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Effects of Flight Speed upon Muscle Activity in Hummingbirds

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1   **Effects of flight speed upon muscle activity in hummingbirds**

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16   **Running Head:** *Muscle activity in hummingbird flight*

17   **Key words:** Rufous hummingbird, *Selasphorus rufus*, electromyography, sonomicrometry, flight

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1   **Summary**

2   Hummingbirds have the smallest body size and highest wingbeat frequencies of all flying

3   vertebrates, so they represent one endpoint for evaluating the effects of body size on sustained

4   muscle function and flight performance. Other bird species vary neuromuscular recruitment and

5   contractile behavior to accomplish flight over a wide range of speeds, typically exhibiting a U-

6   shaped curve with maxima at the slowest and fastest flight speeds. To test whether the high

7   wingbeat frequencies and aerodynamically-active upstroke of hummingbirds lead to different

8   patterns, we flew rufous hummingbirds (*Selasphorus rufus*, 3 g body mass, 42 Hz wingbeat

9   frequency) in a variable-speed wind tunnel (0 – 10 m s^{-1}). We measured neuromuscular activity

10   in the pectoralis (PECT) and supracoracoideus (SUPRA) muscles using electromyography

11   (EMG, N = 4 birds), and we measured changes in PECT length using sonomicrometry (N = 1).

12   Differing markedly from the pattern in other birds, PECT deactivation occurred before the start

13   of downstroke and the SUPRA was deactivated before the start of upstroke. The relative

14   amplitude of EMG signal in the PECT and SUPRA varied according to a U-shaped curve with

15   flight speed; additionally, the onset of SUPRA activity became relatively later in the wingbeat at

16   intermediate flight speeds (4 and 6 m s^{-1}). Variation in the relative amplitude of EMG was

17   comparable to that observed in other birds but the timing of muscle activity was different. These

18   data indicate the high wingbeat frequency of hummingbirds limits the time available for flight

19   muscle relaxation before the next half stroke of a wingbeat. Unlike in a previous study that

20   reported single-twitch EMG signals in the PECT of hovering hummingbirds, across all flight

21   speeds we observed 2.9 $\pm$ 0.8 spikes per contraction in the PECT and 3.8 $\pm$ 0.8 spikes per

22   contraction in the SUPRA. Muscle strain in the PECT was 10.8 $\pm$ 0.5%, the lowest reported for

23   a flying bird, and average strain rate was 7.4 $\pm$ 0.2 muscle lengths s^{-1}. Among species of birds,

24   PECT strain scales proportional to body mass to the 0.2 power ($\propto M^{0.2}$) using species data and

1    $\propto M^{0.3}$ using independent contrasts. This positive scaling is likely a physiological response to an

2   adverse scaling of mass-specific power available for flight.

3

4   **Introduction**

5   The function of muscle during animal flight is of particular interest because flying

6   requires higher power output than other forms of locomotion (Schmidt-Nielsen, 1972). Efforts to

7   measure mechanical work and power produced by the primary downstroke muscle in birds, the

8   pectoralis (hereafter PECT), have involved surgical implantation of sonomicrometry and strain-

9   gauge transducers (Biewener et al. 1998; Hedrick et al. 2003; Tobalske et al. 2003b, Soman et

10   al. 2005, Tobalske and Biewener, 2008) or sonomicrometry coupled with ergometry (Askew and

11   Ellerby, 2007). From these studies, it is clear that mechanical power output varies with flight

12   speed according to a U-shaped curve (Tobalske et al. 2003; Askew and Ellerby, 2007). To

13   modulate work and power across flight speeds, birds may vary total muscle strain (proportional

14   length change), contractile velocity and the timing and magnitude of motor unit recruitment

15   (Hedrick et al. 2003; Tobalske et al. 2005; Askew and Ellerby, 2007; Tobalske and Biewener,

16   2008). In cockatiel (*Nymphicus hollandicus*), work and power in the PECT is modulated

17   primarily using motor-unit recruitment.

18   Due to their small size, hummingbirds (Trochilidae) are generally hypothesized to

19   represent an extreme among extant birds for their adaptations for powered flight (Altshuler and

20   Dudley, 2002). They are the smallest flying vertebrates, and they are the only birds capable of

21   sustained hovering. Their hovering ability is due, at least in part, to their use of an

22   aerodynamically-active, lifting upstroke (Stolpe and Zimmer, 1939; Tobalske et al. 2007; Warrick

23   et al. 2005, 2009). Consistent with this, their primary upstroke muscle, the supracoracoideus

24   (hereafter, SUPRA) is proportionally larger than it is in other bird species (Stolpe and Zimmer,

25   1939; Greenewalt, 1962).

1   The available evidence for neuromuscular function during hummingbird flight, revealed

2   using electromyography (EMG), suggests that hovering hummingbirds exhibit a unique twitch

3   contraction in their PECT (Hagiwara et al. 1968) consisting of a single, alternating-current EMG

4   spike per wingbeat. In contrast, other bird species exhibit EMG's in their flight muscles that

5   consist of multiple spikes per contraction (Hagiwara et al. 1968, Dial, 1992a; Tobalske and Dial,

6   1994, 2000; Tobalske, 1995; Tobalske et al. 1997; 2005; Hedrick et al. 2003; Ellerby and

7   Askew, 2007). To accomplish fast forward flight, Hagiwara et al. (Hagiwara et al. 1968) report

8   that hummingbirds increase PECT recruitment: the amplitude of EMG increases and the

9   number of spikes per burst increases from 2-5. However, flight velocity was not measured, so it

10   is not possible to interpret this observation in relation to existing models of flight costs (Rayner,

11   1979) or to empirical measures of metabolic power input (Berger, 1985; Clark and Dudley,

12   2009).

13   It is possible that the small number of spikes per burst is due to the limited time for

14   muscle activation and deactivation during high-frequency wingbeats. Hagiwara et al. (Hagiwara

15   et al. 1968) report 8 ms time to peak force during isometric contraction in hummingbirds with

16   wingbeat frequencies near 40 Hz. Assuming downstroke lasts 50% of a wingbeat (Tobalske et

17   al. 2007), these birds would have only 12.5 ms to develop tension and relax their primary flight

18   muscles unless some tension is present in each muscle at wing turnaround (Tobalske and

19   Biewener, 2008).

20   Another unique aspect of hummingbird flight is that they do not regularly engage in

21   intermittent flight. Other small birds routinely use intermittent bounds, glides or both (Tobalske,

22   2001; Tobalske et al. 2007). It has been hypothesized that intermittent pauses are a

23   mechanism for modulating power output in lieu of varying contractile velocity in the flight

24   muscles (Rayner, 1985). Although sonomicrometry has revealed that contractile velocity varies

25   significantly in the pectoralis of flap-bounding birds (Tobalske et al. 2005), the magnitude of this

1   variation has not been compared with an alternative model such as the hummingbird that does

2   not use (or only rarely uses) intermittent flight.

3   Given the small size and unusual flight morphology and style of the hummingbird, we

4   undertook the present study to test whether patterns of neuromuscular recruitment and

5   contractile behavior in hummingbirds were different from those observed in larger bird species.

6

7   **Materials and Methods**

8   Birds and experimental design

9   We measured muscle activity during flight in five female rufous hummingbirds

10 *Selasphorus* rufus Gmelen 1788 (body mass 3.4 g, wingspan 10 cm). We previously reported

11 on the three-dimensional wing and body kinematics along with additional morphological data for

12 the same study subjects (Tobalske et al. 2007). We caught the animals in the wild under

13 permits from the US Fish and Wildlife Service and Oregon Department of Fish and Wildlife. All

14 housing and experimental protocols were approved by the University of Portland Institutional

15 Animal Care and Use Committee. During captivity, birds were housed in 1 m × 1 m × 1 m flight

16 cages with *ad libitum* access to food and water in the form of Nektar-Plus (NEKTON®; Günter

17 Enderle, Pforzheim, Baden-Württemberg, Germany) or a 20% sucrose solution (mass:volume).

18   We flew the birds in an open-circuit, variable-speed wind tunnel, the properties of which

19 have been described previously (Tobalske et al., 2005). The working section of the tunnel is 85

20 cm in length, square in cross section, 60 cm × 60 cm at the inlet and increases to 61.5 × 61.5 at

21 the outlet to accommodate boundary-layer thickening. Maximum deviations in velocity within a

22 cross-section are <10% of the mean, the boundary layer is < 1 cm thick, and turbulence is 1.2%.

23 Wind speed during experiments and for reporting in this paper is equivalent air velocity rather

24 than true air velocity (Pennycuick et al. 1997).

25   Birds were fully acclimated to the flight chamber because we completed unimplanted 3D

26 kinematic studies (Tobalske et al. 2007) prior to beginning the electromyography (EMG) and

1   sonomicrometry experiments that are described herein. We allowed the birds to rest on a perch

2   between trials, and the birds were allowed to feed periodically from a 1-ml syringe containing

3   20% sucrose solution. Although unimplanted rufous hummingbirds can easily sustain flight in

4   the tunnel at 12 m s^{-1} (Tobalske et al. 2007), the birds exhibited diminished performance

5   following implantation of electrodes and transducers (see below) so that the maximum speed

6   was 10 m s^{-1} during EMG experiments (achieved in 3 out of 4 birds) and 8 m s^{-1} during

7   sonomicrometry experiments.

8   To measure the timing of wing motion in relation to muscle activity, and to limit our

9   samples to phases of flight during which velocity and altitude were held approximately constant,

10   we used two synchronized Redlake cameras, a PCI-2000 and PCI-500 (Redlake MASD LLC,

11   San Diego, CA, USA) sampling at 500·frames·s–1 and with a shutter speed of1/2500·s.

12   Flights were illuminated from outside the flight chamber using four 650-W halogen lights (Lowel

13   Tota-light, Lowel-Light Manufacturing, Inc., Brooklyn, NY, USA).

14   Digitizing and 3D reconstruction were accomplished as in Tobalske et al. (Tobalske et al.

15   2007) using custom M-files (Hedrick 2008; available: http://www.unc.edu/~thedrick/)) in

16   MATLAB v7.6.5 (The Mathworks, Inc., Natick, MA, USA), Subsequent analyses of wingtip and

17   wrist trajectories and chord angle (deg) relative to the frontal plane of the body were

18   accomplished using MATLAB and IGOR Pro. (v.6.12a, Wavemetrics, Inc., Beaverton, OR,

19   USA). Downstroke started with wrist depression and upstroke began with wrist elevation. Wing

20   chord was a line connecting the wrist and the distal tip of the 1st secondary, and chord angle

21   was the cranially-oriented acute angle formed between this lead line and the mid-frontal plane of

22   the bird.

23

24   Electromyography and sonomicrometry

25   We measured electromyographic (EMG) activity from the pectoralis (PECT) and

26   supracoracoideus (SUPRA) muscles in four birds. In a different bird, we measured pectoralis

1   strain (Length / Resting length; L / L_{rest}) using sonomicrometry (Tobalske and Biewener, 2008)

2   and EMG activity. The tiny size of the birds precluded the use of sonomicrometry with multiple

3   EMG's.

4   To accomplish implantation, birds were anesthetized using isoflurane inhalant and

5   maintained at a surgical plane. Feathers were removed from the skin over the left pectoralis.

6   An alcohol solution was used to clean the skin. EMG electrodes (Cooner Wire, Inc., Chatsworth,

7   CA, USA; twisted pair,bipolar 0.5·mm exposed tips, 100·µm silver wire) and sonomicrometry

8   transducers (Sonometrics,London, Ontario, Canada; omnidirectional, 0.7·mm, 42·Gauge

9   insulated copper wire) were implanted through the skin and into the muscles. The EMG

10   electrodes were implanted percutaneously using a 25·Gauge hypodermic needle to push the

11   electromyography electrodes into the sternobrachialis (SB) portion of the PECT and through the

12   SB portion of the PECT into the mid-belly of the SUPRA. For sonomicrometry, two holes, 6·mm

13   apart, were made through the overlying skin into the muscle, parallel to the longest muscle

14   fascicles of the SB of the PECT (Tobalske et al, 2005; Welch and Altshuler, 2009). The

15   sonomicrometry crystals were placed ~1.5·mm deep into the holes. We sutured all wires near

16   the point of exit from the PECT to superficial fascia of the muscle and to the skin using using 6-0

17   braided silk.

18   Electrode and transducer leads were woven together and extended 60 cm from the

19   animal before being connected to insulated recording cables using a miniature connector. The

20   total mass of all EMG and transducer leads carried by the birds during experiments was ~0.2·g

21   (6 % of body mass).

22   The animal was allowed to recover from surgery, with experiments beginning within 1.5

23   hrs and lasting approximately 30 min. Following the experiments, a recovery surgery was

24   performed in which all recording equipment was removed from the bird. All of the animals fully

25   recovered from the experiments.

1   Electromyographic signals for the combined PECT and SUPRA measurements were

2   amplified (1000x) and filtered (60·Hz notch, 100·Hz low pass, 5000·Hz high pass) using an AM

3   Systems (Carlsborg, WA, USA) Model 1700 differential AC amplifier. These analog signals were

4   imported to computer using a Digidata 1322 16-bit A/D converter sampling at 5000 Hz and

5   Axoscope v10 software (Axon Instruments, Union City, CA, USA). Video was synchronized

6   using a trigger pulse from the cameras sent to the Digidata converter. Sonomicrometry signals

7   were created and recorded at 1050 Hz using a Sonometrics TRX Series 4 Digital Ultrasonic

8   Measurement System and Sonosoft 3.2.1 software. Synchronization between the

9   sonomicrometry, video and EMG data was obtained by sending a trigger pulse from the video

10 cameras and voltage output from the AM Systems amplifier to a separate channels on the

11 Sonometrics A/D converter.

12   Subsequent analysis of the recorded signals was accomplished using IGOR software.

13 EMG signals remained as recorded in volts, and sonomicrometry data were calibrated to strain

14 (L/L_{rest}) using the resting, perched values for crystal separation as L_{rest}. EMG bursts were

15 identified as one or more spikes with rectified amplitude at least twice the amplitude of baseline

16 electrical noise (Tobalske et al. 2005; Tobalske and Biewener, 2008). For each contractile cycle

17 (=wingbeat cycle), we measured the amplitude (mV) and duration (ms) of EMG activity in the

18 PECT and SUPRA from onset to offset. Average EMG amplitude was obtained by dividing the

19 EMG by duration of activity. We counted the number of spikes within each bout of muscle

20 activity as individual peaks of raw EMG signal that exceeded baseline. We calculated the

21 percentage of the wingbeat cycle in which each muscle was active, with the start of downstroke

22 defined using the onset of wrist depression as in Tobalske et al. (Tobalske et al. 2007). Lastly,

23 we computed relative EMG amplitude as in previous studies (Tobalske et al. 2005; Tobalske

24 and Biewener 2008): average amplitude for each burst within a given bird and muscle was

25 divided by the maximum value exhibited for the muscle.

1   Fractional lengthening (%) and fractional shortening (%) were the proportions of PECT

2   strain in which the PECT was longer than or shorter than resting length, respectively. Average

3   strain rate (muscle·L·s^{-1}) was PECT strain divided by the duration of time between maximum

4   and minimum length as the muscle shortened during a contractile cycle.

5   We analyzed 287 wingbeats for EMG data from the combined measurements of

6   pectoralis and supracoracoideus activity and 82 wingbeats from the combined measurements of

7   pectoralis fascicle strain and EMG activity. This represented at least 10 wingbeats from each

8   speed for each bird used in an experiment.

9

10   Statistical and comparative analysis

11   For each variable, amplitude of EMG, duration of EMG, relative offset of PECT, relative

12   onset and offset of SUPRA and spike number per EMG burst, we computed the mean value

13   within each bird for each flight speed. We then tested for a significant effect of speed upon each

14   variable using a univariate repeated-measures analysis of variance (StatView version 5.0.1,

15   SAS Institute, Inc., Cary, NC,USA). Values throughout are presented as means·±·s.d.

16   To place the effects of velocity upon relative amplitude of EMG in the rufous

17   hummingbird within a comparative context, we used coefficients of variance (*CV* = among-flight

18   speed mean / s.d.). These data were compared with EMG data from a variety of larger bird

19   species that have also been studied over comparable ranges in speed in a wind tunnel (zebra

20   finch *Taeniopygia guttata,* 13 g*,* Tobalske et al., 2005, Ellerby and Askew, 2007; budgerigar,

21   *Melopsittacus undulatus*, 45 g, Ellerby and Askew, 2007; cockatiel, 78.5 g, Hedrick et al., 2003;

22   black-billed magpie, *Pica hudsonica,* 82 g (Tobalske et al. 1997). For this analysis, we divided

23   relative amplitude of EMG exhibited by a given bird and muscle at each flight speed by the

24   maximum value exhibited for that bird and muscle among flight speeds.

25   We also added our present data to data from earlier studies to explore the scaling of

26   PECT strain and average strain rate among species. We computed means from the species that

1   have been studied using sonomicrometry over a wide range of speeds in a wind tunnel or in

2   different flight modes including take-off, level flight and landing: zebra finch (Tobalske et al.

3   2005, Ellerby and Askew, 2007); budgerigar (Ellerby and Askew, 2007); cockatiel (Hedrick et al.

4   2003); black-billed magpie (Warrick et al. 2001), blue-breasted quail, *Coturnix chinensis*, 44 g

5   (Askew et al. 2001); northern bobwhite, *Colinus virginianus*, 200 g, chukar, *Alectoris chukar*,

6   492 g, ring-necked pheasant, *Phasianus colchicus, 943 g, and wild turkey, Meleagris gallopavo,*

7   *5.3 kg,* (Tobalske and Dial, 2000), rock dove 605 g (Biewener et al. 1998; Soman et al. 2005,

8   Tobalske and Biewener, 2008) and mallard, *Anas platyrhynchos*, 1 kg (Williamson et al. 2001).

9   If a species was studied more than once, we used means among studies. The data we selected

10   were always from the anterior sternobrachialis portion of the PECT.

11   We log-transformed these data and tested for a significant effect of body size upon strain

12   and average strain rate using reduced-major axis regressions of independent contrasts

13   (Felsenstein, 1985; Garland et al. 1992, 2005) in the PDAP package (Midford et al. 2003) for

14   Mesquite (v2.5; Maddison and Madison, 2008). Contrasts were positivized prior to regression.

15   We developed a phylogenetic tree (Supplement 2) using trees based on molecular data in

16   Hackett et al. (Hackett et al.2008) for among-family relationships and Kimball and Braun

17   (Kimball and Braun, 2008) for relationships within the Phasianidae. We set all branch lengths =

18   1 (i.e., punctuated equilibrium model), and we confirmed that the contrasts were adequately

19   standardized (Garland et al. 2005). We also confirmed that our conclusions regarding scaling

20   exponents and statistical significance were robust when changes in the phylogeny were

21   introduced using alternative trees based on molecular (Ericson et al. 2006) or anatomical

22   (Livezey and Zusi, 2007) evidence. To aid in visual interpretation of the scaling patterns, we

23   include graphs of species (tip) data along with graphs of independent contrasts.

24

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1   **Results**

2   The PECT and SUPRA each exhibited a multi-spike burst of EMG activity per wingbeat

3   (Figs 1 and 2). There were significantly more spikes in the SUPRA signal (3.8 ± 0.2, range 1 -

4   8) compared with the PECT (2.9 ± 0.2 bursts, range 1 -6; paired t-test, $P = 0.02$, d.f. = 3).

5   Although SUPRA EMG duration was slightly longer (5.4 ± 0.6 ms) than PECT EMG duration

6   (4.9 ± 7 ms) the difference was not significant ($P = 0.11$). These durations represented $21 \pm 2\%$

7   and $23 \pm 2\%$ of the wingbeat cycle, respectively (Fig. 3A). Flight speed did not have a

8   significant effect upon spike number per EMG (repeated-measures ANOVA, $P = 0.95$ for PECT

9   and $P = 0.7593$ for SUPRA, d.f. = 2,5 per test) or duration of EMG ($P = 0.31$ for PECT and $P =$

10   0.19 for SUPRA, d.f. = 2, 5).

11   In the bird for which we measured PECT length using sonomicrometry (Fig. 2), average

12   strain was $10.8 \pm 0.5\%$. Fractional lengthening (relative to perched, resting length) was $4.4 \pm$

13   1.6% and fractional shortening was $-6.4 \pm 1.8\%$ (Fig. 4). Average strain rate during PECT

14   shortening was 7.4 ± 0.2 muscle lengths s^{-1} (Supplement 2). Although total strain and average

15   strain rate did not exhibit consistent trends with flight speed, there was a tendency for fractional

16   lengthening to decrease, and fractional shortening to increase, as speed increased (Fig. 4). For

17   example, fractional lengthening was $6.9 \pm 1.2\%$ during hovering and 2.6 ± 0.7 during flight at 6

18   m s^{-1}.

19   The onset and offset of EMG activity in the PECT occurred during kinematic upstroke,

20   prior to depression of the wrist relative to the frontal plane of the body, and EMG activity in the

21   SUPRA occurred during kinematic downstroke (Figs. 1 – 3). These patterns were different from

22   those in other birds in which PECT activation extends into kinematic downstroke and SUPRA

23   activation extends into kinematic upstroke (Fig. 3B, Tobalske and Biewener, 2008). In the

24   hummingbird PECT activity was in the middle of pronation phase of the mid-wing chord relative

25   to the frontal plane of the body, and SUPRA activity was concurrent with a transition from

26   pronation to supination of the wing chord (Figs. 1 and 2). Average wingbeat frequency was 42

1    $\pm$ 2 Hz, so wingbeat duration was 24 $\pm$ 1 ms. The transition from downstroke to upstroke

2   occurred at 51 $\pm$ 5% of the wingbeat cycle. PECT onset preceded the onset of depression of

3   the wrist by 29 $\pm$ 4% of the cycle and offset of PECT EMG preceded wrist depression by 9 $\pm$

4   2%. SUPRA onset and offset preceded wrist elevation by 39 $\pm$ 3% and 16 $\pm$ 2% of cycle

5   duration. The only significant effect of flight speed upon these variables was for relative onset

6   of SUPRA activity, which was earliest during flight at 0 and 2 m s^{-1} (38% of cycle) and increased

7   wing increasing flight speed to be at 43% of cycle during flight at 8 and 10 m s^{-1} (repeated-

8   measures ANOVA, P =0.002, d.f. = 2, 5).

9   There was a time lag between wrist movement and tip movement potentially due to

10   inertial or aerodynamic loading of the wing (Fig. 1), and there was also a time lag between the

11   onset of PECT shortening and the onset of wrist depression relative to the frontal plane of the

12   body (Fig. 2). The duration of these lags was near or less than the time resolution of our 500 Hz

13   video (i.e., $\pm$ 2 ms). For example, average lag time between wrist depression and wingtip

14   depression in the wingbeats in Figure 1 was 2 $\pm$ 1 ms, and average lag time between PECT

15   shortening and wrist depression for the wingbeats in Figure 2 was 3.3 $\pm$ 0.3 ms (14% of

16   wingbeat cycle).

17   The relative amplitude of EMG signal from the PECT and SUPRA varied according to a

18   U-shaped curve with flight speed (Fig. 5A). Values for relative amplitude of EMG were strongly

19   correlated between the two muscles (r = 0.99). The effect of flight speed was statistically

20   significant for the relative amplitude of EMG in the SUPRA (P = 0.014, d.f., 2, 5) and marginally

21   non-significant in the PECT (P = 0.059, d.f., 2, 5). For both muscles, the largest relative

22   amplitude of EMG was exhibited during flight at 10 m s^{-1} (PECT = 0.75 $\pm$ 0.02, SUPRA = 0.83 $\pm$

23   0.03) and the smallest amplitude was during flight at 6 m s^{-1} (PECT = 0.46 $\pm$ 0.04, SUPRA = 0.4

24   $\pm$ 0.1).

25   We observed a positive correlation between relative amplitude of EMG and average

26   wingbeat amplitude (deg, Tobalske et al. 2007; Fig. 5B) and relative amplitude of EMG and

1   average angular velocity of the wing (rad s^{-1}; Tobalske et al. 2007). For wingbeat amplitude and

2   PECT EMG amplitude, r = 0.77 and, for SUPRA EMG amplitude, r = 0.79. Trends were weaker

3   for regressions on angular velocity of the wing. We compared PECT EMG with angular velocity

4   during downstroke and SUPRA EMG with angular velocity during upstroke (Tobalske et al.

5   2007). Correlations were 0.49 for PECT EMG and 0.70 for SUPRA EMG.

6   Comparing the effects of flight speed upon relative amplitude of EMG in the PECT, it is

7   clear that the range of variation exhibited by hummingbirds is in the middle of the range

8   exhibited by other bird species (Fig. 6A). Coefficient of Variance (*CV*) for the hummingbird

9   PECT was 25%. This is similar to the *CV* for the 79-g cockatiel (28%; Hedrick et al. 2003). In

10   contrast, *CV* for the 13-g zebra finch is less (8%, Tobalske et al. 2005; 11%, Ellerby and Askew,

11   2008) and *CV* in the 182-g black-billed magpie is greater (44%). Fewer comparative data are

12   available for the SUPRA (Fig. 6B), but, consistent with the pattern for the PECT, *CV* for the

13   SUPRA in the black-billed magpie is greater (70%) than in the hummingbird (27%).

14   Also in a comparative context (Fig. 7), we observed a significant effect of body mass

15   upon PECT strain and average strain rate. For the log-transformed species (tip) data shown in

16   Fig. 6, strain scales with body mass to the 0.2 power ($M^{0.2}$) ^{strain}, r = 0.69, and average strain

17   rate $\propto M^{-13}$, r = -0.59.   Statistical analyses using independent contrasts of log-transformed data

18   showed PECT strain $\propto M^{0.26}$, r = 0.78, *P* = 0.003, d.f. = 10, and average PECT strain rate $\propto M$

19   $^{0.13}$, r = -0.63, *P* = 0.04, d.f. = 9.

20

21   **Discussion**

22   Our results provide novel insight into the effects of small body size upon neuromuscular

23   activity and the muscle strain during bird flight. It was surprising that rufous hummingbirds

24   exhibited multiple spikes per EMG burst during hovering (Fig. 1), that timing of PECT and

25   SUPRA activity preceded the half stroke of the wingbeat for which the muscles are shortening

1   (Figs 1 -3), and that PECT strain, at 10.8%, was much less than PECT strain in other species

2   (Figs. 2 and 7).

3   A previous study (Hagiwara et al. 1968) reported single spikes of EMG activity in the

4   PECT per wingbeat for three different species of hummingbird during hovering, although a

5   recording from an Allen's hummingbird (*Selasphorus sasin*) exhibited some multiple-spike EMG

6   bursts along with single-spike bursts immediately preceding a transition from hovering to

7   forward flight. The rufous hummingbirds in our study exhibited multiple spikes per EMG burst in

8   the PECT and SUPRA at all flight speeds (Figs. 1 and 2). Moreover, while data from the other

9   species showed the birds increased the number of spikes per wingbeat to accomplish forward

10   flight, our results did not indicate a significant effect of flight speed in rufous hummingbirds. The

11   species studied by Hagiwara et al. (Hagiwara et al. 1968) are 1.2 to 1.3 times larger than the

12   rufous hummingbirds in our study (4 – 4.5 g versus 3.4 g), which may indicate an effect of body

13   size on neuromuscular recruitment. Given our small sample size, the statistical power for our

14   tests was often low, so any lack of significant effect of flight speed upon spike number must to

15   be interpreted with caution. For example, the power for our test of an effect of flight speed upon

16   spike number per PECT burst was 0.081 and for the SUPRA it was 0.132. Thus, these

17   differences among species need to be tested using comparative analysis (e.g. Altshuler et al.

18   2004) probably involving wind tunnel measurements to accomplish flight over a range of

19   speeds.

20   Regardless of differences among hummingbird species, our study supports a general

21   conclusion that activity of the flight muscles of hummingbirds features fewer spikes per EMG

22   burst than are exhibited in other birds (Tobalske and Dial, 1994; 2000; Tobalske, 1995;

23   Tobalske et al. 1997, 2003, 2005; Hedrick et al., 2003, Soman et al. 2005; Tobalske and

24   Biewener, 2008). As in other birds, however, the presence of multiple spikes per burst reveals

25   temporal or spatial variation in motor-unit recruitment (Loeb and Gans, 1986). Raw EMG

26   signals from species within the Phasianidae (pheasants and allies) suggest that spike number

1   may decrease as body size decreases (Tobalske and Dial, 2000). Collectively, these data

2   appear consistent with a hypothesis that there is a positive relationship between body size, time

3   available for neuromuscular activation and spike number.

4   Our results indicate that hummingbirds, due to their high frequency of wingbeat, are

5   approaching a limit for the time necessary for contraction and relaxation of the fast-oxidative

6   glycolytic fibers that make up their flight muscles (Welch and Altshuler, 2009). We observed an

7   offset between PECT and SUPRA activity and kinematic downstroke and upstroke, respectively

8   (Figs 1 – 3). Sonomicrometry shows that the PECT is deactivated approximately concurrent

9   with the onset of PECT shortening, whereas EMG activity is present during the first third of

10   PECT shortening in many larger birds including the pigeon (Tobalske and Biewener, 2008; Fig.

11   3B). In isometric stimulation (Hagiwara et al., 1968) hummingbird muscle has a time to peak

12   force of 8 ms and a decay time of 20 ms or more. These values may be different during cyclical

13   contractions (e.g. Askew and Marsh, 1998), but their magnitude relative to the 12-ms duration of

14   each half stroke during the hummingbird wingbeat helps explain why neuromuscular

15   deactivation precedes wing motion (Figs. 1 - 3).

16   Although superficially suggested by the EMG activity and kinematics, it is unlikely that

17   the electrical activity and muscle force development are completely out of phase. The

18   beginning of wrist elevation and depression almost certainly do not represent the onset of all

19   kinematic or biomechanical effects of the PECT and SUPRA, because, as in other birds, it is

20   likely these muscles do more than elevate and depress the wing. The forces from the PECT

21   and SUPRA are used to decelerate the wing, stabilize the shoulder and effect long-axis rotation

22   of the humerus at transitions between half strokes (Poore et al. 1997; Tobalske and Biewener,

23   2008). Furthermore, at least during hovering, hummingbirds produce rotational circulation and

24   lift at wing turnaround using long-axis pronation and supination of the wing (Warrick et al. 2009).

25   The increasing delay in relative onset of activity in the SUPRA with increasing flight

26   speed is likely related to changes in the magnitude of wing supination during upstroke. During

1   hovering, chord angle (supination) is 93°, and this angle decreases with each increase in

2   forward flight speed to reach 23° during flight at 10 m s^{-1} (Tobalske et al. 2007). With less

3   supination, then, it appears that the SUPRA is recruited later in the cycle. The decrease in

4   upstroke supination with increasing flight speed may also help account for decreasing fractional

5   lengthening of the PECT as speed increases (Fig. 4).

6   Our data from hummingbirds extend the range of body mass that supports an emerging

7   trend for the scaling of muscle strain with body mass in flying birds (Figs. 2 and 7). Within the

8   Phasianidae, Tobalske and Dial (2000) observed that muscle strain scales $\propto M^{0.23}$, but data

9   from a small species, blue-breasted quail, was contradictory to this trend (Askew et al. 2001).

10   Placing data from hummingbirds in a broad comparative context (including the blue-breasted

11   quail) again suggests that PECT strain scales positively with increasing body mass. The

12   observed positive scaling of strain with body size likely represents a response to an adverse

13   scaling of mass-specific power available from the flight muscles for sustained flight (Hill, 1950;

14   Pennycuick, 1975; Tobalske and Dial, 2000). Assuming isometric scaling of other muscle

15   quantities and wingbeat frequency $\propto M^{0.33}$, a scaling of muscle strain $\propto M^{0.33}$ would be sufficient

16   to keep mass-specific power output constant under these classical models. However, given the

17   inter-relationship between strain rate and stress, scaling of one quantity with no change to the

18   others is unlikely and the observed scaling probably represents a compromise among various

19   factors. A negative scaling of average strain rate with increasing body mass ($\propto M^{0.}13$) also

20   merits further study (Fig. 7C, D). The pattern suggests a departure from dynamic similarity in

21   the use of the PECT.

22   It is important to note that the largest species in our comparative analysis (e.g. mallard,

23   phasianids including the ring-necked pheasant and wild turkey) were measured during take-off

24   or escape flight (Tobalske and Dial, 2000; Williamson et al. 2001; Supplement 1), and our

25   sample from the literature therefore likely includes some bias toward higher-power forms of

1   flight in the larger birds. Nonetheless, when comparing average PECT strain in birds that were

2   flown over a wide range of speeds, the trend of an increase in strain with increasing body mass

3   remains. The 10.8% strain we observed in hummingbirds is much less than the 33% and 39%

4   strains reported for black-billed magpies and cockatiels when flying over a wide range of speeds

5   (Warrick et al. 2001; Hedrick et al. 2003).

6   The humeral morphology in hummingbirds likely contributes to their ability to accomplish

7   flight using relatively small strain in the PECT. Their humerus is hypothesized to be specifically

8   adapted for hovering flight (Stolpe and Zimmer, 1939; Karhu, 1992; Mayr, 2003). Hummingbirds

9   have a relatively short humerus with a proportionally massive deltopectoral crest and a medially-

10   directed humeral head with a medio-cranial oriented condyle; this anatomy is thought to permit

11   a strongly-adducted humeral posture compared with the posture in other birds (Stolpe and

12   Zimmer, 1939; Karhu, 1992). A prediction that follows is that much of wing sweep (amplitude) is

13   accomplished using long-axis rotation of the humerus in hummingbirds; whereas, the same

14   movement is accomplished using humeral elevation and depression in other species (Stolpe

15   and Zimmer, 1939; Karhu, 1992). It is also thought that the medio-cranial condyle facilitates

16   sweep of the humerus to accomplish pronounced wing supination during upstroke when

17   hovering or flying slowly (Stolpe and Zimmer, 1939; Kardhu, 1992; Mayr, 2003). These

18   anatomical features of hummingbirds may create proportionally small moment arms for the

19   PECT and SUPRA so that small muscle strains generate large amplitude wingbeats.

20   A lack of significant variation in spike number and duration of EMG burst might suggest

21   limited scope in hummingbirds for modulating muscle activity compared with other species of

22   small birds such as zebra finch (Tobalske et al. 2005), but hummingbirds exhibit greater

23   variation in EMG amplitude than do zebra finch and budgerigar (Tobalske et al. 2005; Ellerby

24   and Askew, 2007). The other small species regularly use intermittent flight styles, with flap-

25   bounding observed in both species and flap-gliding in the budgerigar (Tobalske and Dial, 1994;

26   Tobalske et al. 1999). All three species have pectoralis muscles made up of the same type of

1   fast-oxidative glycolytic fibers (Rosser and George, 1986; Welch and Altshuler, 2009). Thus, the

2   pattern in hummingbirds, which typically use continuous flapping, may indicate the magnitude of

3   modulation of motor unit recruitment that is required to fly over a range of speeds without using

4   intermittent flight.

5   Relative amplitude of EMG is positively correlated with muscle force, work and power in

6   cockatiels (Hedrick et al. 2003). If the same correlation is present in hummingbirds, our

7   measurements of EMG amplitude from the PECT and SUPRA indicate that the hummingbird's

8   minimum power speed (V_{mp}; Rayner, 1979; Hedenström and Alerstam, 1995) is 6 m s^{-1} and

9   maximum range speed (V_{mr}) is 10 m s^{-1} (Figs. 4 and 5). Of course, such conclusions are

10   speculative and require further study. However, recent metabolic measurements of Anna's

11   hummingbirds (*Calypte anna*, controls with intact tails) exhibit the same V_{mp} at 6 m s^{-1} (Clark

12   and Dudley, 2009).

13   As amplitude of EMG is positively correlated with wingbeat amplitude (Fig. 5B), it is also

14   tempting to conclude that the pattern supports the 'mechanical-oscillator' hypothesis , a model

15   for hummingbird wing motion (Greenewalt, 1960) in which it is predicted that muscle force and

16   strain are correlated. However, among flight speeds in the one bird for which we measured

17   muscle strain using sonomicrometry, we observed a negative correlation between EMG

18   amplitude and muscle strain (r = -0.57) as well as between muscle strain and wingbeat

19   amplitude (r = -0.20). This is quite different from the positive correlation between direct

20   measurements of force, strain and EMG amplitude in the cockatiel (Hedrick et al. 2003).

21   The skeletal anatomy of the hummingbird wing may potentially account for this

22   discrepancy. In addition to the hypothesized contribution of the humerus to wing sweep and

23   long-axis rotation described above, it is thought that relatively open or flat articular surfaces at

24   the elbow and wrist facilitate long-axis rotation even when the wing is extended (Stolpe and

25   Zimmer, 1939). In contrast, in most other birds, the forearm is thought to represent a four-bar

26   linkage with limited long-axis rotation (Dial 1992b; Vazquez, 1994) and ridges and grooves in

1   the articular surfaces at the wrist are hypothesized to serve as a locking mechanism, preventing

2   rotation of the distal wing when the wing is extended (Vazquez, 1992). Thus, if hummingbirds

3   vary wrist excursion, in part, using rotation at the elbow and twist in the forearm, some

4   modulation in wingbeat amplitude could be accomplished without direct variation in humeral

5   rotation and PECT strain.

6   Caution is necessary in interpreting this result from one hummingbird. In addition to

7   uncertainties due to a sample $N = 1$, the bird may have been relatively fatigued due to the more-

8   extensive surgery required for implantation of sonomicrometry crystals. Compared with the

9   other birds that were implanted only with EMG electrodes, the bird implanted with

10   sonomicrometry crystals would not fly at 10 m s^{-1} and, in general, its flights were of shorter

11   duration.

12

16

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26

1   **Figure Legends**

2   Figure 1. Wing kinematics (amplitude of wingtip and wrist, chord angle at mid-wing) and

3   electromyographic (EMG) activity in the PECT and SUPRA of a rufous hummingbird

4   (*Selasphorus rufus*) during hovering flight in a wind tunnel. The shaded areas represent

5   downstrokes defined using motion of the wrist. Arrows indicate supination and pronation as

6   measured at mid-wing.

7

8   Figure 2. Contractile activity in the PECT and wing kinematics (wrist amplitude and chord angle

9   of mid-wing) of a rufous hummingbird (*Selasphorus rufus*) during flight at 6 m s^{-1} in a wind

10   tunnel. Muscle length was measured using sonomicrometry. The shaded areas represent

11   downstrokes as indicated by wrist motion. Wrist amplitude and chord angle are with reference

12   the frontal plane of the body. Arrows indicate the direction of supination and pronation as

13   measured at mid-wing. The electromyography (EMG) recorded with sonomicrometry was

14   limited to a 1050-Hz recording frequency and was not typical of the EMG data from other birds

15   (e.g. Fig. 1).

16

17   Figure 3. Relative timing of activation in the PECT and SUPRA of (A) rufous hummingbirds

18   (*Selasphorus rufus*, $N = 4$) and (B) comparable data from rock dove *Columba livia* (from

19   Tobalske and Biewener, 2008). Data are pooled from all flight velocities (0 – 10 m s^{-1}) in

20   hummingbirds and among flight modes (ascending, level and descending) in pigeons.

21   Downstroke is defined using wrist motion for the hummingbird and pectoralis length in the

22   pigeon.

23

24   Figure 4.   Muscle strain and fractional length changes in the PECT according to flight velocity.

25   Resting length is indicated by the origin. Values are mean $\pm$ s.d. among wingbeats ($N \geq 10$ per

26   velocity) for one rufous hummingbird (*Selasphorus rufus*).

1

2   Figure 5. Electromyographic (EMG) activity in the PECT and SUPRA of rufous hummingbirds

3   (*Selasphorus rufus*, $N = 4$) flying at velocities of 0-10 m s^{-1}. (A) Relative amplitude of EMG as a

4   function of flight velocity and (B) relative amplitude of EMG as a function of wingbeat amplitude

5   (Tobalske et al. 2007). Formulas are for reduced-major axis regressions. Filled circles = PECT,

6   Open circles = SUPRA.

7

8   Figure 6. Comparisons among bird species for relative amplitude of EMG in (A) PECT and (B)

9   SUPRA as a function of flight speed in a wind tunnel. Values shown are means. Red = rufous

10   hummingbird *Selasphorus rufus* (this study); orange = zebra finch *Taeniopygia guttata*

11   (Tobalske et al., 2005) and (2) (Ellerby and Askew, 2007); blue = Budgerigar *Melopsittacus*

12   *undulatus* (Ellerby and Askew, 2007); green = cockatiel (Hedrick et al., 2003); black = black-

13   billed magpie *Pica hudsonica* (Tobalske et al. 1997).

14

15   Figure 7. Reduced-major axis regressions and correlation coefficients describing the effects of

16   body mass upon (A and B) strain in the PECT and (C and D) average strain rate in the PECT.

17   Variables are log-transformed. A and C show species (tip) data and B and D are for

18   independent contrasts. For A and C, open circles = species engaged in take-off and escape,

19   filled-circles = species engaged in level flight, a range of flight modes or velocities.
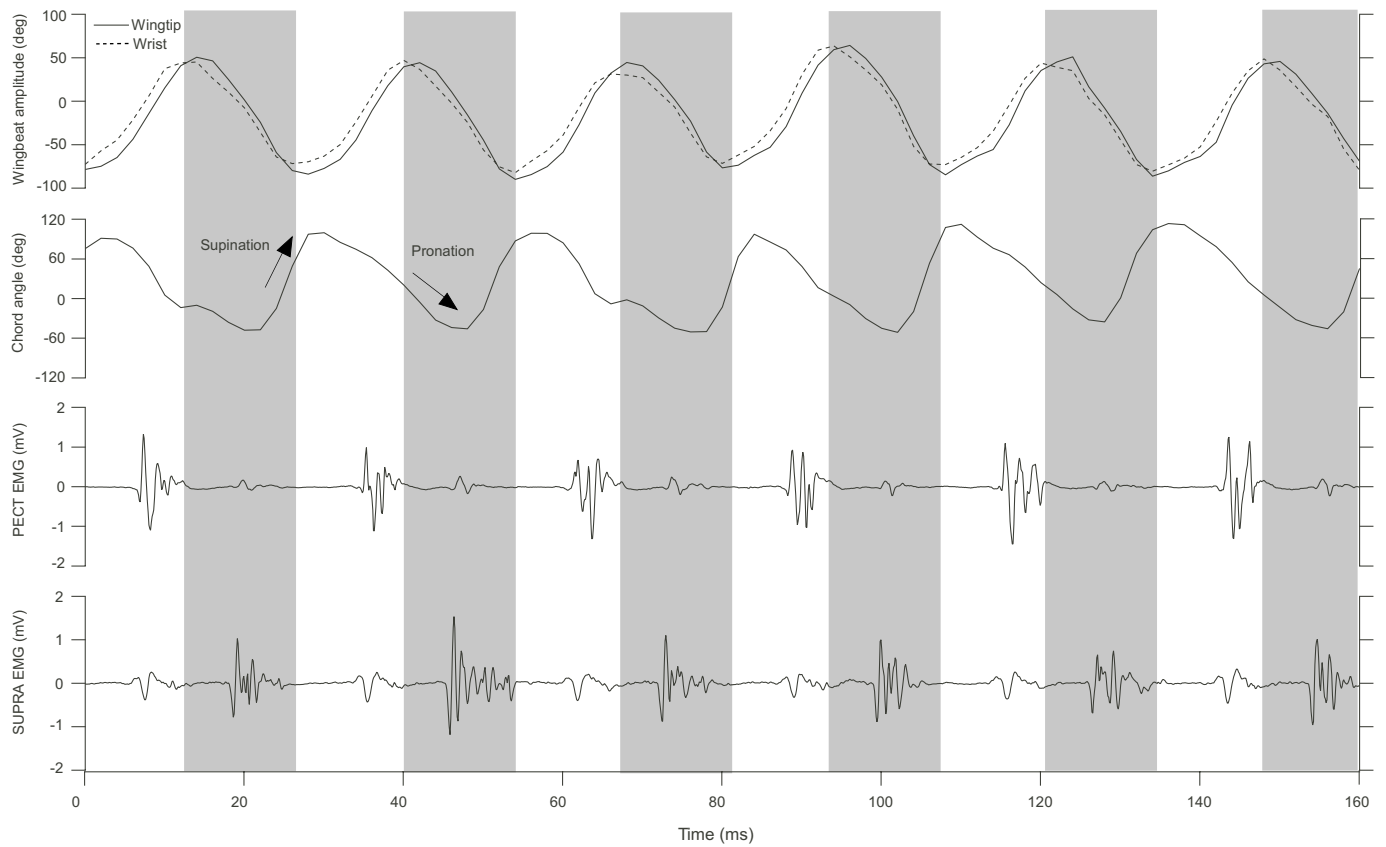
Figure 1. Wing kinematics (amplitude of wingtip and wrist, chord angle at mid-wing) and electromyographic (EMG) activity in the PECT and SUPRA of a rufous hummingbird (*Selasphorus rufus*) during hovering flight in a wind tunnel. The shaded areas represent downstrokes defined using motion of the wrist. Arrows indicate supination and pronation as measured at mid-wing.
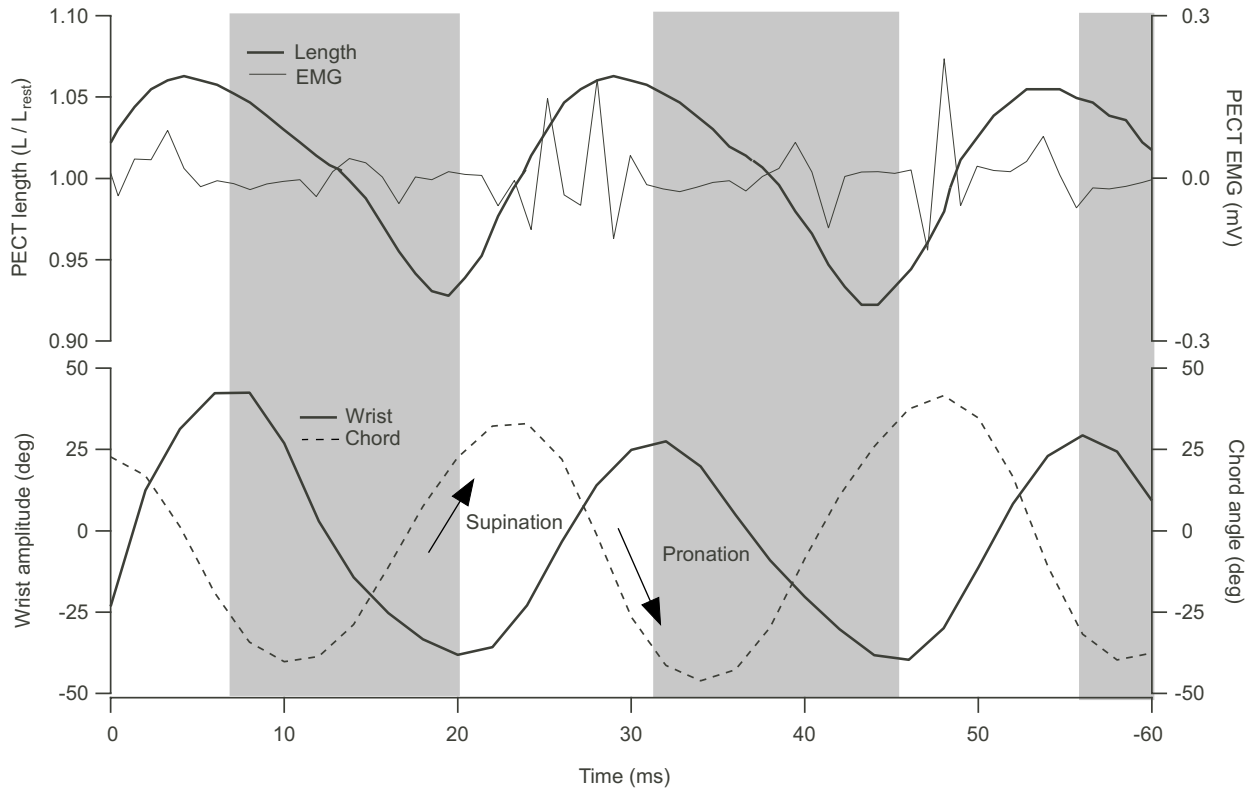
Figure 2. Contractile activity in the PECT and wing kinematics (wrist amplitude and chord angle of mid-wing) of a rufous hummingbird (*Selasphorus rufus*) during flight at 6 m s^{-1} in a wind tunnel. Muscle length was measured using sonomicrometry. The shaded areas represent downstrokes as indicated by wrist motion. Wrist amplitude and chord angle are with reference the frontal plane of the body. Arrows indicate the direction of supination and pronation as measured at mid-wing. The electromyography (EMG) recorded with sonomicrometry was limited to a 1050-Hz recording frequency and was not typical of the EMG data from other birds (e.g. Fig. 1).
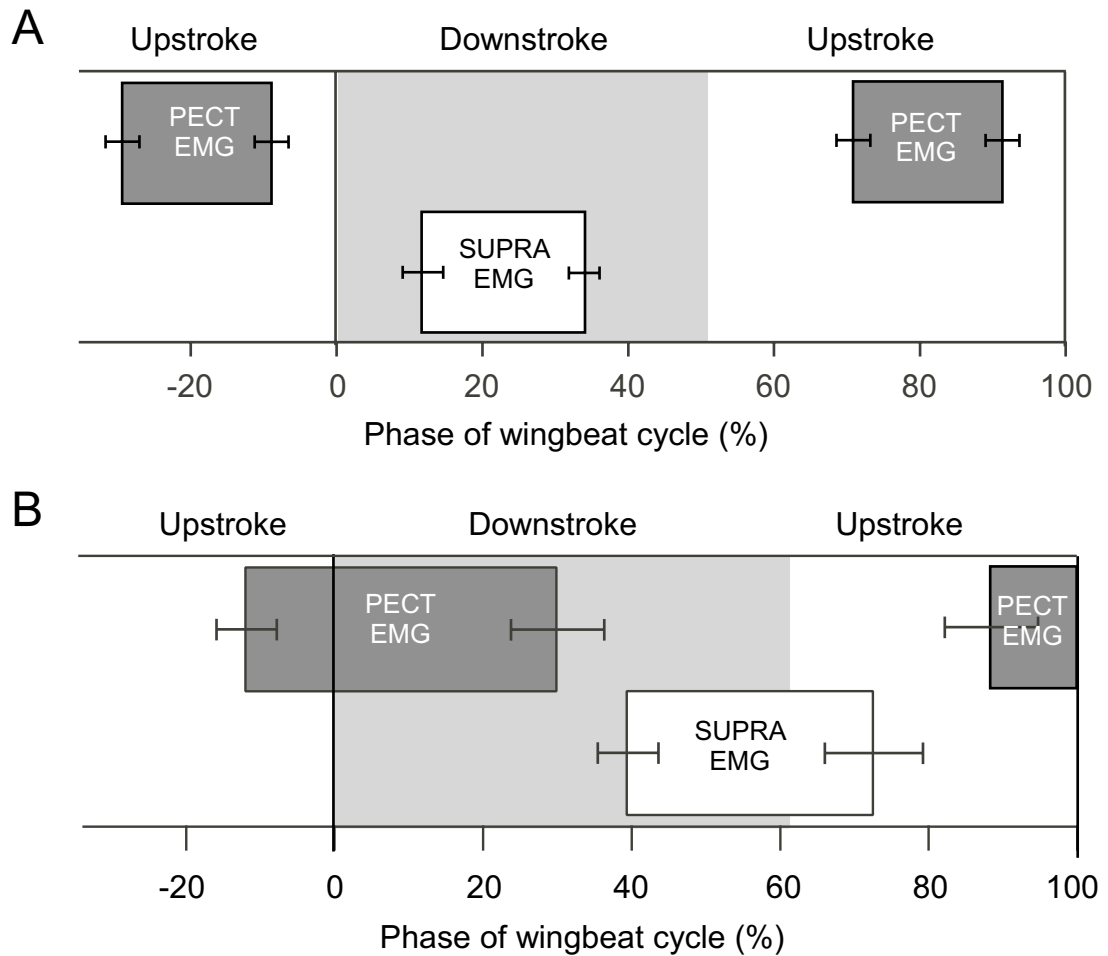
Figure 3. Relative timing of activation in the PECT and SUPRA of (A) rufous hummingbirds (*Selasphorus rufus*, *N* = 4) and (B) comparable data from rock dove *Columba livia* (from Tobalske and Biewener, 2008). Data are pooled from all flight velocities (0 – 10 m s^{-1}) in hummingbirds and among flight modes (ascending, level and descending) in pigeons. Downstroke is defined using wrist motion for the hummingbird and pectoralis length in the pigeon.
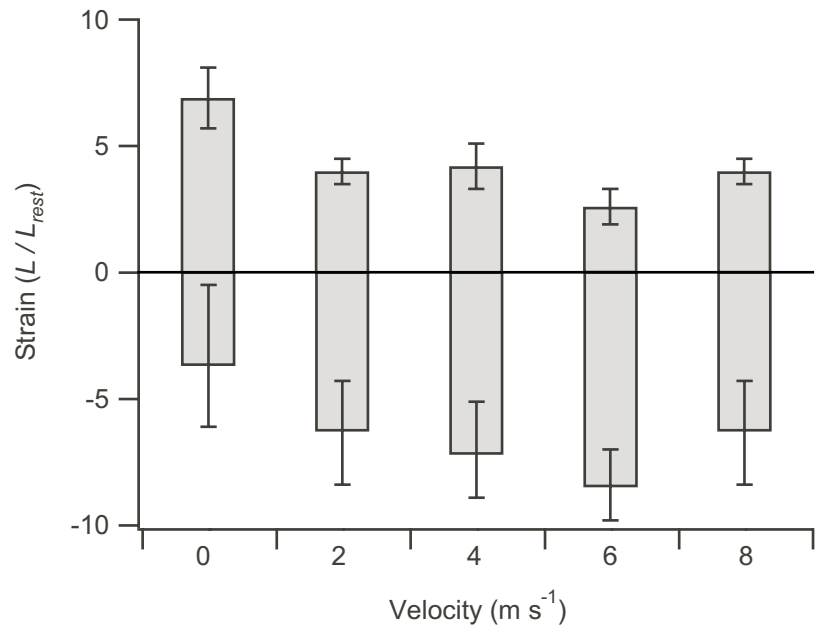
Figure 4. Muscle strain and fractional length changes in the PECT according to flight velocity. Resting length is indicated by the origin. Values are mean ± s.d. among wingbeats ($N \geq 10$ per velocity) for one rufous hummingbird (*Selasphorus rufus*).
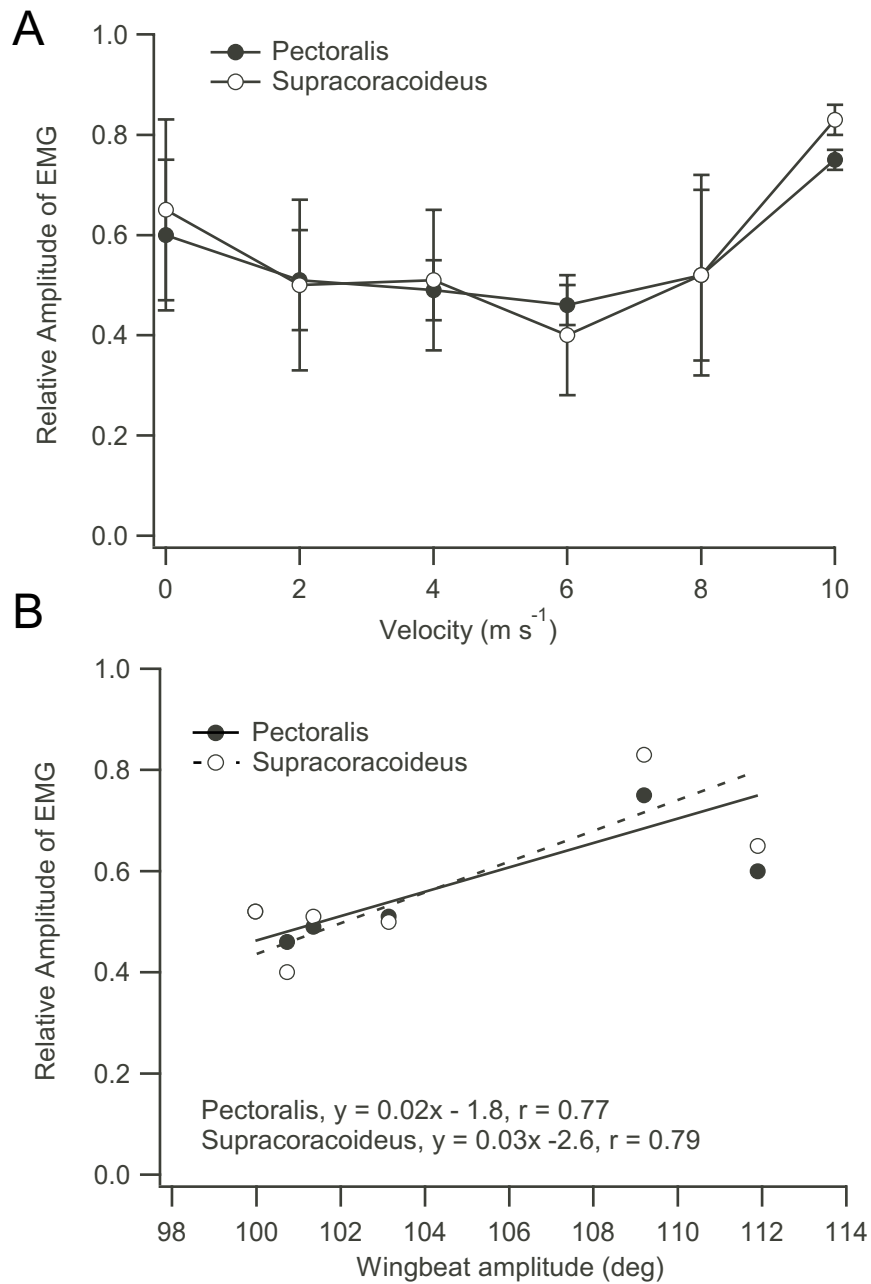
Figure 5. Electromyographic (EMG) activity in the PECT and SUPRA of rufous hummingbirds (*Selasphorus rufus*, $N = 4$) flying at velocities of 0-10 m s^{-1}. (A) Relative amplitude of EMG as a function of flight velocity and (B) relative amplitude of EMG as a function of wingbeat amplitude (Tobalske et al. 2007). Formulas are for reduced-major axis regressions. Filled circles = PECT, Open circles = SUPRA.
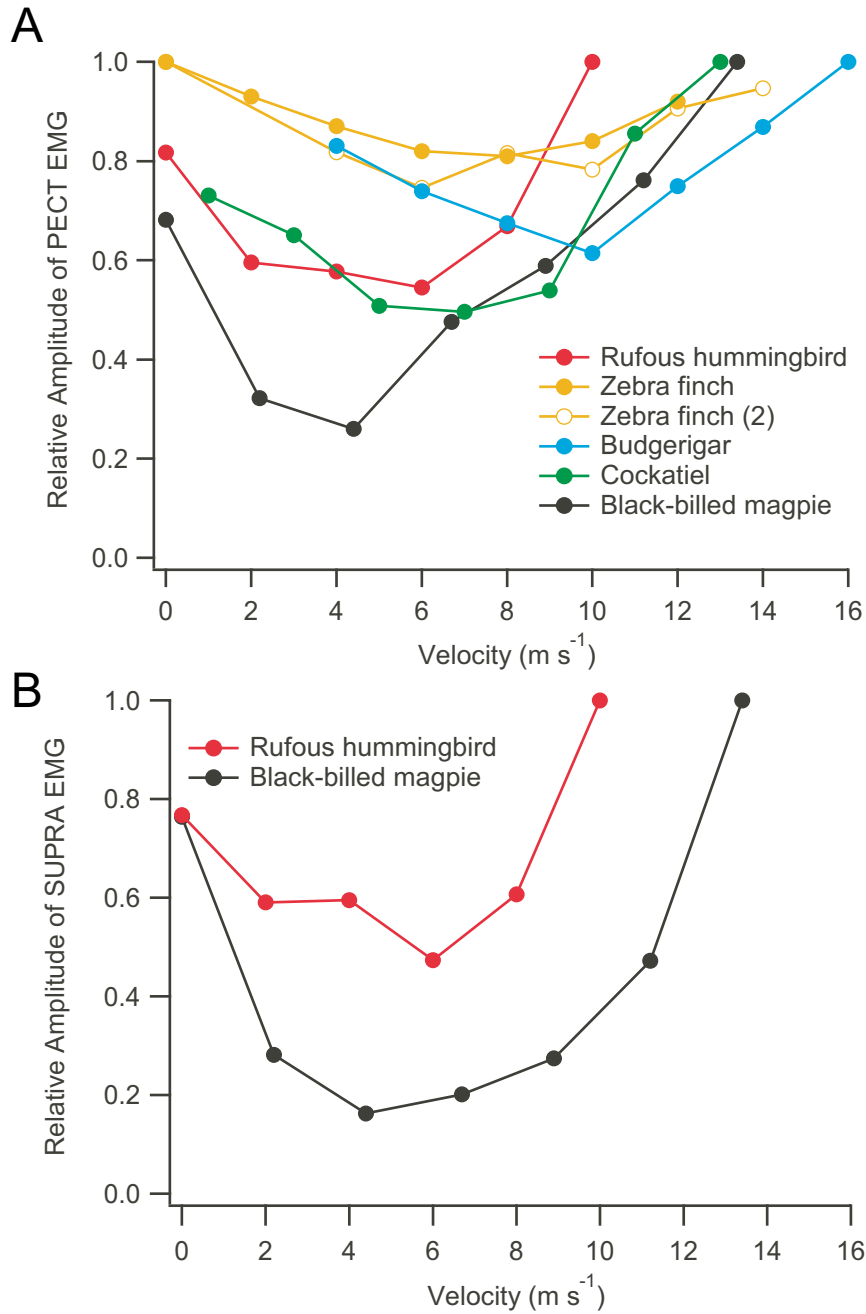
Figure 6. Comparisons among bird species for relative amplitude of EMG in (A) PECT and (B) SUPRA as a function of flight speed in a wind tunnel. Values shown are means. Red = rufous hummingbird *Selasphorus rufus* (this study); orange = zebra finch *Taeniopygia guttata* (Tobalske et al., 2005) and (2) (Ellerby and Askew, 2007); blue = Budgerigar *Melopsittacus undulatus* (Ellerby and Askew, 2007); green = cockatiel (Hedrick et al., 2003); black = black-billed magpie *Pica hudsonica* (Tobalske et al. 1997).
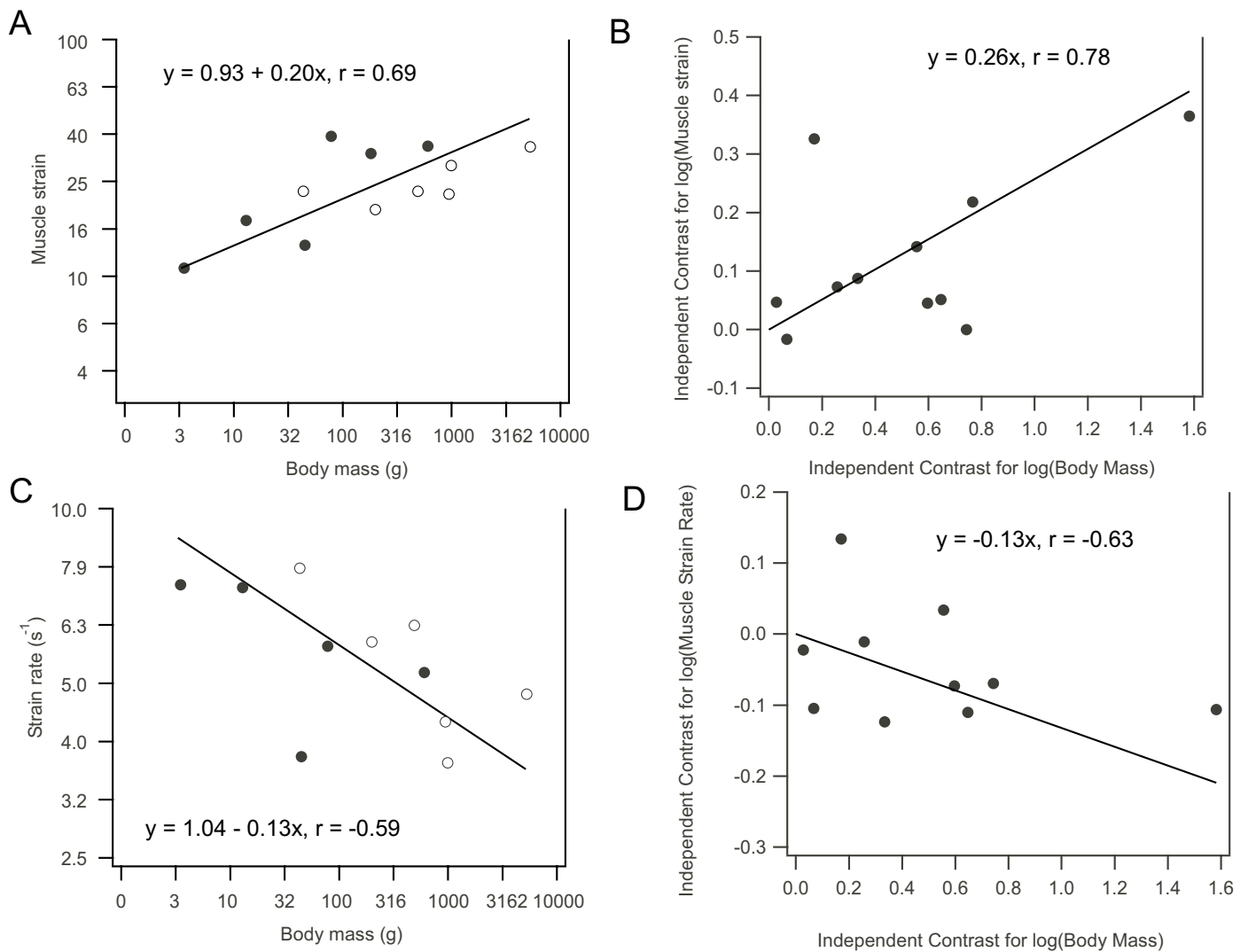
Figure 7. Reduced-major axis regressions and correlation coefficients describing the effects of body mass upon (A and B) strain in the PECT and (C and D) strain rate in the PECT. Variables are log-transformed. A and C show species (tip) data and B and D are for independent contrasts. For A and C, open circles = species engaged in take-off and escape, filled-circles = species engaged in level flight, a range of flight modes or velocities.