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Wingbeat kinematics and energetics during weightlifting in hovering hummingbirds across an elevational gradient

Derrick J. E. Groom[1,2] · M. Cecilia B. Toledo[3] · Kenneth C. Welch Jr.[1,2]

Abstract Hummingbirds differentially modify flight kinematics in response to the type of challenge imposed. Weightlifting is associated with increases in stroke amplitude (the angle swept by the wings) to increase the angular velocity of the wings and generate the requisite lift, but only up to 160°. Conversely, flight in hypodense air is accomplished by increasing the angular velocity of the wing through increases in wingbeat frequency and stroke amplitudes, with larger increases in amplitude than seen in weightlifting flight. The kinematic differences between these two challenges may be facilitated by the lower energetic costs associated with overcoming drag and inertial forces over the wing during hypodense flight. Thus, we hypothesized that energetic expenditure is what limits the kinematics of weightlifting flight, with lower air densities permitting increases in angular velocity at comparatively lower costs. To explore the kinematic and energetic effects of air density and weightlifting on hovering flight performance, video and respirometric recordings of weightlifting were performed on four species of hummingbirds across an elevational gradient. Contrary to our hypothesis, wingbeat frequency did not vary due to elevation. Instead, wingbeat frequency seems to increase depending on the power requirements for sustaining hovering flight. Furthermore, metabolic rates during hovering increased with angular velocity alone, independent of elevation. Thus, it appears that the differential responses to flight challenges are not driven by variation in the flight media.

Keywords Hovering · Hummingbirds · Metabolism · Elevation · Load-lifting · Kinematics

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Introduction

Sustained hovering is one of the most demanding forms of flight a volant animal is capable of performing. In addition to maintaining the large power output necessary to hold position while hovering, animals that sustainably employ this mode of flight must also possess large power reserves to perform even more costly behaviors such as vertical ascent or to support increasing mass associated with feeding or egg formation. Production of elevated aerodynamic power output for such flight behaviors is achieved by changes in flight kinematic variables, primarily stroke amplitude and wingbeat frequency (Dudley 1995; Chai and Dudley 1995; Altshuler and Dudley 2003; Roberts et al. 2004; Altshuler et al. 2005; Vance et al. 2009; Dillon and Dudley 2014; Vance et al. 2014). These changes increase angular and tip velocities of the wings to enhance the aerodynamic forces generated over the wingbeat cycle. However, such increases in power generation are associated with a rise in the mechanical and metabolic demands on the flight musculature (Wells 1993a; Chai and Dudley 1995). As such, either mechanical or metabolic

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constraints, or both, can limit capacities for additional power generation.

Many hovering organisms are able to briefly modify both stroke amplitude and wingbeat frequency during intense, transient challenges [for example, asymptotic load-lifting (Chai et al. 1997, 1999; Altshuler and Dudley 2003)]. However, when increased power must be sustained, hovering fliers (both insects and hummingbirds) primarily vary stroke amplitude (Wells 1993a; Dudley 1995; Chai and Dudley 1995, 1996; Lehmann and Dickinson 1997; Altshuler and Dudley 2003; Roberts et al. 2004; Altshuler et al. 2005; Vance et al. 2009; Altshuler et al. 2010; Mahalingam and Welch 2013; Vance et al. 2014). Further evidence for the importance of stroke amplitude is related to maximum aerodynamic performance, as it has been suggested that maximum aerodynamic force production is limited in part by the maximum excursion angle of the wing (Chai and Dudley 1996; Chai et al. 1996, 1997, 1999; Altshuler and Dudley 2003; Dillon and Dudley 2004; Buchwald and Dudley 2010).

In contrast, wingbeat frequency appears to be a more tightly controlled kinematic variable. This is likely due to metabolic or mechanical constraints that limit the cycling frequency the flight musculature can sustain. Various groups have suggested that wings operate at an optimal cycling frequency during sustainable activities caused by a metabolic or oxygen delivery limitation (Altshuler and Dudley 2003) or to maintain optimal efficiency (Pennycuick 1996). For example, orchid bees have demonstrated a constant wingbeat frequency regardless of hovering flight challenge (Dudley 1995). Other hovering species, such as hummingbirds and carpenter bees, have been shown to vary wingbeat frequency slightly ($\leq$10 %) with increasing power output during hypodense flight challenges (Wells 1993a; Chai and Dudley 1995, 1996; Lehmann and Dickinson 1997; Altshuler and Dudley 2003; Roberts et al. 2004; Altshuler et al. 2010; Mahalingam and Welch 2013).

Studies that have examined hummingbird weightlifting have been primarily concerned with the extremes of flight performance, unweighted hovering and maximum burst performance as elicited by asymptotic load-lifting experiments (Chai et al. 1997; Chai and Millard 1997; Segre et al. 2015). These studies have shown that wingbeat frequency and stroke amplitude both increase during maximum burst hovering relative to unweighted hovering flight, with stroke amplitude reaching morphological maximum excursion angles. However, the lengths of these flights are short (approximately 1 s), and they are likely powered, in part, by anaerobic metabolism (Chai et al. 1997). In the few studies that have examined aerobically sustainable, weighted flight, wingbeat frequency was found to be invariant with the amount of weight being lifted by the animal, while stroke amplitudes varied but peaked well below

maximum morphologically permissible amplitudes (Wells 1993a; Mahalingam and Welch 2013). Thus, there appears to be discontinuous variation in how flight kinematics are controlled during weighted flight, and it is not understood when and if hummingbirds are capable of modulating wingbeat frequency or why stroke amplitude is unable to increase further during aerobically sustainable load-lifting.

The differential maximum flight kinematics of hovering in hypodense gases and while sustainably carrying additional weights may be due to the characteristics of the flight media itself. Reductions in air density change the aerodynamic characteristics of flight, such as profile drag and the inertial requirements for accelerating the wing over a wingbeat (Norberg 1990; Chai et al. 1996; Dudley 2000), potentially facilitating larger excursion angles and greater wingbeat frequencies, resulting in the necessary higher angular velocities. This may also reduce the energetic cost of generating these angular velocities at low air densities relative to normodense air. Thus, metabolic rates may not increase with increasing angular velocity of the wingtip during flight in low-density air to the same extent they do when lifting weights under normodense conditions. Further, metabolic rates when sustaining the highest angular velocities observed under hypodense conditions might not be as high as when maximum angular velocities are achieved under normodense conditions, and instead, morphology may limit flight performance. In contrast, aerobically sustainable flight performance during weightlifting may be constrained by the metabolic capacity of the flight musculature. At normodensity, profile and inertial power requirements to accelerate the wing are relatively high compared to low air densities (Ellington 1984b). To overcome this, greater muscle recruitment is necessary to achieve a given angular velocity (Tobalske et al. 2010). Thus, we hypothesize that the aerobic capacity of the flight muscles limits further increases in flight performance during sustainable weight lifting. Alternatively, the density of the flight media may not have any direct influence on the metabolic costs associated with hovering flight. Mahalingam and Welch (2013) found that relative muscle recruitment necessary to achieve a given angular velocity was independent of air density. It is possible that only angular velocity of the wings sets the metabolic rate. To address this question, we examined the influence of an elevational gradient upon hovering flight metabolism and kinematics and how these varied while sustainably lifting added masses.

This study examines the kinematics and energetic costs of hovering in hummingbirds in relation to variation in wingbeat kinematics as a consequence of lifting weights at various elevations. First, we hypothesize that wingbeat frequency variation is driven by changes in air density. We do not expect to observe variation in wingbeat frequency as a function of loading within an elevation. Instead, we

predict that the elevational gradient will be responsible for any wingbeat frequency variation. Rather, hummingbirds will meet these weightlifting challenges through changes in stroke amplitude. Further, we wish to shed light onto the interplay of mechanics and metabolism in hovering flight in hummingbirds by combining two approaches to understand energetics during sustained flight activities. This will allow us to elucidate the fundamental limitations that may constrain performance during flight in some of the larger hovering animals. We hypothesize that flight performance during sustained weight lifting is primarily limited by the metabolic capacity of the musculature.

Materials and methods

Experimental sites and animals

All data collection occurred in August–September 2012 and May–June 2013. Three field sites were used for this study, Ubatuba, SP; Guainumbi Reserve, Santa Virginia District, São Luiz do Paraitinga, SP; and Campos do Jordao, SP, Brazil. These three sites lie within the Atlantic Forest region of southeastern Brazil, and correspond to 0, 1000, and 1800 m asl, respectively. Hummingbirds were captured from within 80 m of elevation from these field stations. The amount of time hummingbirds could be maintained in captivity was limited (24–36 h), and some birds did not complete the entire protocol. As such, sample sizes for each of the analyses varied and are listed in Table 1.

Four species of hummingbirds were used for this study at three different elevations, *Clytolaema rubricauda* (Boddaert 1783), *Thalurania glaucopis* (Gmelin 1788), *Leucochloris albicollis* (Vieillot 1818), and *Lophornis chalybeus* (Temminck, 1821). Only males were used for this study, but due to the lack of dimorphic characteristics for *Le. albicollis*, sex could not be determined for this species. *Le. albicollis* was only found at 1000 and 1800 m asl, and *Lo. chalybeus* was studied at sea level only. Hummingbirds

were captured by mist net or by modified box trap and immediately transported to the nearest field station and rapidly trained to feed from artificial feeders. All hummingbirds were housed in $61 \times 61 \times 61$ cm mesh cages (Bioquip, Rancho Dominguez, CA, USA), fed 25 % w/v sucrose solutions and maintained on the natural photoperiod. Following completion of the study, all hummingbirds were released at the site of capture or terminally sampled for use in a separate study. All procedures were approved by the University of Toronto Animal Care Committee and the Instituto Brasileiro do Meio Ambiente e dos Recursos Naturais Renováveis (SISBIO Permit Number: 33158-5).

Wingbeat kinematics

To determine the kinematics during hovering flight, high-speed videography was employed as described in Mahalingam and Welch (2013). Briefly, hummingbirds hover fed within a $61 \times 61 \times 61$ cm cage fitted with a clear acrylic top. Birds were trained to regularly feed every 10–20 min from a 1-mL syringe containing a 25 % w/v sucrose solution, which served as an artificial feeder. A high-speed camera (S-PRI, AOS Technologies AG, Baden Daettwil, Switzerland) positioned directly above the feeder filmed hovering bouts at 1000 frames s^{-1} and a shutter speed of 250–400 μs.

Hovering flight recordings were made during (a) unweighted hovering flight, (b) weighted, sustainable hovering flight while lifting progressively heavier loads of plastic beads, and (c) brief hovering of maximum loads. Plastic beads of known masses were attached to a small yoke and suspended from the neck of the hummingbird. The weights were positioned such that they did not interfere with the excursion of the wing over the wingbeat cycle. Masses were increased in a step-wise manner until hover-feeding could no longer be maintained for longer than 2 s. Following sustainable weighted flight failure, hummingbirds were allowed to recover for at least 20 min before commencing with the maximal burst trials. Max trials were performed as

Table 1 Samples sizes of the experimental trials

	Clytolaema rubricauda			*Leucochloris albicollis*		*Thalurania glaucopis*			*Lophornis chalybeus*
Elevation (m)	1800	1000	0	1800	1000	1800	1000	0	0
Morphology	5	7	6	4	4	4	4	7	4
Sustained lifting trials	5	4	4	4	4	4	4	7	4
Kinematic max lifting trials	5	5	4	3	4	4	4	5	4
Unweighted metabolic rate	5	6	6	4	4	4	4	7	4
Weightlifting trials and maximum metabolic rates	4	5	5	3	4	4	4	4	3
Efficiency	4	5	5	4	4	4	4	7	4

described by Chai and colleagues ([1997]; Chai and Millard [1997]). Briefly, an elastic harness attached to a long chain of beaded thread was placed over the head of each hummingbird. The birds were released on from the floor of the chamber, and would immediately fly upwards lifting progressively greater weight until reaching a maximum height and load. Environmental temperature, barometric pressure and humidity were recorded using a nearby respirometer (TurboFox, Sable Systems International, Las Vegas, Nevada), and air density was calculated using standard equations (Denny [1993]).

Wingbeat frequency was determined by dividing the frame rate (1000 frames s^{-1}) by the number of frames that compose a single wingbeat cycle, and the average was taken over 15 consecutive wingbeat cycles. Stroke amplitude was determined by measuring the difference in angular position of the wing at the point of wrist supination (bottom of the downstroke) and wrist pronation (top of the upstroke). The wings appear as thin lines when viewed from overhead during these transitions. All recordings were analyzed using ImageJ 64 (v1.47, US National Institutes of Health, Maryland, USA, http://imagej.nih.gov/ij/).

Respirometry

Hummingbird metabolic rate during hovering flight was measured using open-flow mask respirometry as previously described (Welch Jr. [2011]). Briefly, hummingbirds were trained to hover at a mask that would capture respired gases. The mask was created from a 25-mL Luerlok syringe barrel (BD, Franklin Lakes, NJ, USA) and supplied with a 25 % w/v sucrose solution from a syringe pump powered reservoir (NE-500; New Era Pump Systems, Farmingdale, NY, USA). Excurrent air was captured approximately half way along the length of the mask and pumped to the respirometry setup for gas analysis (Turbofox, Sable Systems International, North Las Vegas, NV, USA) via Bev-a-Line IV tubing. The flow rate of the Turbofox sealed pump was monitored by an in-line mass flow meter integrated into the respirometry unit and calibrated by the manufacturer with a stated accuracy of ± 2 % (Turbofox, Sable Systems International, North Las Vegas, NV, USA). An infrared emitter and detector were placed at the entrance of the mask and were used to determine the length of the feeding event by measuring the length of time the head of the hummingbird occluded the IR beam. Hummingbirds were challenged similarly by sustainable weightlifting as described above.

Flow rate from the mask was maintained at 2500 mL min^{-1} and pumped into a manifold. The gases from the manifold were then subsampled at 500 mL min^{-1}. Subsampled air was first analyzed by a water vapor meter (Turbofox, Sable Systems) to measure water vapor pressure

before being dried by Drierite (W.A. Hammond Drierite, Xenia, OH, USA). Immediately afterward, the sampled air was drawn into the O_2 and CO_2 analyzers. Temperature was continuously monitored by a thermistor probe placed close to the mask, and barometric pressure was measured by an in-line sensor. Voltage outputs from the IR detector, barometer, flow meter, water vapor analyzer, and gas analyzers were recorded every 0.1 s using Expedata (v 1.7.2, Sable Systems International, North Las Vegas, NV, USA) on a laptop computer.

O_2 consumption and CO_2 production rates were calculated similarly to Chen and Welch Jr. ([2014]). Briefly, the primary flow rate through the mask was corrected for water vapor content. The raw O_2 and CO_2 traces during each feeding event were used to calculate the fractional change in O_2 and CO_2 content following baseline and drift correction. Volumes of O_2 consumed or CO_2 produced were calculated by integrating the peaks of each gas over time and using standard equations (Withers [1977]; Lighton [2008]; Welch Jr. [2011]). Rates of O_2 consumption and CO_2 production were calculated by dividing the volume of gas consumed or produced by the length of time of the feeding event. Respiratory exchange ratios (RER) were calculated by dividing CO_2 production rate by the O_2 consumption rate. Only feeding events longer than 2 s were included in the analyses. The feeding event duration means and ranges for each species were 11.03 (2.10–73.80), 9.82 (2.10–46.70), 9.01 (2.00–41.90), and 9.23 (2.00–38.00) seconds for *C. rubricauda*, *L. albicollis*, *T. glaucopis*, and *L. chalybeus*, respectively.

Morphology

The body mass of the birds was determined to 0.01 g precision before and after all kinematic trials using an electronic balance. The mean value was used as the estimated body mass. Wings from each hummingbird were photographed against graph paper while held outstretched in a position approximating that during flight. Images were analyzed using a custom Matlab script (courtesy of Dr. Douglas Altshuler, Matlab v. 7.12, MathWorks, Natick, MA) to measure wing area, wing length, aspect ratio, and non-dimensional morphological variables associated with hovering flight (Ellington [1984a]). Wing loading was calculated as the ratio of body weight (measured in Newtons) divided by the area of the wing pair.

Mechanical power output

Together with kinematic, morphological and environmental parameters, mechanical power output was calculated using a model of animal hovering (Ellington [1984b], [c]). Stroke plane angle was assumed to be zero, based upon previous

measurements of stroke plane angle made on humming-birds during both weighted and unweighted hovering flight (Chai and Dudley 1996; Altshuler et al. 2004b), and simple harmonic motion was assumed for wing movements within the stroke plane (Chai and Dudley 1995).

Mechanical power requirements for hovering were estimated by calculating the profile (P_{pro}) and induced (P_{ind}) power during hovering. Profile power represents the energy required to overcome drag forces over the wings during the wingbeat, and induced power represents the requirement for imparting downward momentum on the air to overcome the body mass of the animal and any additional mass being lifted (Ellington 1984b). A profile drag coefficient of 0.139 was assumed, based on empirical measurements previously made on *Selasphorus rufus* hummingbird wings (Altshuler et al. 2004a). Because we could not determine wing mass for individuals, inertial power requirements were ignored in all analyses (i.e., we assumed perfect elastic storage of kinetic energy). Previous work has suggested that hummingbirds do possess a capacity to store energy in their elastic components (Weis-Fogh 1972; Wells 1993b); however, the contribution to mechanical power is currently unknown. Thus, mechanical power output was estimated by summing P_{pro} and P_{ind}.

Analyses and statistics

Mean metabolic rates and mechanical power output of each lifted mass for each individual were used for the calculation of efficiency. As RER approximated 1.0, metabolic rate was converted to watts using 21.1 J mL O_2^{-1} throughout (Brobeck and Dubois 1980). Efficiency was calculated as $P_{per}/(0.9 \times P_{metabolic})$, similar to Chai and Dudley (1995). Maximum-recorded metabolic rates were calculated using the mean of the three highest oxygen consumption rates for each individual.

All statistical analyses were performed in R (v.3.0.2) (R Core Team 2013). Linear mixed effects models were used to determine the effect of total mass and elevation upon hovering metabolic rates, and angular velocities and elevation upon hovering metabolic rates using the nlme v.3.1-118 package (Pinheiro et al. 2014). The effect of total mass and elevation (only total mass for *Lo. chalybeus*) upon hovering metabolic rates and flight kinematics was determined using competing models, which were compared using Akaike Information Criterion corrected for low sample sizes (AICc) scores. The initial full model included an interaction term between the total mass and elevation. If the difference in AICc scores of competing models was less than 2, the model with fewer predictors was considered to be the best-fitting model (Arnold 2010). AICc scores for all competing models and the parameter estimates for the best-fitting models can be found in the Online Resources.

Normality and homogeneity of variances were visually compared using residuals and quantile–quantile plots (Zuur et al. 2009). Competing variance structures were compared, and the best-fitting structure was used in the final reported model (Zuur et al. 2009). Efficiency data for *C. rubricauda* was log-transformed to satisfy the normality assumption.

Differences across elevations in unweighted and maximum sustainable hovering flight kinematics and power outputs, unweighted whole-animal metabolic rates, maximum aerobic whole-animal metabolic rates, and VO_2 reserves were analyzed using one-way ANCOVAs with body mass as a covariate. Normality and homogeneity of variances were tested using the Shapiro–Wilk test and Levene's test, respectively. If violations of normality were detected, a Kruskal–Wallis test was performed instead, and body mass was omitted from the analyses. Due to the small number of species examined in this study, interspecies comparisons were not performed due to the lack of statistical power (Felsenstein 1985). All values are reported as mean ± SD.

Results

Morphology and body mass

Wing morphology and body mass were not significantly different across elevations for *C. rubricauda* and *Le. albicollis* (Table 2). Conversely, there was significant variation in morphology in *T. glaucopis* across the elevations, with higher-elevation individuals being smaller and having a different wing shape relative to their low-elevation counterparts (Table 2).

Unweighted hovering metabolic rate, kinematics and power output across elevations

Stroke amplitude during unweighted hovering flight was similar across elevations within each species (*C. rubricauda* $F_{2,10} = 0.30$, $p = 0.7502$; *Le. albicollis* $F_{1,5} = 1.16$, $p = 0.3298$; *T. glaucopis* $F_{2,11} = 3.22$, $p = 0.0792$; Table 3). Likewise, wingbeat frequency did not vary significantly with elevation (*C. rubricauda* $F_{2,10} = 1.09$, $p = 0.3745$; *Le. albicollis* Kruskal–Wallis Test $p = 0.3298$; *T. glaucopis* $F_{2,11} = 1.40$, $p = 0.2863$; Table 3). Angular velocity did not vary significantly with elevation in *C. rubricauda* and *Le. albicollis* [*C. rubricauda* ($F_{2,10} = 2.00$, $p = 0.1862$); *Le. albicollis* (Kruskal–Wallis Test, $p = 0.1489$)], but did vary significantly with elevation in *T. glaucopis* ($F_{2,11} = 5.88$, $p = 0.0183$; Table 3). The mechanical power output required to maintain unweighted hovering flight was found to significantly increase with elevation for two species (*C. rubricauda* $F_{2,10} = 6.60$, $p = 0.0149$; *T. glaucopis* $F_{2,11} = 6.26$, $p = 0.0153$; Table 3). Mechanical power

Table 2 Morphological measurements of three hummingbird species across an 1800-m elevational gradient

|	Elevation (m)	Body mass (g)	Wing length (mm)	Wing area (cm^2)	Aspect ratio	Wing loading (N m^{-2})
Clytolaema rubricauda	1800	8.91 ± 0.33	69.08 ± 4.28	26.39 ± 3.26	7.28 ± 0.70	33.47 ± 4.01
	1000	8.42 ± 0.61	68.79 ± 5.39	26.67 ± 3.71	7.13 ± 0.45	31.50 ± 5.47
	0	9.21 ± 0.49	72.42 ± 3.14	29.88 ± 1.67	7.03 ± 0.44	30.25 ± 1.66
Leucochloris albicollis	1800	6.17 ± 0.31	58.50 ± 1.73	19.71 ± 0.30	6.95 ± 0.37	30.70 ± 1.70
	1000	6.36 ± 0.33	58.44 ± 3.02	19.45 ± 1.49	7.04 ± 0.32	32.17 ± 2.59
Thalurania glaucopis	1800	4.73 ± 0.26[a]	54.97 ± 2.33[a]	18.64 ± 0.46[a,b]	6.49 ± 0.51[a]	24.83 ± 0.85[a]
	1000	5.08 ± 0.19[a,b]	57.43 ± 1.04[a,b]	17.54 ± 0.84[a]	7.53 ± 0.18[b]	28.39 ± 1.49[b]
	0	5.17 ± 0.26[b]	59.77 ± 2.55[b]	19.64 ± 1.25[b]	7.28 ± 0.22[b]	25.92 ± 2.24[a,b]
Lophornis chalybeus	0	3.27 ± 0.31	44.11 ± 4.07	9.32 ± 0.70	8.03 ± 0.49	33.50 ± 6.06

Different letters indicate significant differences following post hoc testing across elevations within a species ($p < 0.05$). See Table 1 for the sample sizes. Data are displayed as mean $\pm$ SD

Table 3 Unweighted hovering flight kinematics and mechanical power outputs in *Clytolaema rubricauda*, *Thalurania glaucopis*, *Leucochloris albicollis* and *Lophornis chalybeus* across three elevations

	Elevation (m)	Wingbeat frequency (Hz)	Stroke amplitude (°)	Angular velocity (rads s^{-1})	Power output (W kg^{-1})
Clytolaema rubricauda	1800	25.51 ± 0.89	154.28 ± 5.18	137.29 ± 3.59	28.32 ± 1.71[a]
	1000	24.88 ± 1.13	149.43 ± 13.85	129.44 ± 9.35	26.13 ± 1.63[a,b]
	0	24.58 ± 0.67	152.86 ± 10.09	130.98 ± 6.64	24.57 ± 1.20[b]
Leucochloris albicollis	1800	29.46 ± 1.18	156.00 ± 5.25	160.26 ± 6.31	27.22 ± 0.44
	1000	28.88 ± 0.43	152.52 ± 4.41	153.70 ± 5.94	26.36 ± 1.06
Thalurania glaucopis	1800	31.38 ± 1.27	134.30 ± 1.83	147.12 ± 6.98[a,b]	26.60 ± 1.52[a]
	1000	32.30 ± 0.46	133.10 ± 6.32	149.93 ± 5.05[a]	25.56 ± 1.78[a,b]
	0	31.09 ± 1.38	126.24 ± 6.50	136.83 ± 6.71[b]	23.84 ± 0.80[b]
Lophornis chalybeus	0	47.97 ± 0.91	133.48 ± 15.83	223.20 ± 23.31	26.04 ± 1.12

Different letters indicate significant differences following post hoc testing across elevations within a species ($p < 0.05$). See Table 1 for sample sizes. Data displayed as mean $\pm$ SD

output requirements in *Le. albicollis* were not significantly different across the two elevations explored ($F_{1,5} = 2.60$, $p = 0.1677$; Table 3). Since *Lo. chalybeus* was found at only one study site, the effect of elevation on hovering kinematics and power output could not be evaluated. Kinematics and power output can be found in Table 3 for *Lo. chalybeus*.

There was no effect of elevation upon unweighted hovering metabolic rates across all three species studied at multiple elevations (*C. rubricauda*: $F_{2,13} = 1.85$, $p = 0.1968$; *T. glaucopis*: $\chi^2 = 2.88$, $p = 0.2367$; *Le. albicollis* $F_{1,5} = 5.52$, $p = 0.0657$; Fig. 1a). Increasing body mass significantly increased unweighted hovering flight metabolic rate in *C. rubricauda* ($F_{1,13} = 6.23$, $p = 0.0268$), but unweighted hovering metabolic rate did not vary significantly with body mass among *Le. albicollis* ($F_{1,5} = 1.27$, $p = 0.3106$). Due to the non-normal distribution of *T. glaucopis* metabolic rates, a Kruskal–Wallis test was, instead, performed, and body mass was removed from the analysis. As *Lo. chalybeus* was only found at a single elevation, elevational comparisons were not made (Fig. 1a).

Sustainable weightlifting and hovering metabolic rates, kinematic and power output

Elevation was not a factor in the best-fitting models predicting variation in wingbeat frequency for any of the species examined. In contrast, total mass lifted was a positive predictor of wingbeat frequency in all four species [*C. rubricauda* ($F_{1,173} = 91.43$, $p < 0.0001$); *Le. albicollis* ($F_{1,123} = 101.08$, $p < 0.0001$); *T. glaucopis* ($F_{1,215} = 494.11$, $p < 0.0001$); *Lo. chalybeus* ($F_{1,57} = 14.64$, $p = 0.0021$); Fig. 2]. Elevation was not a factor in the models that best predicted variation in stroke amplitude in *C. rubricauda* and *Le. albicollis*, but stroke amplitude increased with elevation in *T. glaucopis* ($F_{2,12} = 10.29$, $p = 0.0025$). Stroke amplitude increased with increasing total mass lifted for all four species [*C. rubricauda* ($F_{1,173} = 96.54$, $p < 0.0001$); *Le. albicollis* ($F_{1,123} = 119.96$, $p < 0.0001$); *T. glaucopis* ($F_{1,215} = 188.40$, $p < 0.0001$); *Lo. chalybeus* ($F_{1,57} = 16.66$, $p < 0.0001$); Fig. 3]. The effect of total mass lifted on stroke amplitude was similar across elevations for *T. glaucopis*, as
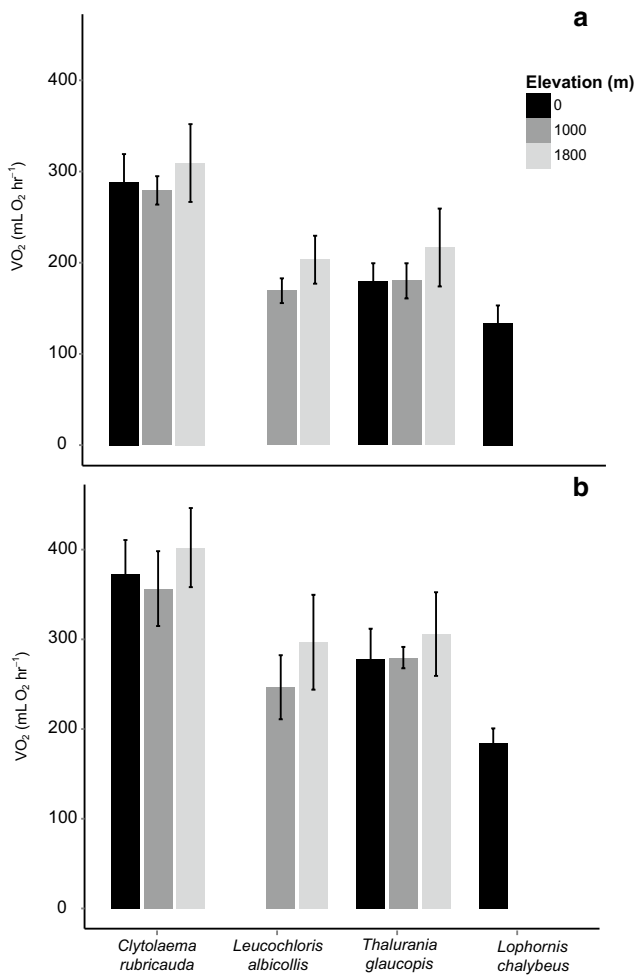
Fig. 1 The relationship of whole-animal (**a**) unweighted hovering and (**b**) maximum-recorded metabolic rates across three elevations in four species of hummingbird (*Clytolaema rubricauda*, *Thalurania glaucopis*, *Leucochloris albicollis*, and *Lophornis chalybeus*). Sample sizes can be found in Table 1

the interaction between total mass lifted and elevation was excluded from the best-fitting model.

Following from the patterns seen in wingbeat frequency and stroke amplitude, total mass lifted was found to significantly increase angular velocity of the wing for all four species [*C. rubricauda* ($F_{1,173} = 213.70$, $p < 0.0001$); *Le. albicollis* ($F_{1,123} = 295.16$, $p < 0.0001$); *T. glaucopis* ($F_{1,215} = 452.88$, $p < 0.0001$); *Lo. chalybeus* ($F_{1,57} = 60.82$, $p < 0.0001$); Fig. 4]. Despite the lack of elevational influence on either major kinematic variable for most species, elevation was included as a factor in the best-fit model describing patterns of variation in angular velocity, as judged by AICc scores. However, ANOVA analysis revealed that elevation was a significant factor in two species [*Le. albicollis* ($F_{1,6} = 7.03$, $p = 0.0380$); *T. glaucopis* ($F_{2,12} = 11.17$, $p = 0.0018$)], but was not in *C. rubricauda* ($F_{2,11} = 3.24$, $p = 0.0804$).

Mechanical power output was found to increase with increasing load lifted in all four species [*C. rubricauda* ($F_{1,173} = 938.75$, $p < 0.0001$); *Le. albicollis* ($F_{1,123} = 659.45$, $p < 0.0001$); *T. glaucopis* ($F_{1,215} = 1582.84$, $p < 0.0001$); *Lo. chalybeus* ($F_{1,57} = 41.05$, $p < 0.0001$); Fig. 5]. Elevation was included as a factor in the models which best predicted mechanical power output required to maintain hovering flight for *C. rubricauda* and *T. glaucopis* [*C. rubricauda* ($F_{2,11} = 17.51$, $p = 0.0004$); *T. glaucopis* ($F_{2,12} = 4.029$; $p = 0.046$), Fig. 5], with higher elevations requiring greater mechanical power output. In contrast, elevation was not included as a factor in the best-fitting model predicting variation in mechanical power requirements for *Le. albicollis*.

Whole-animal metabolic rates increased significantly with total mass lifted for all four species (*C. rubricauda*: $F_{1,330} = 6.37$, $p = 0.0120$; *T. glaucopis*: $F_{1,393} = 31.30$, $p < 0.0001$; *Le. albicollis*: $F_{1,176} = 7.37$, $p = 0.0073$, *Lo. chalybeus*: $F_{1,66} = 51.30$, $p < 0.0001$; Fig. 6). Metabolic rate increased with elevation for two of the three species found at multiple elevations (*C. rubricauda*: $F_{2,14} = 4.06$, $p = 0.0407$; *T. glaucopis*: $F_{2,12} = 5.16$, $p = 0.0242$). The best-fitting model for *Le. albicollis* included elevation, but this was not found to be a significant factor by ANOVA ($F_{1,6} = 4.58$, $p = 0.0761$; Fig. 6b). There was a significant interaction effect between elevation and total mass lifted for *C. rubricauda* ($F_{2,330} = 3.75$, $p = 0.0246$; Fig. 6a). This interaction was caused by the near-zero slope of the 0 m asl population of *C. rubricauda*.

Angular velocities and metabolic rates

Whole-animal metabolic rate significantly increased with angular velocity of the wing for three of the four species (*T. glaucopis*: $F_{1,62} = 49.27$, $p < 0.0001$; *Le. albicollis*: $F_{1,43} = 5.15$, $p = 0.0283$; *Lo. chalybeus*: $F_{1,16} = 9.97$, $p = 0.0061$; Fig. 7), but elevation was not found to be a significant predictor. In contrast, for *C. rubricauda*, only elevation was retained in the best-fitting model, but was not a significant factor ($F_{2,11} = 3.91$, $p = 0.0522$; Fig. 7).

Maximum sustainable kinematics, mechanical power output, metabolic rate and scope

As the amount of time hummingbirds could be maintained in captivity was limited, not all hummingbirds completed the entire weightlifting protocol. Only the hummingbirds that had completed the weightlifting protocol were included in the analysis of maximum sustainable kinematics, mechanical power output, metabolic rate, and scope. Sample sizes can be found in Table 1.

The maximum sustainable kinematics and mechanical power output that the animals produced were
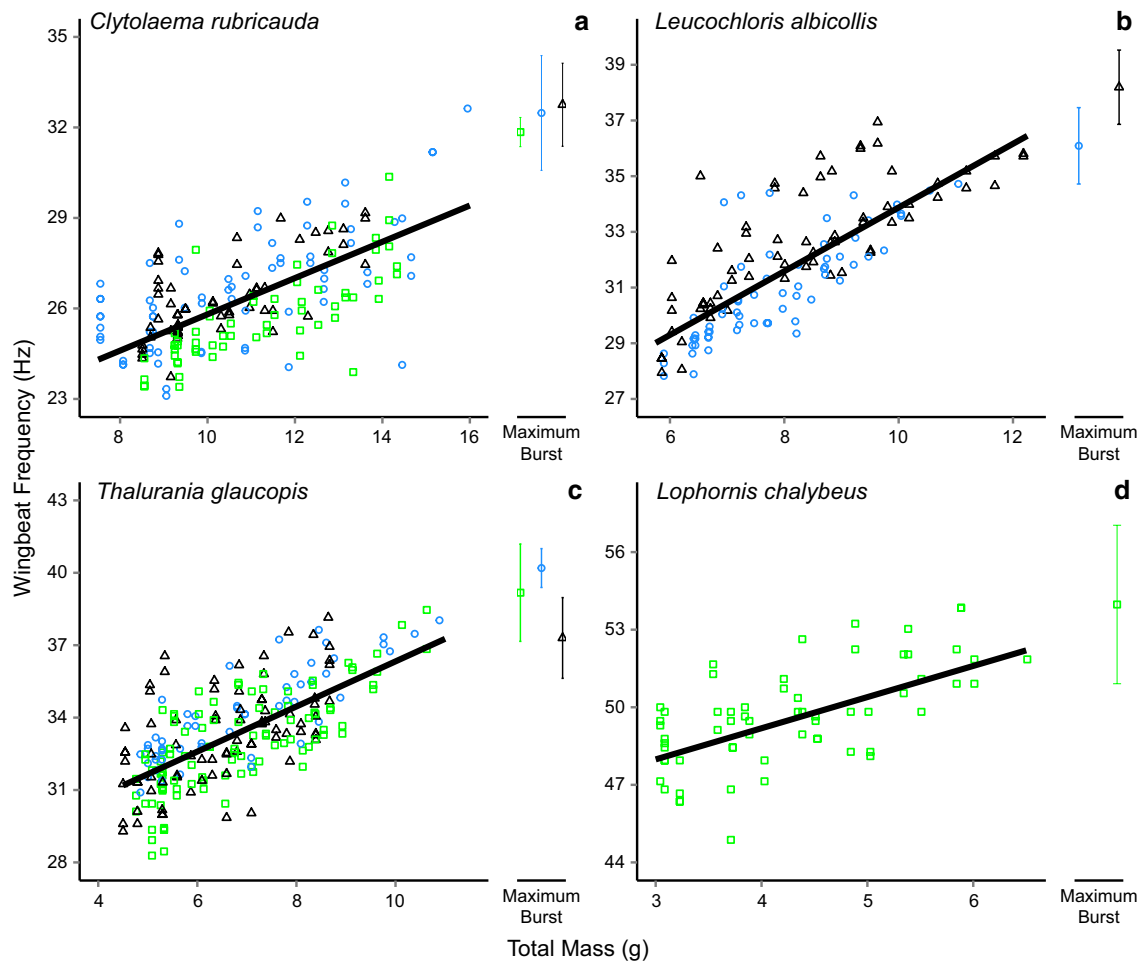
Fig. 2 The effect of sustainable and maximum burst weightlifting upon wingbeat frequency in **a** *Clytolaema rubricauda*, **b** *Thalurania glaucopis*, **c** *Leucochloris albicollis*, and **d** *Lophornis chalybeus*. *Green squares* represent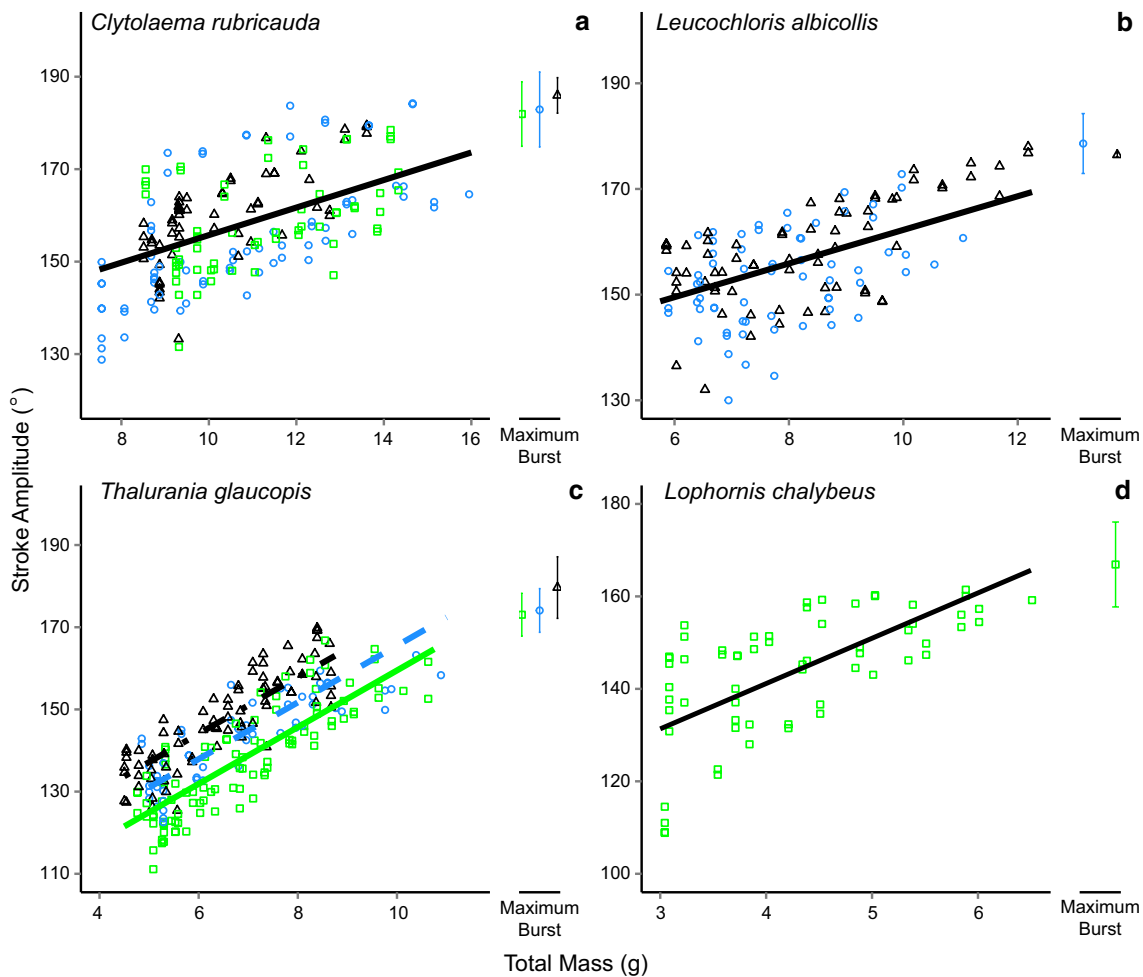 0 m, *blue circles* represent 1000 m and *black* *triangles* represent 1800 m. The *regression lines* represent the best-fitting models, according to AICc model selection. Data for maximum burst weightlifting are mean ± SD. See Table 1 for sample sizes (color figure online)

examined across elevations (Figs. 2, 3, 4, 5). Neither maximum sustainable stroke amplitude nor wingbeat frequency varied significantly across elevations [Wingbeat frequency: *C. rubricauda* ($F_{2,10} = 1.31$, $p = 0.3117$); *Le. albicollis* ($F_{1,4} = 2.82$, $p = 0.1681$); *T. glaucopis* ($F_{2,9} = 1.94$, $p = 0.1988$)]. Stroke amplitude: *C. rubricauda* ($F_{2,10} = 0.006$, $p = 0.9941$); *T. glaucopis* ($F_{2,9} = 2.39$, $p = 0.1468$); *Le. albicollis* (Kruskal–Wallis Test; $p = 0.2888$)]. Maximum sustainable angular velocity did not vary across elevations for *C. rubricauda* ($F_{2,10} = 0.55$, $p = 0.5925$) or *T. glaucopis* (Kruskal–Wallis Test, $p = 0.3431$), but did increase significantly with elevation for *Le. albicollis* ($F_{1,4} = 8.24$, $p = 0.0455$). Maximum sustainable mechanical power output was not found to differ significantly across elevations for any species [*C. rubricauda* ($F_{2,10} = 1.80$, $p = 0.2143$); *T. glaucopis* (Kruskall–Wallis test, $p = 0.1925$); *Le. albicollis* ($F_{1,4} = 2.33$, $p = 0.2018$)]. Sustainable mechanical scopes (the ratio

between maximum sustained weighted and unweighted hovering power output) were not significantly different across elevations for *Le. albicollis* (1000 m: 1.22 ± 0.03; 1800 m: 1.26 ± 0.03) and *T. glaucopis* (0 m: 1.29 ± 0.04; 1000 m: 1.29 ± 0.03; 1800 m: 1.25 ± 0.04) ($p > 0.1$), but was found to significantly vary with increasing elevation in *C. rubricauda* [0 m: 1.23 ± 0.07; 1000 m: 1.26 ± 0.07; 1800 m: 1.16 ± 0.05 ($F_{1,11} = 5.22$, $P = 0.04315$)]. The mechanical scope of *Lo. chalybeus* at 0 m was 1.24 ± 0.04.

According to one-way ANCOVA analysis, whole-animal maximum-recorded metabolic rates did not vary across elevations for each species (*C. rubricauda*: $F_{2,10} = 1.50$, $p = 0.2684$; *T. glaucopis*: $F_{2,8} = 1.34$, $p = 0.31435$; *Le. albicollis*: $F_{1,4} = 1.85$, $p = 0.2459$, Fig. 1b). Body mass had no significant effect on maximum hovering metabolic rate for two of the reported species (*C. rubricauda*: $F_{1,10} = 0.73$, $p = 0.4144$; *Le. albicollis*: $F_{1,4} = 0.03$, $p = 0.8665$). However, larger body mass significantly

Fig. 3 The effect of sustainable and maximum burst weightlifting upon stroke amplitude in **a** *Clytolaema rubricauda*, **b** *Thalurania glaucopis*, **c** *Leucochloris albicollis*, and **d** *Lophornis chalybeus*. *Green squares* represent 0 m, *blue circles* represent 1000 m and *black triangles* represent 1800 m. The *regression lines* represent the best-fitting models, according to AICc model selection. *Color* and *style* of the *regression lines* correspond to the different elevations for *T. glaucopis*, with *solid green*, *dashed blue*, and *dot-dash black* representing 0, 1000 and 1800 m, respectively. Data for maximum burst weightlifting are mean ± SD. See Table 1 for sample sizes (color figure online)

increased maximum metabolic rate in *T. glaucopis* ($F_{1,8} = 6.38$, $p = 0.0355$).

VO$_2$ reserve was not significantly different across elevations for each species (*C. rubricauda*: $F_{2,10} = 0.30$, $p = 0.7465$; *T. glaucopis*: $F_{2,8} = 0.54$, $p = 0.6005$; *Le. albicollis*: $F_{1,5} = 0.06$, $p = 0.8234$). The mean VO$_2$ reserve was 1.28 ± 0.14, 1.48 ± 0.26, 1.54 ± 0.23, and 1.46 ± 0.05 for *C. rubricauda*, *Le. albicollis*, *T. glaucopis*, and *Lo. chalybeus*, respectively.

Maximum burst load-lifting trials

Flight kinematics during maximum load-lifting were similar across elevations for the three species with multiple elevational representation, with stroke amplitude approaching 180° for all species at each elevation [Wingbeat frequency: *C. rubricauda* ($F_{2,10} = 0.42$, $p = 0.6699$); *T. glaucopis* ($F_{2,10} = 3.16$, $p = 0.0863$); *Le. albicollis* ($F_{1,4} = 3.30$, $p = 0.1433$) (Fig. 2). Stroke amplitude: *C. rubricauda* ($F_{2,10} = 0.45$, $p = 0.6482$); *T. glaucopis* ($F_{2,10} = 1.67$, $p = 0.2366$); *Le. albicollis* ($F_{1,4} = 0.043$, $p = 0.5486$) (Fig. 3). Angular velocity: *C. rubricauda* ($F_{2,10} = 2.86$, $p = 0.1040$); *T. glaucopis* ($F_{2,10} = 1.18$, $p = 0.3468$); *Le. albicollis* ($F_{1,4} = 1.97$, $p = 0.2333$) (Fig. 4)]. The total mass *T. glaucopis* was able to lift significantly varied with increasing elevation [0 m: 9.03 ± 1.12 g; 1000 m: 9.13 ± 0.67 g; 1800 m: $7.42 \pm .57$ g ($F_{2,10} = 4.65$, $p = 0.0374$)], while *C. rubricauda* displayed a non-significant negative trend with increasing elevation [0 m: 14.53 ± 1.00 g; 1000 m: 13.64 ± 2.08 g; 1800 m: 11.49 ± 1.56 g ($F_{2,10} = 4.02$, $p = 0.0523$)]. The maximum mass-lifting capacity of *Le. albicollis* was not significantly affected by elevation [1000 m: 8.10 ± 1.52 g; 1800 m: 9.19 ± 1.19 g ($F_{1,4} = 0.92$, $p = 0.3908$)]. Furthermore,
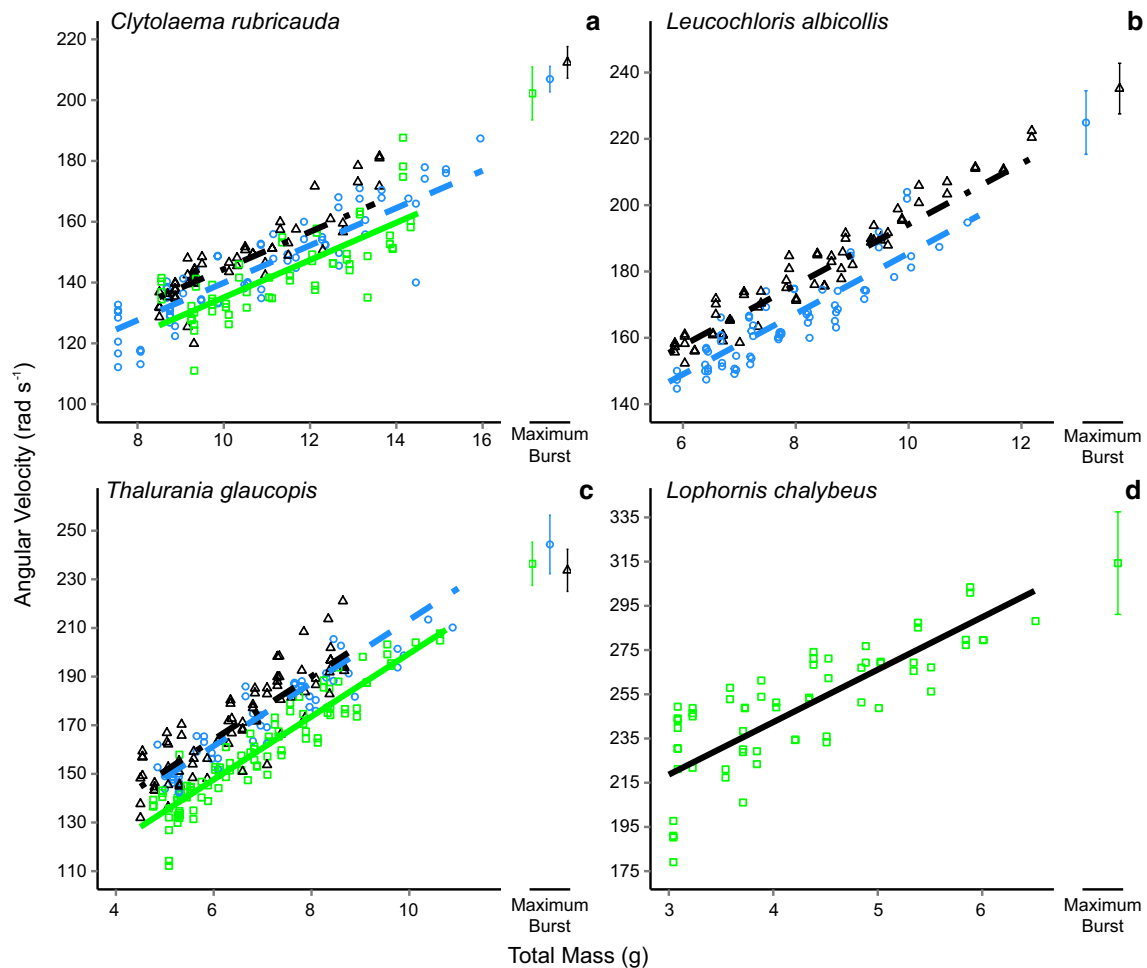
Fig. 4 The effect of sustainable and maximum burst weightlifting upon angular velocity in **a** *Clytolaema rubricauda*, **b** *Thalurania glaucopis*, **c** *Leucochloris albicollis*, and **d** *Lophornis chalybeus*. *Green squares* represent 0 m, *blue circles* represent 1000 m and *black triangles* represent 1800 m. The *regression lines* represent the best-fitting models, according to AICc model selection. *Color* and *style* of the *regression lines* corresponding to the different elevations, with *solid green*, *dashed blue*, and *dot-dash black* representing 0, 1000 and 1800 m, respectively. Data for maximum burst weightlifting are mean ± SD. See Table 1 for sample sizes (color figure online)

there were no differences in the maximum burst mechanical power output across the elevations within any of the three species [*C. rubricauda* ($F_{2,10} = 2.51$, $p = 0.1300$); *T. glaucopis* ($F_{2,10} = 1.35$, $p = 0.3030$); *Le. albicollis* ($F_{1,4} = 2.39$, $p = 0.1972$) (Fig. 5)].

When comparing maximum burst with maximum sustainable performance, all species generally displayed higher wingbeat frequencies, stroke amplitudes, angular velocities and mechanical power outputs at all elevations during maximum load-lifting trials, except for stroke amplitude at 0 m in *C. rubricauda* and 1800 m in *Le. albicollis*, and angular velocity in *Le. albicollis* at 1800 m (See Online Resource 3 for statistics). The stroke amplitudes of *Lo. chalybeus* were not significantly different between the two trial types. There was no significant effect of elevation on the difference between maximum sustainable or burst flight kinematics or mechanical power output across elevations.

The only exception was angular velocity in *T. glaucopis*, which increased with higher elevation ($F_{2,10} = 4.7793$, $p = 0.0349$).

Efficiency

Efficiency during lifting and across elevations was compared, and best-fitting models were selected using AICc (Fig. 8). The best-fitting model for *T. glaucopis* only included elevation. However, elevation was not a significant factor by ANCOVA ($F_{2,12} = 3.28$, $p = 0.0729$). The null model without fixed effects was found to be the best-fitting model for *Lo. albicollis* and *C. rubricauda* and *Lo. chalybeus*. The mean efficiency over the entire range of masses were 15.46 ± 2.74, 18.42 ± 3.82, 12.26 ± 1.92, and 11.41 ± 0.25 % for *C. rubricauda*, *Le. albicollis*, *T. glaucopis*, and *Lo. chalybeus*, respectively.
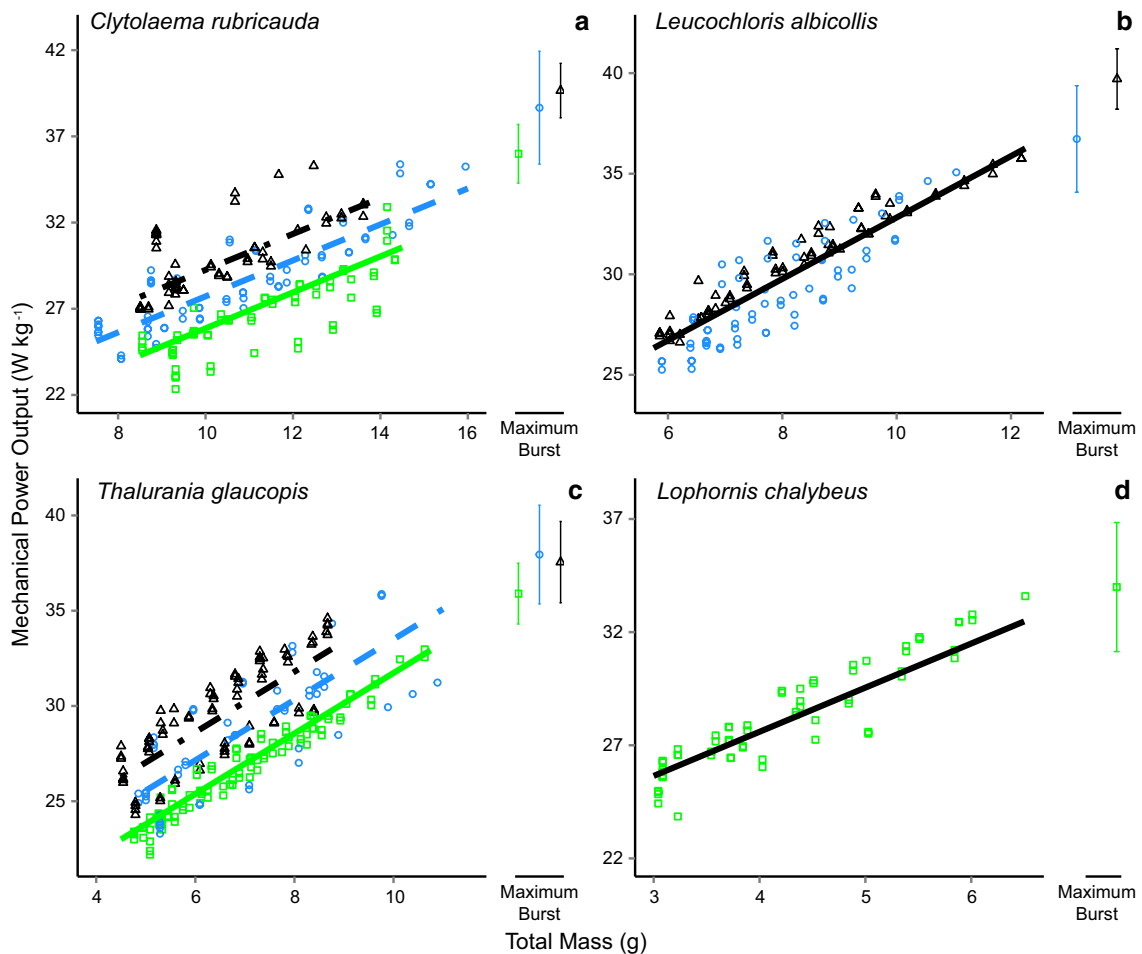
Fig. 5 The effect of sustainable and maximum burst weightlifting upon mechanical power output in **a** *Clytolaema rubricauda*, **b** *Thalurania glaucopis*, **c** *Leucochloris albicollis*, and **d** *Lophornis chalybeus*. *Green squares* represent 0 m, *blue circles* represent 1000 m and *black triangles* represent 1800 m. The *regression lines* represent the best-fitting models, according to AICc model selection. *Color and style* of the *regression lines* corresponding to the different elevations, with *solid green*, *dashed blue*, and *dot-dash black* representing 0, 1000 and 1800 m, respectively. As elevation was excluded from *Le. albicollis*, a *regression line* for each elevation was excluded. Data for maximum burst weightlifting are mean ± SD. See Table 1 for sample sizes (color figure online)

Discussion

Metabolic rate, elevation, and weightlifting

In contrast to previous studies (Wells 1993a; Chai and Dudley 1995, 1996; Altshuler and Dudley 2003; Altshuler et al. 2010; Mahalingam and Welch 2013), we find that hummingbirds are able to modify wingbeat frequency and stroke amplitude in concert when responding to changing lift conditions. Moreover, the increase in wingbeat frequency was not due to changes in air density with elevation, even in unweighted and maximum flight capacities, as elevation was frequently dropped from the best-fitting model. This indicates that wingbeat frequency variation was more strongly tied to aerodynamic requirements to sustain hovering rather than air density in particular. This is unlike other studies, which have shown a lack of variation in wingbeat frequency in the face of increasing lift demands during sustainable weight lifting, as an increase in only one kinematic variable (primarily stroke amplitude) is necessary to overcome the additional weight added to the animals (Wells 1993a; Mahalingam and Welch 2013).

Wingbeat frequency is seen as less malleable to changes in flight requirements across many taxa, and has been found to change from slightly to not at all. In insects, the wingbeat frequency may be largely constrained by the mechanical resonance of the pterothorax, with operation at its resonant frequency thought to reduce the energetic costs of flapping the wings (Greenewalt 1960; Dudley 2000). When hovering wingbeat frequency was found to vary with mechanical demand in hummingbirds and carpenter bees, wingbeat
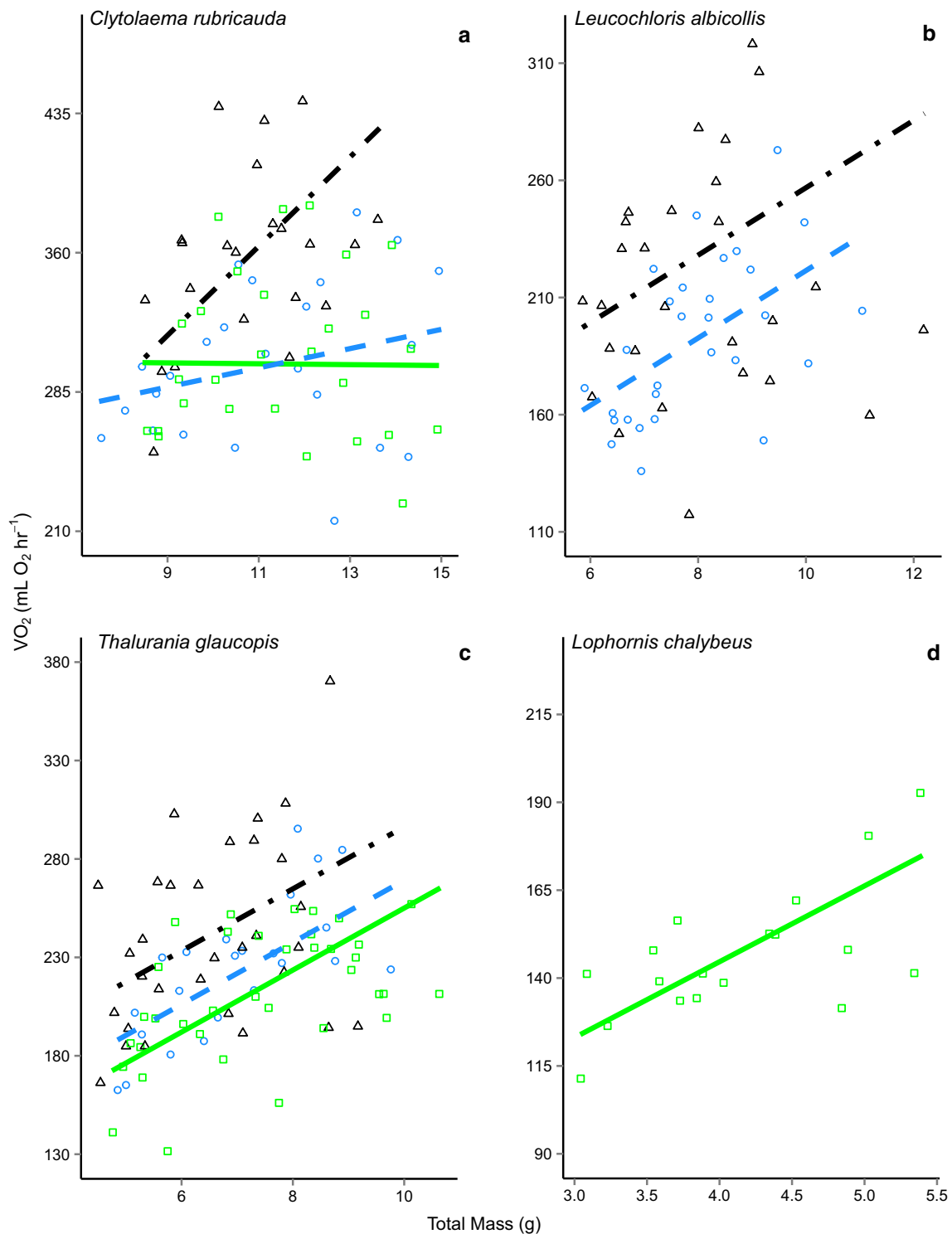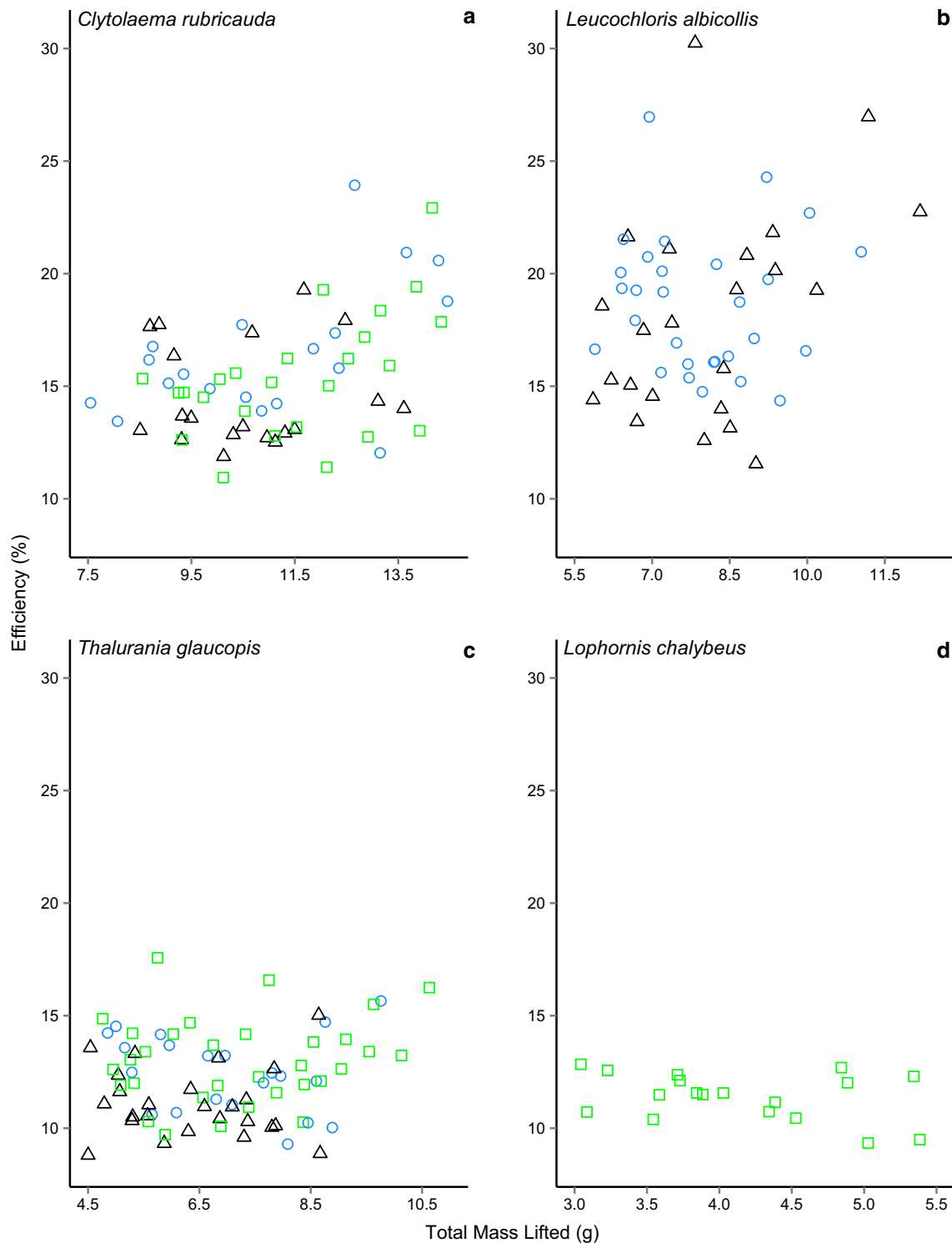
Fig. 6 The relationship between metabolic rate and total mass (sum of body mass and added mass) during sustained hovering flight across three elevations in four species of hummingbird **a** *Clytolaema rubricauda*, **b** *Thalurania glaucopis*, **c** *Leucochloris albicollis*, **d** *Lophornis chalybeus*. *Symbols* represent average metabolic rates for an individual at a given mass. *Green squares*, *blue circles*, and *black triangles* correspond to 0, 1000, and 1800 m asl, respectively. *Color* and *style* of the *regression lines* corresponding to the different elevations, with *solid green*, *dashed blue*, and *dot-dash black* representing 0, 1000 and 1800 m, respectively. Sample sizes can be found in Table 1 (color figure online)
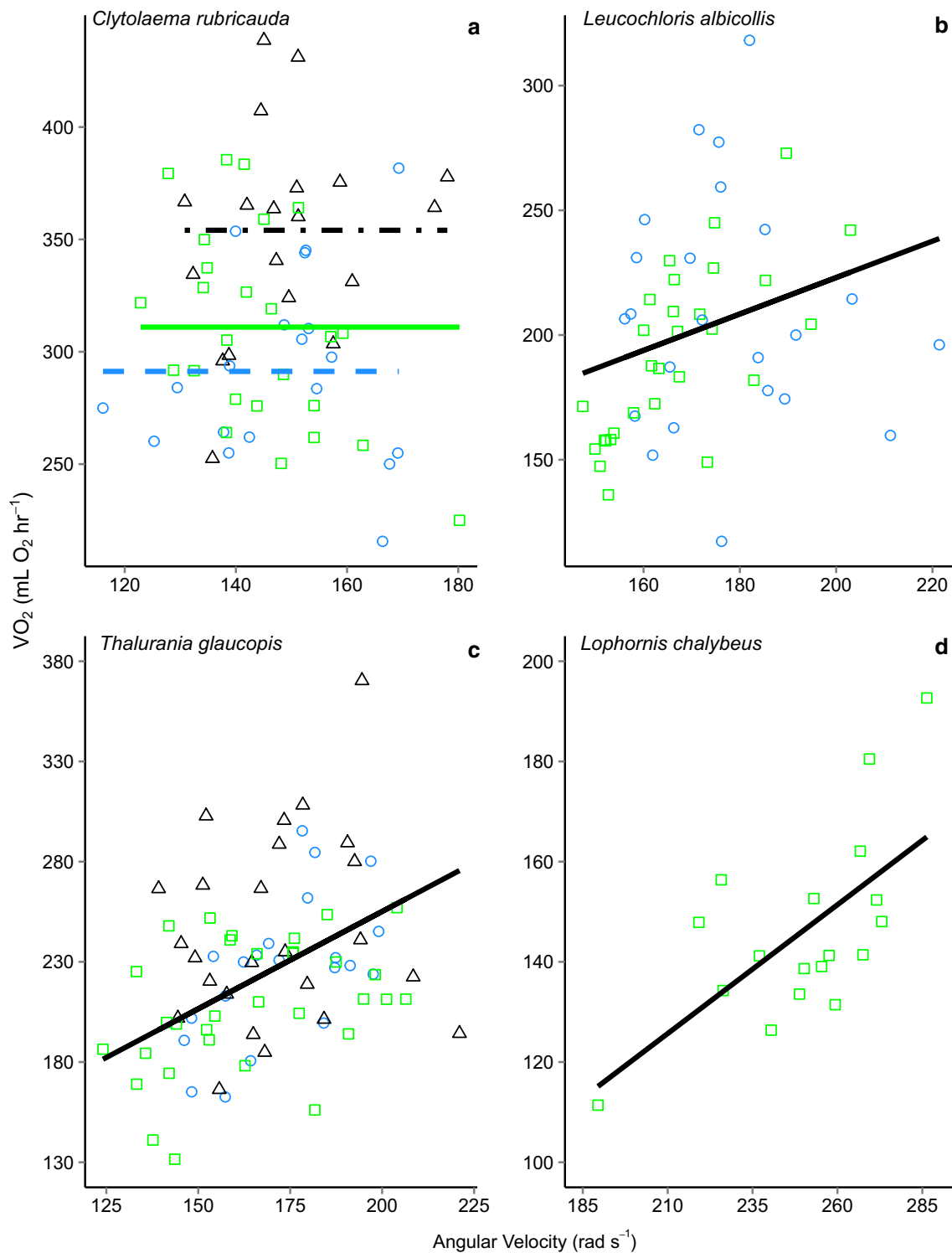
Fig. 7 The relationship between mean angular velocity of the wing and whole-animal metabolic rate for **a** *Clytolaema rubricauda*, **b** *Leucochloris albicollis*, **c** *Thalurania glaucopis*, **d** *Lophornis chalybeus*. *Green squares*, *blue circles*, and *black triangles* correspond to 0, 1000, and 1800 m asl, respectively. A *single regression line* is pre-sented for 3 of the species, as metabolic rate did not vary with eleva-tion. *Clytolaema rubricauda* varied due to elevation alone, thus each *regression line* corresponds to the respective elevational color. Sam-ple sizes can be found on Table 1 (color figure online)

Fig. 8 The relationship between efficiency and total mass during sustained hovering across three elevations in four species of hummingbird **a** *Clytolaema rubricauda*, **b** *Leucochloris albicollis*, **c** *Thalurania glaucopis*, and **d** *Lophornis chalybeus*. *Green squares*, *blue circles*, and *black triangles* correspond to 0, 1000, and 1800 m asl, respectively. Sample sizes can be found in Table 1 (color figure online)

frequency tended to increase only by as much as 10 % (Chai and Dudley 1995, 1996; Altshuler and Dudley 2003;

Roberts et al. 2004; Altshuler et al. 2010; Mahalingam and Welch 2013), but these studies employed density reduction

which reduced profile drag and inertial costs of accelerating the wing. However, our results indicate these changes in environmental conditions may not exert a particularly strong effect on wingbeat frequency. Instead, it seems that increasing wingbeat frequency across a range of mechanical power demands may be an overall strategy that hummingbirds use regardless of air density. Further, given the difference in morphology and musculoskeletal dynamics, it is perhaps not surprising that the mechanical resonance of the flight structures may not play as large of a role in setting wingbeat frequencies in birds when compared to the exoskeletons of flying insects.

Similar to previous studies in both hummingbirds and insects (Wells 1993a; Dudley 1995; Chai and Dudley 1995, 1996; Lehmann and Dickinson 1997; Altshuler and Dudley 2003; Roberts et al. 2004; Altshuler et al. 2005; Vance et al. 2009; Altshuler et al. 2010; Mahalingam and Welch 2013; Vance et al. 2014), we found that stroke amplitude is an important driver of variation in the angular velocity of the wing during hovering flight. Stroke amplitude increased in concert with increasing total mass being lifted, to help generate the necessary lift. Stroke amplitudes approached 160°, which is similar to other failure stroke amplitudes observed during sustainable weight-lifting trials (Wells 1993a; Mahalingam and Welch 2013), but much lower than what is achieved during air density reduction challenges which reach stroke amplitudes of 180° (Chai and Dudley 1996). The difference in peak stroke amplitudes may be related to environmental variation, with the reduced air density possibly permitting greater excursion angles during hypodensity experiments when compared to the constant air density of weightlifting. This may be related to the drag and inertial requirements of hovering in normodense air when compared to extremely low air densities achieved by heliox replacement. However, there was no evidence to support this assertion in our study. Though we saw no significant variation in stroke amplitude during maximal sustained hovering flight across an almost 2000 m elevational gradient, such variation may be evident when examined across a broader real or simulated elevational range (e.g. 0–4000 m). Further studies across a greater elevational range or by using heliox gas mixtures during weightlifting may provide some insight into this.

Despite the lack of elevational influence upon wingbeat frequency and stroke amplitude, we, nonetheless, observed significant increases in the angular velocity of the wings with increasing elevation. We conclude that changes in both variables that exhibit no significant pattern with elevation can still significantly alter the kinematics they collectively define. Individual birds did not show a clear preference for modulation of one kinematic variable over another, given the variance found within each individual. Increases in angular velocity resulted in greater mechanical power output and metabolic expenditure in most of the species examined.

Variation in the relationship of angular velocity and metabolic rate with increasing elevation could suggest that declines in air density reduce the metabolic requirements associated with achieving given wingtip velocities during hovering flight. However, we find that metabolic rates are determined only by the angular velocities for most species presented and that this relationship is not different among elevations tested. Similarly, Mahalingam and Welch (2013) found that relative muscular recruitment at a given angular velocity is the same, regardless of air density. Taken together, this suggests that muscle recruitment is solely dependent upon the tip velocities that are required to sustain hovering flight and that other effects associated with air density, such as reduced drag and inertial costs, do not play a role in setting the required amount of muscle recruitment. Thus, it appears that metabolic capacity does not constrain the variation in stroke amplitude that is seen during weight-lifting challenges. Instead, other aspects of hovering flight may be preventing the utilization of the remaining stroke amplitude reserve, such as the nature of the weightlifting protocol.

Clytolaema rubricauda displays very unusual patterns in oxygen consumption rates that are vastly different from the other species. It is currently unknown as to why *C. rubricauda* did not vary their metabolic response to their unweighted hovering flight across elevations (Fig. 1a) or the unusual pattern across weight lifting (Fig. 6a). It appears that the unweighted hovering metabolic rates of the sea-level population are higher than expected when compared to 1000 and 1800 m. In regards to weightlifting, birds at the sea-level site showed very little effect of weightlifting upon metabolic rate, whereas the other elevations show a pattern more similar to the other species. It is unlikely that this species at sea-level is relying primarily upon anaerobic sources of energy to support weighted hovering flight, as the hovering durations are all much longer than 2 s. Given the reproducibility of the trend relating mass lifted and metabolic rate during hovering at other elevations and in other species, we suspect that the near-zero slope of this relationship in *C. rubricada* at sea level is artifactual. Though we cannot be certain that this is the case, we suspect that *C. rubricada* engaged in unweighted hovering at sea-level displayed unusually elevated metabolic rates due to the effects of handling stress.

Maximum sustained and burst flight performance

Asymptotic load-lifting in conjunction with air density manipulation has become an increasingly common method of assessing the maximum mechanical power output that a hovering animal is capable of producing. Results

comparing mechanical power output during sustained and burst loading lifting suggest that the latter elicits much higher maximum power production, as has been found previously in bees (Buchwald and Dudley 2010).

Kinematics and mechanical power output of the three species during burst load-lifting did not significantly vary across the elevations examined (Figs. 2, 3, 4, 5). This suggests that maximum stroke amplitude and maximum wingbeat frequency during burst flight are species-dependent rather than environmentally determined (Buermann et al. 2011), since muscle strain rates and trajectories are likely at their peak and cannot increase further. The maximum stroke amplitude that a hummingbird is able to attain is constrained by the morphology of the wing and shoulder. Stroke amplitudes usually peak around 180°, as seen in this and other studies (Chai et al. 1997; Altshuler and Dudley 2003; Altshuler et al. 2010; Mahalingam and Welch 2013), as at values above this, wings interfere with each other during supination. Wingbeat frequency is limited by the capacity a hummingbird possesses to increase muscle shortening velocity and cycling frequency (Altshuler et al. 2010; Mahalingam and Welch 2013). As such, the maximum burst mechanical power output of the flight apparatus remained largely similar across elevations for each of the three species.

For hummingbirds, asymptotic load-lifting led to larger stroke amplitudes and wingbeat frequencies than those observed when lifting maximum sustainable loads (Figs. 2, 3). The differences in performance are likely due to the energetic strategies employed during these trials: primarily aerobic sources during maximum sustained lifting and aerobic plus substantial anaerobic sources during asymptotic load-lifting (Chai et al. 1997). The short duration of these asymptotic trials is likely due to the limited support anaerobic metabolism can provide for highly intense activities, as only anaerobic metabolism can power the high angular velocities necessary for burst flight. The two-second threshold to delineate between anaerobic and aerobic flight selected by this study may be robust enough to ensure flight is primarily aerobically powered (Hochachka and Matheson 1992). As efficiency did not change across masses being lifted or across elevations, oxygen consumption rates and mechanical power output must be increasing proportionally. Invariable efficiency during hovering flight challenges has been documented before in heliox or with a weightlifting challenge (Wells 1993a; Chai and Dudley 1995; Chai et al. 1996; Chai and Dudley 1999). Even when hummingbirds modified their kinematics due to temperature or the effects of floral morphology, efficiency was maintained at ~10 % (Evangelista et al. 2010; Wells 1993a, b). Only when oxygen is a limiting resource, does efficiency begin to increase with challenge, as flight durations decline, and there is a greater reliance upon supplemental

anaerobic sources of energy which is not accounted for in respirometry without measuring post-exercise recovery (Chai and Dudley 1996). However, follow-up studies examining lactate production during weightlifting activities needs to be performed to ensure anaerobic energy stores are not being utilized at the higher sustainably lifted masses.

Generally, maximum-recorded aerobic metabolic rates within a species did not vary across the different elevations. Given that different populations of hummingbirds were sampled across elevations, which, in turn, affects thermoregulatory costs and metabolic expenditure during hovering flight, variation in maximum-recorded metabolic rates during hovering flight were expected. This lack in variability across the elevations may be related to the profile drag and inertial costs of accelerating and decelerating the wings. Any reduction in profile drag or inertia due to low air density may be offset by an increased energetic requirement associated with decelerating the wing at the end of the half wingbeat. This would maintain the metabolic requirements for a specific angular velocity, regardless of air density. The lack of variation could also be related to the weightlifting protocol, as the beads that were available defined the amount of weight that could be added at each interval. Smaller weightlifting intervals may be able to provide finer resolution to the limits of metabolic and energetic expenditure across environmental gradients. However, time limitations prevented us from exploring finer gradations in sustainable weightlifting performance.

The ratio between maximum-recorded metabolic rate and metabolic rate during unweighted hovering flight is much higher within three of the four species than has been previously reported (Chai and Dudley 1999), with ruby-throated hummingbirds (*Archilochus colubris*) capable of increasing metabolic rate by around 25 % during maximal power output (Chai and Dudley 1999). Re-analysis of data recorded from rufous hummingbirds (*Selasphorus rufus*) hovering at various elevations from sea level to ~3000 m asl and at a broad range of temperatures reveals that they can increase metabolic rates by a further ~40 % over sea level hovering at warm temperatures (Welch and Suarez

Table 4 VO_2 reserves of rufus hummingbirds (*Selasphorus rufus*) hovering at 12–3089 m asl

Age	AHY		HY	
	Male	Female	Male	Female
N	4	5	3	1
VO_2 reserve	1.41 ± 0.10	1.45 ± 0.16	1.47 ± 0.16	1.49

Data calculated and reproduced from (Welch and Suarez 2008)

AHY after hatch year, *HY* hatch year, *N* sample size

2008; Table 4). However, these measurements occurred across a natural elevational gradient, birds were not challenged to the point where flight failure occurred, and variation in metabolic rate reflect both changes in lift as well as thermoregulatory costs. Thus, it is difficult to directly compare these data, as rufous hummingbirds may maintain VO$_2$ reserves that are still higher. One possibility for the discrepancy in VO$_2$ reserves across species may be inherent species-specific differences, particularly wing morphology and kinematics. This would cause some species to operate at a greater proportion of their maximum metabolic ceiling than others. Further studies examining VO$_2$ reserve variation across species would provide valuable insight into this.

Conclusions

Kinematics during hovering flight vary depending upon the challenge imposed and the size of the organism. Previous studies have suggested that wingbeat frequency is highly constrained for all hovering organisms, and stroke amplitude is the preferred kinematic variable to be modified (Chai and Dudley 1996; Chai et al. 1997; Altshuler and Dudley 2003; Buchwald and Dudley 2010). This has been thought to limit the necessary increases in energetic cost associated with more demanding hovering flight. However, the hummingbirds examined here exhibited different kinematic patterns, with both wingbeat frequency and stroke amplitude increasing in concert with a sustainable load challenge. These hummingbirds likely modulate both kinematic variables in response to any change in the power output that is required for flight. The increase in wingbeat frequency occurred independent of elevation, suggesting that lower profile drag is not responsible for the increased wingbeat frequencies observed. Further, metabolic rates appear to be set by the angular velocity required to maintain hovering flight, and any mechanical or energetic savings attributable to environmental variation (such as the influence of air density on drag or inertial costs) do not exist.

Compliance with ethical standards

Conflict of interest No competing interests declared.

Ethical approval All procedures performed in studies involving animals were in accordance with the ethical standards of the institution or practice at which the studies were conducted.

References

Altshuler DL, Dudley R (2003) Kinematics of hovering hummingbird flight along simulated and natural elevational gradients. J Exp Biol 206:3139–3147. doi:10.1242/jeb.00540

Altshuler DL, Dudley R, Ellington CP (2004a) Aerodynamic forces of revolving hummingbird wings and wing models. J Zool 264:327–332. doi:10.1017/S0952836904005813

Altshuler DL, Dudley R, McGuire JA (2004b) Resolution of a paradox: Hummingbird flight at high elevation does not come without a cost. Proc Natl Acad Sci USA 101:17731–17736. doi:10.1073/pnas.0405260101

Altshuler DL, Dickson WB, Vance JT et al (2005) Short-amplitude high-frequency wing strokes determine the aerodynamics of honeybee flight. Proc Natl Acad Sci USA 102:18213–18218. doi:10.1073/pnas.0506590102

Altshuler DL, Welch KC, Cho BH et al (2010) Neuromuscular control of wingbeat kinematics in Anna's hummingbirds (Calypte anna). J Exp Biol 213:2507–2514. doi:10.1242/jeb.043497

Arnold TW (2010) Uninformative parameters and model selection using Akaike's information criterion. J Wildl Manag 74:1175–1178. doi:10.1111/j.1937-2817.2010.tb01236.x

Brobeck JR, Dubois AB (1980) Energy exchange. In: Mountcastle VB (ed) Medical physiology. C.V. Mosby Co., St. Louis, pp 1351–1365

Buchwald R, Dudley R (2010) Limits to vertical force and power production in bumblebees (Hymenoptera: Bombus impatiens). J Exp Biol 213:426–432. doi:10.1242/jeb.033563

Buermann W, Chaves JA, Dudley R et al (2011) Projected changes in elevational distribution and flight performance of montane Neotropical hummingbirds in response to climate change. Glob Change Biol 17:1671–1680. doi:10.1111/j.1365-2486.2010.02330.x

Chai P, Dudley R (1995) Limits to vertebrate locomotor energetics suggested by hummingbirds hovering in heliox. Nature 377:722–725. doi:10.1038/377722a0

Chai P, Dudley R (1996) Limits to flight energetics of hummingbirds hovering in hypodense and hypoxic gas mixtures. J Exp Biol 199:2285–2295

Chai P, Dudley R (1999) Maximum flight performance of hummingbirds: capacities, constraints, and trade-offs. Am Nat 153:398–411. doi:10.1086/an.1999.153.issue-4

Chai P, Millard D (1997) Flight and size constraints: hovering performance of large hummingbirds under maximal loading. J Exp Biol 200:2757–2763

Chai P, Harrykissoon R, Dudley R (1996) Hummingbird hovering performance in hyperoxic heliox: effects of body mass and sex. J Exp Biol 199:2745–2755

Chai P, Chen JS, Dudley R (1997) Transient hovering performance of hummingbirds under conditions of maximal loading. J Exp Biol 200:921–929

Chai P, Altshuler DL, Stephens DB, Dillon ME (1999) Maximal horizontal flight performance of hummingbirds: effects of body mass and molt. Physiol Biochem Zool 72:145–155. doi:10.1086/316652

Chen CCW, Welch KC (2014) Hummingbirds can fuel expensive hovering flight completely with either exogenous glucose or fructose. Funct Ecol 28:589–600. doi:10.1111/1365-2435.12202

Denny MW (1993) Air and water: the biology and physics of life's media. Princeton University Press, Princeton

Dillon ME, Dudley R (2004) Allometry of maximum vertical force production during hovering flight of neotropical orchid bees (Apidae: Euglossini). J Exp Biol 207:417–425. doi:10.1242/jeb.00777

Dillon ME, Dudley R (2014) Surpassing Mt. Everest: extreme flight performance of alpine bumble-bees. Biol Lett 10:20130922. doi:10.1098/rsbl.2013.0922

Dudley R (1995) Extraordinary flight performance of orchid bees (Apidae: Euglossini) hovering in heliox (80 % He/20 % O2). J Exp Biol 198:1065–1070

Dudley R (2000) The biomechanics of insect flight: form, function, evolution. Princeton University Press, Princeton

Ellington CP (1984a) The aerodynamics of hovering insect flight. VI. Lift and power requirements. Philos Trans R Soc Lond B Biol Sci 305:145–181. doi:10.1098/rstb.1984.0054

Ellington CP (1984b) The aerodynamics of hovering insect flight. II. Morphological parameters. Philos Trans R Soc Lond B Biol Sci 305:17–40. doi:10.1098/rstb.1984.0050

Ellington CP (1984c) The aerodynamics of hovering insect flight. V. A vortex theory. Philos Trans R Soc Lond B Biol Sci 305:115–144. doi:10.1098/rstb.1984.0053

Evangelista D, Fernández MJ, Berns MS, Hoover A, Dudley R (2010) Hovering energetics and thermal balance in Anna's hummingbirds (Calypte anna). Physiol Biochem Zool 83:406–413. doi:10.1086/605107

Felsenstein J (1985) Phylogenies and the comparative method. Am Nat 125:1–15

Greenewalt CH (1960) The wings of insects and birds as mechanical oscillators. Proc Am Philos Soc 104:605–611

Hochachka PW, Matheson GO (1992) Regulating ATP turnover rates over broad dynamic work ranges in skeletal muscles. J Appl Physiol 73:1697–1703

Lehmann FO, Dickinson MH (1997) The changes in power requirements and muscle efficiency during elevated force production in the fruit fly Drosophila melanogaster. J Exp Biol 200:1133–1143

Lighton JRB (2008) Measuring metabolic rates: a manual for scientists. Oxford University Press, New York

Mahalingam S, Welch KC (2013) Neuromuscular control of hovering wingbeat kinematics in response to distinct flight challenges in the ruby-throated hummingbird, Archilochus colubris. J Exp Biol 216:4161–4171. doi:10.1242/jeb.089383

Norberg U (1990) Vertebrate flight: mechanics, physiology, morphology, ecology and evolution. Springer-Verlag, Berlin

Pennycuick C (1996) Wingbeat frequency of birds in steady cruising flight: new data and improved predictions. J Exp Biol 199:1613–1618

Pinheiro J, Bates D, DebRoy S et al (2014) nlme: linear and nonlinear mixed effects models

R Core Team (2013) R: a language and environment for statistical computing

Roberts SP, Harrison JF, Dudley R (2004) Allometry of kinematics and energetics in carpenter bees (Xylocopa varipuncta) hovering in variable-density gases. J Exp Biol 207:993–1004. doi:10.1242/jeb.00850

Segre PS, Dakin R, Zordan VB et al (2015) Burst muscle performance predicts the speed, acceleration, and turning performance of Anna's hummingbirds. eLife. e11159. doi:10.7554/eLife.11159

Tobalske BW, Biewener AA, Warrick DR et al (2010) Effects of flight speed upon muscle activity in hummingbirds. J Exp Biol 213:2515–2523. doi:10.1242/jeb.043844

Vance JT, Williams JB, Elekonich MM, Roberts SP (2009) The effects of age and behavioral development on honey bee (Apis mellifera) flight performance. J Exp Biol 212:2604–2611. doi:10.1242/jeb.028100

Vance JT, Altshuler DL, Dickson WB et al (2014) Hovering flight in the honeybee Apis mellifera: kinematic mechanisms for varying aerodynamic forces. Physiol Biochem Zool 87:870–881. doi:10.1086/678955

Weis-Fogh T (1972) Energetics of hovering flight in hummingbirds and in Drosophila. J Exp Biol 56:79–104

Welch KC Jr (2011) The power of feeder-mask respirometry as a method for examining hummingbird energetics. Comp Biochem Physiol A Mol Integr Physiol 158:276–286. doi:10.1016/j.cbpa.2010.07.014

Welch KC, Suarez RK (2008) Altitude and temperature effects on the energetic cost of hover-feeding in migratory rufous hummingbirds, Selasphorus rufus. Can J Zool 86:161–169. doi:10.1139/Z07-127

Wells DJ (1993a) Ecological correlates of hovering flight of hummingbirds. J Exp Biol 178:59–70

Wells DJ (1993b) Muscle performance in hovering hummingbirds. J Exp Biol 178:39–57

Withers PC (1977) Measurement of VO2, VCO2, and evaporative water loss with a flow-through mask. J Appl Physiol 42:120–123

Zuur AF, Ieno EN, Walker NJ et al (2009) Mixed effects models and extensions in ecology with R. Springer Science, New York