking errors
to have a disproportionate effect on the analysis, for each performance metric I removed values that were more extreme than five standard deviations from the pooled mean. The total number of extreme values removed was 77 out of 242,074 data points for the transplant experiment, and 48 out of 278,191 for the gas substitution experiment, or < 0.04% of both datasets. With the remaining data, I calculated a mean value for each bird-trial combination (elevation: n = 48; 8 birds in 16 solo trials and 16 competition trials; gas substitution: n = 48; 8 birds in 24 solo trials and 12 competition trials). The calculated means were used in the subsequent statistical analyses.

The transplant experiment was designed to test the effects of elevation on maneuvering performance metrics. I fit a separate linear mixed effects model for each performance metric that included fixed effects of elevation, competitor presence, body mass, mass lifted during load-lifting, and capture-group, as well as a random effect of bird identity to account for repeated measures of individuals. I used Wald's test to evaluate the effect of elevation.

The gas mixture experiments were designed to test how reduction in oxygen availability and in air density affected maneuvering performance metrics. I followed a similar procedure, fitting a separate model with fixed effects of gas treatment (nitrogen, heliox, normal air), competitor presence, body mass, mass lifted, number of days since capture, and a random effect of bird identity for each performance metric. I used Dunnett’s tests to evaluate the independent effects of air density (heliox mixture vs. air) and hypoxia (nitrogen mixture vs. air) in the gas substitution experiment.

I accounted for multiple testing of the seven performance metrics using the Benjamini–Hochberg procedure. I used a false discovery rate of q = 0.05 within each of the following comparison sets: (i) high vs. low elevation, (ii) nitrogen vs. air, and (iii) heliox vs. air.
5.3 Results

Morphology and flight performance of individual Anna's hummingbirds are given in table 5.1. The birds from California and British Columbia that were used in the elevation and gas mixture experiment, respectively, differed in wing morphology, and some features of hovering and load-lifting performance values.

The hummingbirds studied in the translocation experiment produced a similar number of maneuvers at both sites. Sample sizes for most maneuvers were lower at high elevation with the only exceptions being total accelerations, which were essentially identical between sites, and arcing turns, which were more frequent at high elevation (table 5.2). In every performance metric, the birds at higher elevation experienced a significant decrease in maneuverability (all $P \leq 0.01$). I did observe individual differences in the performance metrics at both sites and in the strength of the declines between sites (figure 5.2a).

The hummingbird studied in the gas mixture experiments produced a large number of trajectories. Sample sizes were similar between air and low oxygen availability trails, but low air density trials had generally fewer samples. Hummingbirds flying in a hypoxic gas mixture with oxygen levels equivalent to 3925 m did not experience a decrease in any of the maneuvering performance metrics I measured (all $P > 0.2$; table 5.2; figure 5.2b). However, flying in a hypodense mixture with a density equivalent to 3800 m significantly decreased horizontal acceleration ($P = 0.024$), horizontal deceleration ($P = 0.006$), pitch-up velocity ($P = 0.003$), and yaw velocity ($P = 0.008$; figure 5.1c, table 5.3). Notably, I found that low air density decreased performance on both translational and rotational maneuvers.
5.4 Discussion

The goal of this study was to determine if the maneuvering performance of flying birds is affected by high elevation, and if so, whether this is due to decreases in oxygen availability or lower air density (figure 5.1). Using a multi-camera tracking system, I demonstrated that all of the metrics I used to quantify maneuvering performance in hummingbirds were lower at high elevation (table 5.2, figure 5.2). Experimental decreases in oxygen availability via nitrogen infusion did not lead to any measurable changes in maneuvering performance (table 5.3). In contrast, experimental decreases in air density via heliox infusion decreased horizontal accelerations and decelerations, yaw velocities, and pitch-up velocities. Collectively, these experiments demonstrate a strong influence of elevation on the maneuvering performance of hummingbirds, which is driven more by changes in air density than oxygen availability.

Hummingbirds translocated to higher elevation experienced a significant reduction in maneuverability. Performance of translational velocities and accelerations, and rotational velocities decreased compared to performance at low elevation. Decreased maneuverability may represent a cost of permanently (Altshuler et al., 2004b; Feinsinger et al., 1979) or temporarily (Altshuler, 2006; Carpenter et al., 1983) living at higher elevations. Hummingbirds rely on their high level of maneuverability to perform a range of important behaviors. Complex displays require high accelerations (Clark, 2009; Hurly et al., 2001), rotations (Felton et al., 2008), and load carrying capabilities (Zusi and Gill, 2009) to obtain mates and to intimidate competitors. Additionally, many hummingbird species depend on aerial insectivory to supplement their nectar based diet (Stiles, 1995; Yanega and Rubega, 2004). Finally, there is evidence that maneuvering performance affects the outcomes of territorial contests. Altshuler et al. (Altshuler, 2006) suggested that rapid vertical ascents may play a role in establishing dominance. Vertical
accelerations and decelerations were not repeatably surveyed using the experimental setup, possibly because of the limited height of the cage (Segre et al.). The influence of maneuvering performance on the outcomes of behaviors such as courtship displays, predatory chases, and territorial competitions has not been tested and remains an area with potential for future work.

I did not find an influence of low oxygen on hummingbird maneuvering performance. Previous studies with hypoxic normodense gas mixtures showed that as oxygen concentration is reduced, hovering hummingbirds slightly decrease their wingbeat frequency (Altshuler, 2006; Altshuler and Dudley, 2003). This is likely the effect of fatigue, since the mechanical cost of hovering does not change. Because burst performance is transient and likely anaerobic (Altshuler and Dudley, 2003), the effects of hypoxia on maximum force generation may be limited, although this remains to be tested. Therefore, it is possible that maneuvering performance does not change because power reserves (the difference between the minimum power required to fly and maximum power generated during burst performance) are unaffected by hypoxia. It is probable that hummingbirds experienced increased fatigue while flying in hypoxia, however I did not test this.

Hummingbirds flying in low air density exhibited decreased performance in several translational and rotational metrics. I found reductions in horizontal accelerations and decelerations, as well as pitch-up and yaw velocities. Interestingly, I did not find any effect on translational velocity, centripetal acceleration, or pitch-down velocity. It is possible that the combined effect of a metabolic challenge and a mechanical challenge would decrease performance more than either one separately, and this could explain why I found a dramatic decrease in performance during the translocation experiment but only a modest decrease during the gas substitution experiment. However, this remains to be tested in future studies, as my two
experiments cannot be directly compared because of the differences in setup and source populations.

A large body of work on hummingbirds has shown that the kinematic changes to hovering flight that occur in response to elevation are being driven by decreased air density, not reduced oxygen availability (reviewed in Altshuler and Dudley, 2006). However, low oxygen availability can exacerbate the challenges caused by low density. This study extends these observations to the more complicated maneuvering flight of hummingbirds. At high elevation where power reserves are low, performance of complex maneuvers decreases. Furthermore, the mechanical challenge imposed by low air density has a greater effect on maneuvering performance than the metabolic challenge of flying in hypoxia.
Figure 5.1. Methods to measure hummingbird maneuvering performance across elevations and in physically variable gas mixtures. (A) Experiments were conducted with males Anna's hummingbirds (*Calypte anna*). (B) A multi camera tracking system measured maneuvering performance in solitary and paired flight trials. Blue circles indicate the body position for a single trajectory and red lines denote body orientation. (C) A translocation experiment was performed to measure the effects of elevation on maneuvering performance (H1). A gas substitution experiment was performed to measure the independent effects of low oxygen (H2) and low density (H3) on maneuvering performance.
Figure 5.2. At high elevation where power reserves are low, hummingbird maneuvering performance decreases. We measured seven maneuvering performance metrics: velocity (AccTot\textsubscript{vel,max}), horizontal acceleration (AccHor\textsubscript{max}) and deceleration (DecHor\textsubscript{max}), arcing turn centripetal acceleration (Arc\textsubscript{cent,max}), hovering pitch-up, pitch-down, and yaw velocities (PitchU\textsubscript{vel,avg}, PitchD\textsubscript{vel,avg}, Yaw\textsubscript{vel,avg}). (A) The mean for each bird at low (326 m) and high (3800 m) elevations. At high elevation there was a significant decrease in every performance metric analyzed. (B) When challenged to fly in a normodense hypoxic gas mixture (oxygen equivalent: 3925 m), there was no decrease in maneuvering performance compared to flying in normal air. (C) When challenged to fly in a hypodense normoxic gas mixture there was a significant decrease in horizontal deceleration, pitch-up velocity, and yaw velocity, relative to flying in normal air.
Table 5.1. Individual morphology and load-lifting parameters (mass $m$ (grams), wing length $L$ (mm), wing area $S$ (mm$^2$), aspect ratio $AR$, and hovering (hov) and load-lifting (ll) performance (lifted mass $load$ (g), wingbeat frequency $f$ (Hz) and wing stroke amplitude $\Phi$ (º)) during hovering and load-lifting). Differences between experiments were assessed using t-Tests with significance indicated by * ($P < .01$) and ** ($P < .0001$).

<table>
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<th>$AR$</th>
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Table 5.2. Maneuvering performance at high and low elevations. The performance metrics are velocity \((\text{AccTot}_\text{vel, max})\), horizontal acceleration \((\text{AccHor}_\text{max})\) and deceleration \((\text{DecHor}_\text{max})\), arcing turn centripetal acceleration \((\text{Arc}_{\text{cent, max}})\), hovering pitch-up, pitch-down, and yaw velocities \((\text{PitchU}_\text{vel, avg}, \text{PitchD}_\text{vel, avg}, \text{Yaw}_\text{vel, avg})\). The model included fixed effects of elevation, competitor presence, body mass, load-lifting performance, and capture-group, as well as a random effect of bird identity. The number of trajectories measured, the mean and standard errors, and the treatment effects are presented for each metric.

<table>
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<th>elevation translocation</th>
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* denotes significance
Table 5.3. Maneuvering performance in normodense hypoxic gas and normal air. The performance metrics are velocity (\(Acc_{\text{Tot}, \text{vel, max}}\)), horizontal acceleration (\(Acc_{\text{Hor}, \text{max}}\)) and deceleration (\(Dec_{\text{Hor}, \text{max}}\)), arcing turn centripetal acceleration (\(Arc_{\text{cent, max}}\)), hovering pitch-up, pitch-down, and yaw velocities (\(Pitch_{\text{U, vel, avg}}, Pitch_{\text{D, vel, avg}}, Yaw_{\text{vel, avg}}\)). The model included fixed effects of gas mixture, competitor presence, body mass, load-lifting performance, and days since capture, as well as a random effect of bird identity. The number of trajectories measured, the mean and standard errors, and the treatment effects are presented for each metric.

<table>
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* denotes significance
Table 5.4 Maneuvering performance metrics in hypodense normoxic gas and normal air.
The performance metrics are velocity ($Acc_{Tot,\text{vel, max}}$), horizontal acceleration ($Acc_{Hor,\text{max}}$) and deceleration ($Dec_{Hor,\text{max}}$), arcing turn centripetal acceleration ($Arc_{cent,\text{max}}$), hovering pitch-up, pitch-down, and yaw velocities ($Pitch_{U,\text{vel, avg}}$, $Pitch_{D,\text{vel, avg}}$, $Yaw_{vel, avg}$). The model included fixed effects of gas mixture, competitor presence, body mass, load-lifting performance, and days since capture, as well as a random effect of bird identity. The number of trajectories measured, the mean and standard errors, and the treatment effects are presented for each metric.

<table>
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</table>

* denotes significance
6. Conclusions

Hummingbirds are a highly successful and diverse avian family found throughout North and South America. They rely on their well-documented agility to perform a wide range of complex behaviors such as territorial competitions, aerial insect captures, and highly coordinated mating displays. The motivation behind the research presented in this dissertation was to further understand the aerodynamic, kinematic, and morphological underpinnings that give hummingbirds their spectacular maneuvering ability.

6.1 Bilateral wake structures

With the proliferation of high speed video and high resolution particle imaging techniques, our understanding of the wake structures created by flying animals has greatly increased in recent years. The general pattern described for birds is that the wake produced by the wings is connected over the body, generating a single vortex loop at slow speeds (Kokshaysky, 1979; Spedding, 1987b). As the speed increases the loops become increasingly merged to form a ladder-like wake structure (Spedding et al., 2003). In contrast, bats generate bilateral vortex rings, with each wing producing independent vortex loops (Muijres et al., 2008). In insects, both single vortex rings (Bomphrey et al., 2005) and bilateral vortex rings (Bomphrey et al., 2009) have been observed. However, recent studies have revealed that more complex flow structures are also found in flying animal wakes, and that the presence of these structures may vary with different taxa and flight modes (Johansson and Hedenström, 2009; Muijres et al., 2008).
Hummingbirds are unique among birds in their high wingbeat frequency, their ability to hover, (Greenewalt, 1960) and their aerodynamically active upstroke that provides ~35% of bodyweight support (Wolf et al., 2013). These features likely have an important influence on the wake structure that hummingbirds generate. In the second chapter of this dissertation, I used flow visualization to test the hypothesis that hovering hummingbirds create bilateral vortex jets. Previously, two vortex flow patterns had been proposed for the wake of hovering hummingbirds: 1) the two wings form a single, merged vortex ring during each stroke (Ellington, 1984a; Rayner, 1979; Rayner and Gordon, 1998); and 2) the two wings form bilateral vortex loops during each stroke (Altshuler et al., 2009). My study resulted in three important contributions. First, I present a novel method of low-tech flow visualization for use in birds or animals that may be sensitive to the smoke or chemicals commonly used in engineering trials. This technique uses plumes of carbon dioxide created from dry ice that can be filmed to identify vortex structures. Secondly, I found two structural features that support the hypothesis that hummingbirds use bilateral vortex jets while hovering: 1) the airflow under the wings forms two distinct jets, and 2) vortex loops around each jet are shed during each upstroke and downstroke. I then used the 3D kinematic patterns of hovering wings to create a detailed model of the vortex pattern shed by hovering hummingbirds.

Since this study has been published another research group has independently confirmed my results and the validity of my proposed bilateral vortex model (Wolf et al., 2013). The aerodynamic implications of using a single vortex ring versus bilateral vortex rings are not well understood. It has been hypothesized that a single vortex loop may be more efficient (Muijres et al., 2012; Norberg et al., 1993), while bilateral vortex loops may enhance the ability for the wings to produce left-right asymmetries and may improve maneuvering performance (Bomphrey...
et al., 2009; Henningsson et al., 2011), but this remains to be tested.

6.2 Constrained maneuvers

Although, much of the seminal work on animal flight aerodynamics and kinematics has focused on the performance of steady state behaviors, attention has recently turned to investigating the initiation and maintenance of maneuvers. It has long been debated whether aerial maneuvers are performed by tilting the body to orient the forces (Wagner, 1986c) or by tilting the wings without adjusting the body posture (Blondeau, 1981; Nachtigall, 1979). To address this question, I filmed hummingbirds as they performed controlled lateral flight while tracking a moving feeder to determine which mechanism was employed. I designed a framework to model, describe, and compare the 3D kinematics of the wings, extracted from the synchronized videos. Kinematic measurements revealed that hummingbirds banked their stroke plane while maintaining an upright body posture. However, the stroke plane was banked against the direction of travel and therefore opposite to what was predicted. This surprising result led to the question: how is the lateral force produced? A quasi-steady aerodynamic analysis suggests that the net lateral force may result from unsteady aerodynamic mechanisms operating during stroke reversal (Dickinson et al., 1999), although this remains to be tested.

There is mounting evidence that many flying animals have the ability to perform maneuvers that are initiated by force vectoring as well as maneuvers that are initiated with complex wing asymmetries. In addition to the work presented in this dissertation, I have collaborated on a series of studies that use the kinematic framework that I developed in chapter three to study hummingbirds performing yaw turns (Altshuler et al., 2012) and banking turns (Read, 2015). Collectively, these three studies have shown that hummingbirds use
body-independent asymmetrical wing kinematics to perform both yaw turns and controlled lateral flight, but they use force vectoring to perform fast, banked turns. Similar results have been demonstrated in other taxa. Fruit bats performed slow turns with a translating yaw and fast turns by banking (Iriarte-Díaz and Swartz, 2008). Likewise, slow moving pigeons relied on wingbeat kinematics to turn (Ros et al., 2015), but faster moving pigeons performed banking turns (Ros et al., 2011). In fruit flies, saccades are used for voluntary exploratory turns (Fry et al., 2003), while rapid escapes are initiated by force vectoring (Muijres et al., 2014). These studies suggest that the ability to perform both force vectoring and wingstroke driven maneuvers may be more common than previously thought.

6.3 Free-flight maneuvering performance

To understand the effects of maneuvering performance on behavior, ecology, and evolution, it is important to measure the breadth of an animal's maneuvering capabilities in a natural or semi-natural setting. Recently, computational approaches have been used to categorize and compare similar maneuvering trajectories (Braun et al., 2010; Geurten et al., 2010; Shelton et al., 2014), although the full potential of this technology is only starting to become apparent. In this dissertation I developed a framework to measure and compare free-flight maneuvering performance in hummingbirds. This approach uses a high-throughput analysis to compare body position and orientation data extracted from synchronized videos. In chapters four and five, I use this method to investigate the morphological and physiological determinants of individual maneuvering performance.

It has been proposed that maneuverability is determined by both intrinsic constraints, such as body mass, wing size and shape, and by facultative capacity, such as muscle power.
output (Warrick and Dial, 1998; Warrick et al., 1998). However, there have been no studies that have correlated individual characteristics with maneuvering performance. To address this question I studied the free-flight of male hummingbirds to determine the extent to which maneuvering performance is 1) repeatable across trials, 2) determined by body and wing morphology or burst muscle capacity, and 3) influenced by presence of a competitor. Using a multi-camera tracking system, I analyzed performance metrics of velocity and acceleration based on body position and orientation. Most of these measures were highly repeatable. Burst power was associated with the majority of the performance metrics, whereas wing shape and size were only associated with a few of the performance metrics measured. Acceleration, deceleration, and mechanical power had lower magnitude when hummingbirds flew in the presence of a competitor. However, body angular velocity during slow flight, especially pitch-down velocity, was higher for competitive flights as was the relative use of arcing versus pitch-roll turns. These results indicate that burst power capacity is a key predictor of maneuverability, and that body angular velocity and arcing turns are associated with competition in flight. Hummingbirds rely on their high level of maneuverability to perform complex mating displays (Clark, 2009; Clark et al., 2012; Felton et al., 2008; Feo and Clark, 2010), capture insects (Stiles, 1995; Yanega and Rubega, 2004), and aggressively defend territories (Altshuler, 2006; Carpenter et al., 1983). However, the extent to which maneuvering performance determines success in these behaviors has not been tested.

Hummingbirds have one of the more expensive forms of flight but are nonetheless common in high elevation habitats (Brown and Kodric-Brown, 1979; Carpenter et al., 1983; Feinsinger and Colwell, 1978; Stiles, 1980). Animals flying at high elevation face the dual challenges of reduced oxygen availability, which constrains metabolic input, and decreased air
density, which constrains mechanical power output. However, how maneuvering performance is affected by the reduced oxygen and decreased air density found at high elevations was not known. I tested the effects of altering power reserves on individual maneuvering performance using natural and simulated elevation gradients. With the multi-camera tracking system and computational framework developed in the fourth chapter, I measured the maneuvering performance of hummingbirds at low and high elevations. I asked the question: how does individual maneuvering performance change with increased elevation where power reserves are low? In a follow-up experiment I asked the question: how do metabolic restrictions imposed by low oxygen availability and mechanical restrictions imposed by low air density independently affect maneuvering performance? To address this question I compared the maneuvering performance of individual Anna's hummingbirds flying in normal air, in a hypoxic normodense mixture of nitrogen and air designed to impose a mechanical challenge to power reserves, and in a normoxic hypodense mixture of helium. At high elevation where power reserves are low, performance of complex maneuvers decreases. Furthermore, the mechanical challenge imposed by low air density has a greater effect on maneuvering performance than the metabolic challenge of flying in hypoxia. A large body of work on hummingbirds has shown that the kinematic changes to hovering flight that occur in response to elevation are being driven by decreased air density, not reduced oxygen availability (Altshuler and Dudley, 2006). However, low oxygen availability can exacerbate the challenges caused by low density. This study extends these observations to the more complicated maneuvering flight of hummingbirds.

The study of self-selected maneuvering performance is a relatively new field with the potential to unite the study of locomotion with questions of behavior, ecology, and evolution, in a way that was not previously possible. In hummingbirds, voluntary maneuvers at low elevation
are primarily influenced by facultative capacity, specifically burst power, and to a lesser extent by intrinsic limits, specifically wing aspect ratio. At higher elevations, maneuvering performance declines due to decreases in air density. This research demonstrates that the remarkable maneuverability of hummingbirds derives from their ability to control their wings independently and from high muscle power reserves for generating aerodynamic force.

6.4 Future directions

In the last few years several research groups have begun to investigate the maneuvering performance of aquatic, terrestrial, and flying animals, and my work adds significantly to this growing body of literature. Many of these studies have focused on laboratory-based performance, but the next step will undoubtedly involve questions dealing with behavior in more natural settings. One of the goals of my dissertation was to develop portable techniques that can be applied to field-based questions. The flow visualization method that I used in the second chapter can be performed almost anywhere with minimal equipment. Although it cannot be used to quantify flow properties, this method allows researchers to view the wake structures of free-flying animals at a high level of spatial and temporal resolution. Historically, the intricate laboratory equipment required to study wake topology has limited the amount of species that have been studied. A technique similar to the one described could be used to conduct a large-scale, field based comparative study of simple wake structures.

The portability of high speed cameras coupled with the ability to calibrate large three dimensional spaces have made natural studies of maneuvering performance possible. The kinematic framework that I developed to measure and compare hummingbird wingbeat kinematics is currently being used to measure a variety of maneuvers both in the lab (e.g. drifting
and studies of wingbeat repeatability by two current graduate students, D. A. Skandalis and B. Goller) and in the field (e.g. elaborate shuttle displays by C. J. Clark from the University of California, Riverside). Once a sufficient repertoire of maneuvers is examined, it may emerge that hummingbirds use combinations of wingbeat kinematic features such as wing elevation angles, elevation amplitudes, and angles of attack that are consistently associated. At the moment, wingbeats measured from hovering (Pournazeri et al., 2012), yaw turns (Altshuler et al., 2012), banked turns (Read, 2015), and lateral flight suggest that this may be a possibility. It remains to be seen if these patterns hold for other flight behaviors, and if they are driven by aerodynamic or anatomical constraints.

Very little is known about variation in the performance of constrained maneuvers. To focus on repeated wingbeat patterns most studies have not looked at variation across wingbeats, trials and individuals (a notable exception being Iriarte-Diaz et al., 2012). Variation in individual wingbeats may prove important for stability and control, variation between trials may reflect influences of motivation, and differences between individuals could be determined by morphological, physiological, or aerodynamic constraints on maneuvering performance. Additionally, it remains to be seen if any universal patterns emerge for the control of specific maneuvers across species. Evidence is mounting that behaviors that rely on rapid accelerations and changes in direction are likely performed by force vectoring whereas displays that involve stability, control, and precision may rely on wingbeat kinematics. However, given the novelty of the field, to date this pattern has only been documented in a few species.

Finally, the automated tracking system designed and built for the fourth and fifth chapters is a powerful tool that will be indispensable for answering questions that require large amounts of data to answer. It has already been used to track hovering responses to optic flow (Goller and
Altshuler, 2014) and is being used to measure a variety of behavioral responses to other types of visual stimuli. In addition, it is currently being used to study forward flight control in response to different visual stimuli (R. L. Dakin, unpublished data). The tracking system is also currently being used to investigate the relative contributions of ecological role, wing morphology, and muscle power reserves to maneuvering performance across a wide range of tropical hummingbird species.

One of the most exciting developments in the field of biomechanics is the increasingly portable technology that makes it possible to conduct rigorous studies in the field. Inexpensive, rugged systems provide the opportunity to take basic biomechanics studies and link them to their relevant environmental contexts. This is the newest frontier in the study of flight maneuvering performance and ensures that it will remain an exciting research field for years to come.
Bibliography


Appendices

Appendix A: Supplementary video captions*

**Video 2.1** provides the image sequence of bird 6 during trial 5 from the rear-left perspective of the hovering hummingbird. The video shows the sequential development of a vortex loop on the left side of the bird. Frames 59-65 are depicted in figure 2.1. Frame rate speed has been reduced by 50 times.

**Video 2.2** provides the image sequence of bird 6 during trial 3 demonstrating the frontal perspective of the bird while hovering. The development of vortex loops on the left and right side of the bird as well as the presence of wingtip and root vortices on the left side of the bird can be observed. This sequence is depicted in figure 2.2, with frame 101 in panels a and b, and frame 165 in panels d and e. Frame rate speed has been reduced by 50 times.

**Video 2.3** provides the image sequence of bird 5 during trial 2 from an off axis rear perspective with a wide field of view of the hovering hummingbird. This video provides a view of the shape of the vortex tube connecting the vortices. Frame 58 is depicted in figure 2.2 (G, H). Frame rate speed has been reduced by 50 times.

**Video 2.4** provides the image sequence of bird 6 during trial 9 from the lateral perspective of the bird while hovering. Evolution of distal vortices and the wingtip paths during the down- and up-strokes can be observed. Frame 37 is depicted in figure 2.2 (J, K). Frame rate speed has been reduced by 50 times.

**Video 2.5** provides the image sequence of bird 4 from the rear perspective during hovering. This bird used the highest wing stroke amplitude and lowest wingbeat frequency when hovering in the plume. This video provides views of the wing tip and reversal vortices on the animal’s left side. Frames 347-355 are depicted in figure 2.3. Frame rate speed has been reduced by 50 times.

**Video 2.6** provides a 3D animation of the merged ring vortex model constructed using the available kinematic data.

**Video 2.7** provides a 3D animation of the indented merged vortex loop model constructed using the available kinematic data.

**Video 2.8** provides a 3D animation of the bilateral vortex loops model constructed using the available kinematic data.

**Video 2.9** provides a 3D animation of the concentric vortex rings model constructed using the available kinematic data.

* videos can be found at: [http://hdl.handle.net/2429/54568](http://hdl.handle.net/2429/54568)
**Video 3.1.** A movie of a bird performing controlled lateral flight to the left. The feeder was moving at 15 cm/second and the video is slowed down 40x.

**Video 3.2** A movie of a bird performing controlled lateral flight to the left through a plume of CO₂. The plume was created by sublimation of dry ice. The feeder was moving at 30 cm/second and the video is slowed down 40x.

**Video 4.1.** The multi-camera, automated tracking system filming two hummingbirds in the flight arena at 200 frames per second. Continuously tracked sequences are assigned an object number (from 0 to 4 over this sequence). Body position and orientation are calculated and reprojected onto the video of four cameras. The videos are saved using a custom designed compression algorithm that only records the sections of the image that are moving. Thus, birds disappear from the video when they land and stop moving. The trajectory shown in figure 4.1 is taken from the bird labeled #2 and begins at 5.1 seconds and ends at 8.05 seconds.
Appendix B: Tracking system parameters

The automated tracking system extracts the 3D coordinates of multiple flying animals and saves each trajectory as a separate object. An object begins when the tracking system picks up new movement and ends when either the object stops moving, the error in the 3D reprojection grows too large, or multiple objects come within 2 cm of each other. In my experiments tracking hummingbird flight led to two problems in determining distinct objects. The first is that very stable hovering can be misidentified as perching. For example, as a bird goes into an extended hovering bout, such as at a feeder, the tracking system considers it to have stopped moving and ends the trajectory. Conversely, when the bird perches at the end of a flight or in between two flights, especially if it continues to move its head or fluff its feathers, the tracking system would consider the bird as moving and would continue tracking. Since my study focuses on identifying and analyzing relatively long, moving trajectories, these types of errors did not cause problems. The second challenge concerns identification of birds during close encounters in competition trials. When two tracked objects get close enough to each other, even if they do not physically touch, the tracking system cannot accurately distinguish them. I used a conservative solution and terminated the trajectories whenever two birds came into close proximity and the tracked objects merged. Birds were later identified manually by a team of digitizers who viewed the videos and assigned each object number to either the marked or unmarked bird.

Smoothing parameters

A simple forward/reverse non-causal Kalman filter was used to minimize the effect of errors in the 3D tracking. The smoothing parameters were chosen so that 7 traces of a tracked, falling object averaged a peak acceleration of 9.8 m/s². The process covariance matrix I used is:
and the observation covariance matrix I used is:

\[
R_{pos} = \begin{bmatrix}
0.000144 & 0 & 0 \\
0 & 0.000144 & 0 \\
0 & 0 & 0.000144
\end{bmatrix}
\]

Figure 4.1c and 4.1d show examples of two trajectories with plots of the unsmoothed data, the data smoothed with \(Q_{pos}\) and \(R_{pos}\), and the effects of two different smoothing parameters (\(R_{pos} \times 10\), \(R_{pos} \times 0.1\)).

Following establishment of the 3D trajectories, the tracking system assigns 3D body orientation vectors to each bird in each frame based on 2D estimates of the long axis of the body. Orientation vector assignments were also smoothed with a Kalman filter using more restrictive smoothing parameters than used to smooth the body position. To determine appropriate smoothing parameters I replotted the smoothed body orientation vectors onto a sample of videos, and visually chose the ones that fit the best. The process covariance matrix used for body orientation is:
and the observation covariance matrix used is:

\[
Q_{\text{ori}} = \begin{bmatrix}
0.0033 & 0 & 0 & 0.005 & 0 & 0 \\
0 & 0.0033 & 0 & 0 & 0.005 & 0 \\
0 & 0 & 0.0033 & 0 & 0 & 0.005 \\
0.005 & 0 & 0 & 0.01 & 0 & 0 \\
0 & 0.005 & 0 & 0 & 0.01 & 0 \\
0 & 0 & 0.005 & 0 & 0 & 0.01 \\
\end{bmatrix}
\]

For my final performance metrics I used instantaneous body orientation and orientation velocity, but not orientation acceleration. Because of this, the choice of smoothing parameters for body orientation had little effect on the final results. Traces of the body orientation and the calculated average orientation velocities at different levels of smoothing (unsmoothed, smoothed, \(R_{\text{ori}} \times 10\), \(R_{\text{ori}} \times 0.1\)) for sample flight trajectories are also shown in figure 4.1c and 4.1d of the main text.

**Orientation assignment**

Once the body orientations were calculated I used a dynamic programming algorithm to decide which end of the vector was the head and which end was the tail. The direction of the head was chosen based on the direction of the previous orientation, the direction of travel, and the vertical up direction. For each frame \((n)\), the “cost” associated with the two possible orientations \((\vec{O}_i, -\vec{O}_i)\) were calculated:
\[ Cost_{\text{Ori}} = \text{Speed} \left( \frac{\vec{Ori}_n \cdot \vec{Vel}_{\text{mod}}}{|\vec{Ori}_n||\vec{Vel}_{\text{mod}}|} \right) + |1 - \text{Speed}| \left( \frac{\vec{Ori}_n \cdot \vec{Up}}{|\vec{Ori}_n||\vec{Up}|} \right) + |1 - \text{Speed}| \left( \frac{\vec{Ori}_n \cdot \vec{Ori}_{n-1}}{|\vec{Ori}_n||\vec{Ori}_{n-1}|} \right) \]

\[ Cost_{\text{Ori}} = \text{Speed} \left( -\frac{\vec{Ori}_n \cdot \vec{Vel}_{\text{mod}}}{|\vec{Ori}_n||\vec{Vel}_{\text{mod}}|} \right) + |1 - \text{Speed}| \left( -\frac{\vec{Ori}_n \cdot \vec{Up}}{|\vec{Ori}_n||\vec{Up}|} \right) + |1 - \text{Speed}| \left( -\frac{\vec{Ori}_n \cdot \vec{Ori}_{n-1}}{|\vec{Ori}_n||\vec{Ori}_{n-1}|} \right) \]

where \( \vec{Ori} \) is the body vector, \( \vec{Vel}_{\text{mod}} \) is the modified velocity vector tipped up 15° towards the vertical direction. \( \vec{Up} \) is the vertical direction vector, \( \vec{Ori}_{n-1} \) is the orientation during the previous frame, and if the magnitude of the velocity is greater than 0.5 m/s:

\[ \text{Speed} = |\vec{Vel}| \]

otherwise:

\[ \text{Speed} = 0.5 \]

This takes into account the propensity of hummingbirds to fly forwards and with an upright posture, but allows for exceptions in the case of backwards flight, inversions, and dives, particularly if they occur at low speeds.
Appendix C: Candidate models of maneuvering performance metrics

Candidate models of maneuvering performance metrics (n = 20 birds in 20 solo trials and 16 paired competition trials). Models are ranked by AICc, with supported models within 2 AICc units of the best-fit model highlighted in bold. Akaike weight is a measure of the probability that a given model is the best-fit model relative to others in that model set.

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