© 2020. Published by The Company of Biologists Ltd | Journal of Experimental Biology (2020) 223, jeb234104. doi:10.1242/jeb.234104

SHORT COMMUNICATION

Accelerometry predicts muscle ultrastructure and flight capabilities in a wild bird

Kristen M. Lalla[1,*], Shannon Whelan[1], Karl Brown[2], Allison Patterson[1], Ana Gabriela Jimenez[2], Scott A. Hatch[3] and Kyle H. Elliott[1]

ABSTRACT

Muscle ultrastructure is closely linked with athletic performance in humans and lab animals, and presumably plays an important role in the movement ecology of wild animals. Movement is critical for wild animals to forage, escape predators and reproduce. However, little evidence directly links muscle condition to locomotion in the wild. We used GPS-accelerometers to examine flight behaviour and muscle biopsies to assess muscle ultrastructure in breeding black-legged kittiwakes (*Rissa tridactyla*). Biopsied kittiwakes showed similar reproductive success and subsequent over-winter survival to non-biopsied kittiwakes, suggesting that our study method did not greatly impact foraging ability. Muscle fibre diameter was negatively associated with wing beat frequency, likely because larger muscle fibres facilitate powered flight. The number of nuclei per fibre was positively associated with average air speed, likely because higher power output needed by faster-flying birds required plasticity for muscle fibre recruitment. These results suggest the potential for flight behaviour to predict muscle ultrastructure.

KEY WORDS: Muscle histology, Myonuclei, Biologging, Wing beat frequency, Laridae, Foraging behaviour

INTRODUCTION

Efficient movement is essential for animals to successfully forage, reproduce and, ultimately, survive (Fraser et al., 2018; Nathan et al., 2008). Although muscle ultrastructure is closely linked with locomotory performance in humans and animals in the lab (Gleeson and Harrison, 1988; Methenitis et al., 2016; Terzis et al., 2010), few studies have examined this link in wild animals, partly because there are few non-invasive techniques for measuring muscle performance. Consequently, although muscle ultrastructure may be of paramount importance to survival and reproduction, muscle physiology and plasticity have largely been ignored as traits linked to individual fitness. Biologgers can provide detailed information on animal movement (Ainley et al., 2003; Gese et al., 2016; Jacobsen et al., 2017), including fine-scale motion such as limb stroke rate or wing beat frequency (Sato et al., 2008). Biologging could reveal details about underlying muscle condition. Using miniature biologgers, we investigated whether there are links between locomotory performance and muscle condition in a wild bird.

[1]Department of Natural Resource Sciences, McGill University, Sainte-Anne-de-Bellevue, QC, Canada H9X 3V9. [2]Department of Biology, Colgate University, Hamilton, NY 13346, USA. [3]Institute for Seabird Research and Conservation, Anchorage, AK 99516-3185, USA.

*Author for correspondence (kristen.lalla@mail.mcgill.ca)

K.M.L., 0000-0003-1422-0672; S.W., 0000-0003-2862-327X; K.B., 0000-0001-8132-1196; A.P., 0000-0001-9931-2693; S.A.H., 0000-0002-0064-8187

Received 31 July 2020; Accepted 11 October 2020

Animal movement is a product of underlying muscle physiology. Muscle tissue includes fast-twitch, slow-twitch and fast-oxidative glycolytic fibres in birds (Burke et al., 1973; Chiarandini and Stefani, 1983; Rosser and George, 1986; Talesara and Goldspink, 1978; Weber, 2009). Movement is critical for predators to overpower prey and for prey to outmanoeuvre their attackers (Wilson et al., 2018). Thus, having good muscle condition is essential for survival in animals, especially flying animals that require efficient flight to forage, find mates and breed. Wing shape and fuselage affect drag, body weight and profile power costs (Bowlin and Wikelski, 2008), but it is the pectoralis muscles that ultimately power flight (Dietz et al., 1999). Contraction of fast-twitch muscle fibres powers the downstroke (Caldow and Furness, 1993; Pennycuick, 2008). In animals, muscle growth is a combination of hyperplasia (an increase in fibre number) and hypertrophy (an increase in fibre diameter), and muscle ultrastructure is plastic across seasons and/or lifetime (Bittner and Traut, 1978; Brown et al., 2019; Jimenez et al., 2019a,b, 2011; Vézina et al., 2019). Muscles play an important role in movement, and muscle ultrastructure may drive individual variation in behaviour. A slight loss of muscle function can lead to a reduction in locomotory performance, with clear fitness implications (Ricklefs, 2008).

Athletic performance depends on both muscle performance and body morphology. For example, hammer throwers have both larger type IIA fibre diameters and lean body mass compared with non-athletes (Terzis et al., 2010), and jumping, sprinting and throwing performance correlate with muscle fibre cross-sectional area and lean body mass (Methenitis et al., 2016). Similarly, flight performance of a wild animal depends on both costs, determined by aerofoil shape, and power generated from the engine (muscles), determined by muscle anatomy (Pennycuick, 2008). Body mass and wingspan together explain most of the variation in mechanical flight costs. Larger animals with shorter wings require more power to stay aloft, while larger animals have wider girths and therefore require more power to overcome drag (Pennycuick, 2008). Thus, larger birds with shorter wings have higher optimal flight speeds to generate sufficient lift; indeed, flight speed increases with body mass within and across species (Pennycuick, 2008; Tennekes, 2009). The power to overcome flight costs is provided in birds by their wing beats. As power cost increases, so must power generated by the wings (Pennycuick, 2008). Across species, wing beat frequency decreases with body mass because the thrust generated by increasing wingspan (and wing beat amplitude) more than offsets the cost associated with increased body mass (Pennycuick, 2008; Sato et al., 2007). However, within an individual bird, where wingspan is constant, an increase in body mass requires an increase in wing beat frequency or amplitude to offset the increased lift needed to keep a heavier body aloft – as has been demonstrated both theoretically and empirically (Pennycuick, 2008; Sato et al., 2008; Schmidt-Wellenburg et al., 2007). Thus, across individuals within a species, where variation in body mass is much larger than variation

Journal of Experimental Biology (2020) 223, jeb234104. doi:10.1242/jeb.234104

in wingspan, we would expect that both flight speed and wing beat frequency increase with body mass.

The power required to keep a body of a given mass and wingspan aloft must be generated by the underlying musculature of the animal. During each wing beat, the pectoralis muscles that power the downstroke complete a work loop (Hill, 1938; Pennycuick, 2008). Increased power needed for increased flight speed should be associated with increased muscle cross-sectional area (fibre diameter), potential new fibre recruitment in the form of satellite cells or increasing potential protein machinery into existing fibres by having satellite cells fuse with existing muscles (nuclei per fibre) and increasing oxygen and nutrient flow to muscles (capillary density). According to Hill's equation, the optimal frequency should be constant for a muscle. Thus, increasing fibre diameter, the main parameter determining power, should increase wing beat amplitude. For a constant air speed, an increase in amplitude should be balanced by a reduction in the number of wing beats (the flap: glide ratio but not the frequency during each flap) (Pennycuick, 2008). Therefore, we expect that average wing beat frequency should decrease with fibre diameter.

Black-legged kittiwakes (*Rissa tridactyla*) are a declining piscivorous colonial seabird species. They must travel hundreds of kilometres and work exceptionally hard to breed successfully (Coulson, 2011; Kitaysky et al., 2001). Here, we paired biologging with measures of pectoral (flight) muscle condition in adult breeding kittiwakes. During commuting flights, kittiwakes use air speeds above minimum power speed, and power requirements therefore increase with flight speed (Elliott et al., 2014; Pennycuick, 2008). Previous work in our study system demonstrated that air speed is independent of wing beat frequency (Collins et al., 2020), and we considered those parameters to be independent of one another. We examined muscle fibre diameter (which we use as interchangeable with the related parameter 'fibre cross-sectional area'), myonuclear domain, number of nuclei per fibre and capillary density. Myonuclear domain is the quantity of cytoplasm in a muscle fibre, measured per nucleus (Qaisar and Larsson, 2014). Larger muscle fibres have higher contraction force as a result of more actin–myosin crossbridges and are cheaper to maintain because the lower surface area to volume ratio requires lower investment in membrane-associated active pumps and supporting structures, such as capillaries (Jimenez et al., 2013; Jimenez and Williams, 2014a). In contrast, smaller muscle fibre diameter can also be advantageous because of faster diffusion rates of metabolites as a result of the shorter diffusion distances (Jimenez et al., 2019a; Kinsey et al., 2011). Likewise, greater capillary density is associated with greater provision of oxygen and nutrients to muscles because there are more capillaries to supply the muscle with these metabolites (Jimenez et al., 2019a). Myonuclei are responsible for producing proteins for the cytoplasm (Brooks et al., 2009). A higher number of nuclei per fibre likely leads to improved muscle efficiency by increasing protein turnover and facilitating the recruitment of new fibres (Brooks et al., 2009; Vézina et al., 2019). Therefore, as described above, we expected that fibres with larger diameters, higher capillary density and higher nuclei per fibre would be linked to higher air speed in kittiwakes. In contrast, we expected that fibre diameter would decrease with average wing beat frequency.

MATERIALS AND METHODS

Field and lab methods

We studied black-legged kittiwakes, *Rissa tridactyla* (Linnaeus 1758), at Middleton Island (59°26′N, 146°20′W), Alaska, where they breed on an abandoned US Air Force radar tower with one-way glass (Gill and Hatch, 2002; Hatch et al., 1993). Some of the breeding kittiwake pairs were food-supplemented with Atlantic capelin as part of a long-term study throughout the breeding season (Gill and Hatch, 2002). Food-supplemented kittiwakes were fed 3 times a day (09:00 h, 14:00 h, 18:00 h) from the time that they began building nests. Individual capelin were fed through holes on the tower, and we fed each nest during each feeding bout until each kittiwake refused food. We do not expect food supplementation to affect muscle ultrastructure as Brown et al. (2019) observed no differences in muscle ultrastructure between food-supplemented and non-supplemented individuals in the kittiwakes at Middleton. We did not measure muscle fat content, as we assumed that food-supplemented kittiwakes did not gain a significant amount of fat or experience drastic changes in muscle. We deployed GPS-accelerometers (9 g, AxyTrek, TechnoSmart Europe) on 37 adult breeding individuals during late incubation (≥15 days after eggs laid) in 2017, recording GPS locations every 3 min and tri-axial acceleration at 25 Hz for 4 days. We attached GPS-accelerometers to the two central retrices below the preen gland on top of the tail using Tesa tape, two cable ties and superglue. We recaptured kittiwakes 4 days after deployment to remove GPS-accelerometers and to take pectoral muscle biopsies (N=24; 14 males, 10 females, 6 of 24 food-supplemented; see Brown et al., 2019, for details).

Muscle biopsies were performed after recapture, on a clean surface. To ensure the kittiwake would lie still, we placed the individual in a sock with a hole over the left pectoralis muscle. One person continuously monitored the condition of the restrained animal, while a second person performed the biopsy. We covered the bird's head with a cloth to reduce stress. We then located the feather tract on the left breast and the thickest area of the pectoralis. The area was cleaned using Hibiclens and the feathers were smoothed away from the biopsy area. We injected 0.05 ml of lidocaine HCl 2% in 3–4 spots under the skin and up to 2 mm intramuscularly using a 1 ml tuberculin syringe with a 26 1⁄2 gauge needle. Ketaprofen (0.10 ml) was injected in the same way and we waited 2 min for it to numb the area. We first used a scalpel to make a paramedian incision with the minimum size required to insert the 6 mm biopsy punch. The fat was lifted with forceps and cut away. We pulled up the biopsy plug with forceps and immediately inserted it into a vial containing 4% paraformaldehyde, and stored it at room temperature. Because of the remoteness of our field site, this was the only available storage method. Lidocaine, in combination with adrenaline (epinephrine), may affect gene transcription and cause vasoconstriction (Trappe et al., 2013; Zhang et al., 2017), though we did not use adrenaline and did not observe any impacts of Lidocaine on our muscle tissue samples. Moreover, impacts on gene transcription are unlikely to influence any of our muscle parameters in the short period from injection to taking the biopsy and fixing the sample.

To close the biopsy site, we used a simple interrupted stitch and applied tissue adhesive (Vetbond™, 3M). Once sure that the incision was closed and tissue adhesive was dry, we smoothed feathers over the biopsy site and released the bird. All birds flew away without noticeable difficulty and returned to attend their nests. We obtained permits from the United States Fish and Wildlife Service (MB01629B) and the Alaska Department of Fish and Game (17-162), and an Animal Use Protocol from McGill University (2016-7814; sample size was justified by a statistical power analysis). We monitored hatching and fledging success via daily nest checks. In the year following our study, we conducted daily resighting by recording colour band combinations of individuals present to determine apparent overwinter survival of birds that bred

in our study year to examine whether muscle biopsies affected survival.

Muscle staining methods can be found in Brown et al. (2019). Briefly, 30 µm muscle sections were cut both in cross-section and longitudinally to study fibre diameter and fibre cross-sectional area, and myonuclear domain, respectively. Sections were mounted on slides and stained using a 250 mg ml^{-1} solution of DAPI to stain nuclei, wheat germ agglutinin with Alexa Fluor 488 to stain the sarcolemma, and *Griffonia simplicifolia* lectin 694 to stain capillaries (Molecular Probes, Inc.) for 30 min, and subsequently rinsed in avian Ringer's solution for 1 h. We examined the stained slides using a Zeiss 710 laser filter confocal microscope and traced polygons around the fibres using ImageJ (https://imagej.nih.gov/ij/) as in Brown et al. (2019). We counted capillaries and myonuclei by hand using split images of each stain. To measure fibre diameter, cross-sectional area and myonuclear domain, we randomly selected 45 fibres across three separate randomly selected areas in the tissue for each individual. Because pectoralis muscles fibres are considered homogeneous, we considered these 45 fibres from three sections to be representative (Caldow and Furness, 1993). Measurements and calculations of fibre diameter, fibre cross-sectional area and myonuclear domain can be found in more detail in Brown et al. (2019). Histochemistry images can also be found in Brown et al. (2019).

Movement parameters and behavioural classification

We calculated wing beat frequency as the peak frequency from a fast Fourier transform on acceleration in the *z*-axis, calculated over a 5 s sliding window (Patterson et al., 2019). GPS-accelerometer files subsampled to 1 s intervals were screened to identify flights of 30 min duration or longer. We calculated average wing beat frequency for each entire flight. We obtained wind speed and direction for each GPS location through the Env-DATA track-annotation service provided by Movebank, from which we calculated wind speed and direction for each flight (Dodge et al., 2013, National Oceanic & Atmospheric Administration: Earth System Research Laboratory, https://www.esrl.noaa.gov/psd/data/gridded/data.narr.html). To calculate distance, direction of travel and speed between GPS points, we used *adehabitatLT* (https://CRAN.R-project.org/package=adehabitatLT) in R (http://www.R-project.org/, version 3.4.3). Finally, we calculated air speed from the bird's ground speed and wind speed.

Statistical analyses

All statistical analyses were performed in R (http://www.R-project.org/, version 3.4.3) and statistical significance was considered at $P<0.05$. To examine effects of the tagging and biopsy combination on hatching and fledging success (0, 1, 2 eggs hatched or chicks fledged), we used 2-way ANOVA with tagging/biopsy (yes/no) and food supplementation (supplemented/control) as 2-level factors with Poisson distribution. To examine the effects of muscle biopsies on apparent interannual survival (yes=resighted in 2018, no=absent in 2018), we used Fisher's exact test for each food-supplemented and non-supplemented control group. We constructed stepwise models with backwards selection for mean wing beat frequency and air speed in response to fixed effects body mass, sex (female/male), food supplementation, maximum distance flown from the colony over the deployment period, and muscle histology variables: myonuclear domain, fibre diameter [removing fibre cross-sectional area (CSA) because it is closely correlated with fibre diameter (FD)=0.0199CSA+24.42, R^2=0.875] and total capillary density. We considered sex and food supplementation as 2-level factors and all other predictors as continuous regression variables and report adjusted R^2. We tested for equal variance and normality of the residuals.

RESULTS AND DISCUSSION

Our 2×2 model indicated supplemental feeding (z=−2.31, $P<0.03$, 0.42±0.18 more chicks per pair for food-supplemented pairs) but not GPS-biopsy treatment (z=−0.11, P=0.91, 0.02±0.21 more chicks per pair for biopsied than control pairs), altered hatching success. Similarly, fledging success of fed birds was higher than that of control birds ($F_{1,129}$=31.3, $P<0.001$) but there was no effect of muscle biopsy ($F_{1,129}$=0.015, P=0.90). Among food-supplemented birds, there was similar apparent interannual survival for biopsied (100%, n=10) and non-biopsied (90.3%, n=124) birds from 2017 to 2018 (Fisher's exact test, P=0.60). Among non-supplemented birds, there was also similar apparent interannual survival for biopsied (81.7%, n=27) and non-biopsied (86.4%, n=103) birds (Fisher's exact test, P=0.55).

The top model of mean wing beat frequency included body mass, food supplementation, myonuclear domain and fibre diameter ($F_{4,19}$=4.91, P=0.007). Of the parameters included in this model, body mass and fibre diameter were significant, where wing beat frequency increased with body mass, and decreased with fibre diameter (Fig. 1, Table 1).

The top model of air speed included body mass, food supplementation, sex, number of nuclei per fibre, capillary density and maximum distance flown from the colony ($F_{7,16}$=4.54, P=0.006). Body mass, food supplementation, number of nuclei per fibre and maximum distance from the colony were significant (Table 1). Air speed increased with body mass and number of nuclei per fibre, but decreased with maximum distance from the colony (Fig. 2).

Conclusions

Heavier kittiwakes had higher wing beat frequency, relative to lighter individuals, likely because heavier kittiwakes must work harder to maintain flight (Elliott et al., 2014; Pennycuick, 2008; Sato et al., 2008). Wing beat frequencies were lower among individuals with larger muscle fibre diameter, after accounting for body mass. Furthermore, previous work showed that body mass does not predict fibre diameter in kittiwakes (Brown et al., 2019) despite correlations between fibre diameter and mass in fish and crustaceans, species which vary much more substantially in body mass than kittiwakes (Jimenez et al., 2013, 2008); therefore, the relationship between wing beat frequency and fibre diameter that we observed is not due to an underlying correlation with body size. It is advantageous to have larger pectoral muscle fibres because larger muscle fibres are cheaper to maintain metabolically as they have a lower surface area to volume ratio, and are thus cheaper for the animal in times of greater workload, and provide greater contraction force (Jimenez et al., 2013; Jimenez and Williams, 2014a). In contrast to the case in large muscle fibres, diffusion of oxygen and ATP are more efficient in smaller muscle fibres because the diffusion distance is shorter, which could facilitate greater wing beat frequency at smaller muscle fibre size (Jimenez et al., 2013). The optimal fibre size hypothesis in fish predicts that fibre size is a balance between diffusion, which is better at smaller fibre diameters, and metabolic costs, which are lower at large fibre sizes (Jimenez et al., 2011; Johnston et al., 2006, 2004, 2003). We assumed that lower average wing beat frequency is linked to larger fibre diameter. With this assumption, kittiwakes with larger fibre sizes are able to sustain higher wing beat frequencies; however, at a larger fibre size, diffusion rates are reduced (Jimenez et al., 2013). To further examine this trade-off between diffusion and metabolic cost, future work could pair instantaneous wing beat frequency with instantaneous air speed to examine how birds are performing in different flight conditions and compare performance with muscle ultrastructure.

Journal of Experimental Biology (2020) 223, jeb234104. doi:10.1242/jeb.234104

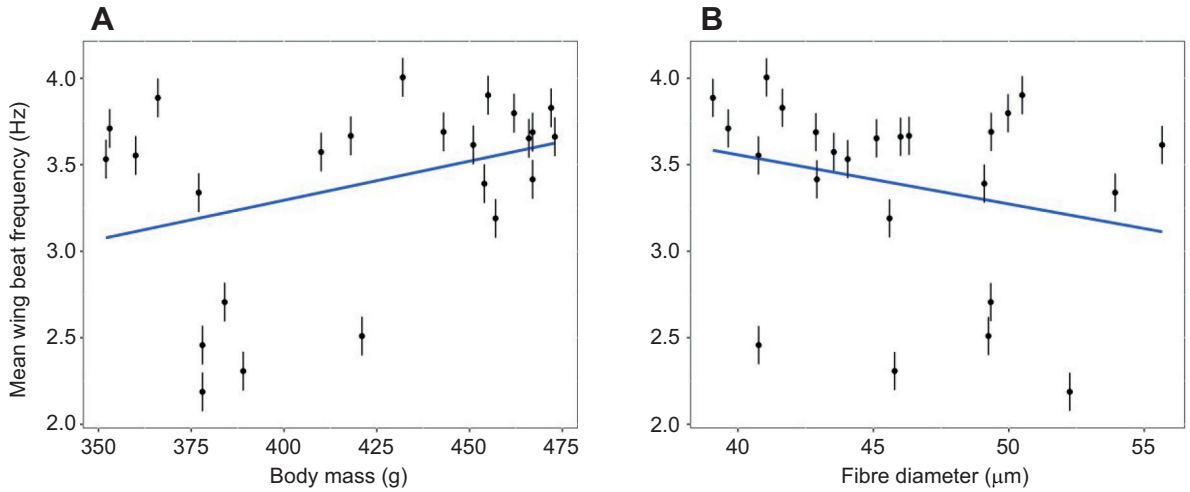

Fig. 1. Wing beat frequency versus body mass and fibre diameter. (A) Mean wing beat frequency increases with body mass and (B) decreases with fibre diameter. Error bars represent s.e.m. *N*=24.

In birds, muscle mass and ultrastructure can change in response to environmental conditions, e.g. grow larger in winter to tolerate the cold (Jimenez and Williams, 2014a,b). However, as we conducted our study during late incubation, a relatively narrow time frame, we expected that any seasonal muscle ultrastructure changes would be similar across our study individuals (Jimenez et al., 2019a). In addition, muscle ultrastructure in adult kittiwakes does not vary with age or between food-supplemented and non-supplemented individuals (Brown et al., 2019), so we do not believe that seasonality, age or food supplementation confounded the relationships we observed between our flight variables and muscle ultrastructure measurements. We were not able to measure muscle mass in the field, and were therefore unable to verify whether heavier kittiwakes had greater muscle mass than lighter individuals. Another limitation of our study is that we did not analyse intramuscular and intermuscular fat distribution.

Body mass correlated with air speed, suggesting that heavier kittiwakes are also flying faster, as expected from flight theory. Heavier kittiwakes have a higher optimal flight speed, as they must fly faster to generate optimal lift to keep aloft (Pennycuick, 2008). Individuals that were food supplemented or that travelled farther from the colony (where prey is likely more abundant) flew more slowly. The food-supplemented individuals did not need to spend as much effort foraging to meet daily energy intake requirements, and thus were able to meet foraging requirements travelling at lower flight speeds than control individuals.

The number of nuclei per millimetre of fibre was positively associated with air speed, independently from mass. As muscles grow hypertrophically (increase in fibre size), they can recruit more nuclei to maintain function (protein turnover), allowing muscles to continue to function for sustained periods, and allowing individuals within a species to sustain greater flight speeds (Rosenblatt and Parry, 1992; Rosenblatt et al., 1994). Past studies demonstrated that the number of nuclei per fibre increases with muscle training, even preceding hypertrophic muscle growth (Brooks et al., 2009; Bruusgaard et al., 2010; Vézina et al., 2019), supporting our hypothesis that a higher number of nuclei per fibre is associated with greater air speeds. The number of nuclei per fibre has been positively linked to fibre diameter and cross-sectional fibre area; however, we found separate effects of fibre diameter and number of nuclei per fibre on wing beat frequency and average air speed, respectively, suggesting they affect different aspects of flight performance (Brown et al., 2019; Burleigh, 1977; Johnston et al., 2004).

We did not find any impact of the biologger/biopsy combination on reproductive success or overwinter survival, implying that a small biopsy did not greatly impair the kittiwakes' ability to forage and care for offspring. There were no immediate lethal effects. Many previous studies on wild birds did not detect negative effects of biopsies on survival and body condition (Baker, 1981; Evans et al., 2009; Westneat, 1986), though one study detected mixed effects on reproductive success and survival (Westneat et al., 1986)

Table 1. Estimates of variables influencing mean wing beat frequency and average air speed from top models determined through backwards stepwise model selection

	Wing beat frequency (Hz)			Air speed (m s^{-1})		
	Estimate±s.e.m.	*F*	*P*	Estimate±s.e.m.	*F*	*P*
Body mass (g)	0.005±0.002	5.06	0.04*	0.010±0.007	6.99	0.02*
Supplementation (control)	−0.28±0.20	1.14	0.30	1.88±0.64	8.66	0.01*
Myonuclear domain (μm^3)	0.00023±0.00008	0.32	0.58	–	–	–
Fibre diameter (μm)	−0.096±0.026	13.13	0.002*	−0.090±0.060	0.80	0.38
Sex (male)	–	–	–	1.58±0.68	0.78	0.39
Number of nuclei per mm fibre	–	–	–	0.025±0.05	5.19	0.04*
Capillary density	–	–	–	857±654	1.51	0.24
Maximum distance (km)	–	–	–	−0.041±0.015	7.84	0.01*
Mean square error	0.17			1.21		
Model	$F_{4,19}$=4.91, *P*=0.007			$F_{7,16}$=4.54, *P*=0.006		
Model fit (R^2)	0.40			0.52		

Supplementation effects are relative to control. Asterisks indicate significant *P*-values.

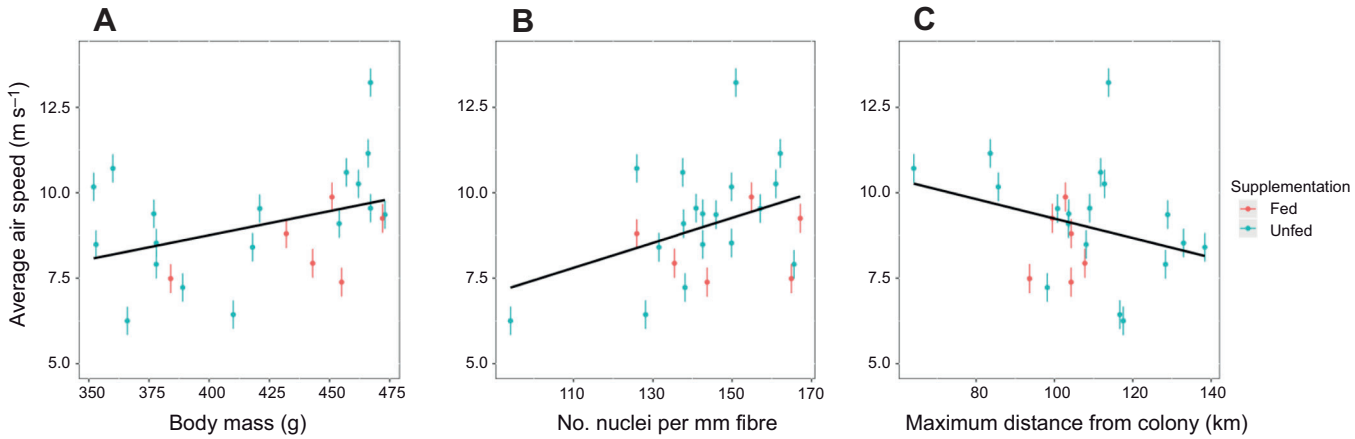

Fig. 2. Air speed versus body mass, nuclei per fibre and distance from colony. Air speed during flight increased with (A) body mass and (B) number of nuclei per fibre, but decreased with (C) maximum displacement distance from the colony. Error bars represent s.e.m. *N*=24.

and another reported abandonment in white storks (*Eudocimus albus*; including one individual operated as a control with no biopsy taken; Frederick, 1986). In our study, there may be effects on long-term survival or fine-scale effects not observed. For instance, GPS loggers twice as large as those used here had an impact on time budgets for kittiwakes at our colony (Chivers et al., 2016); it is possible that muscle biopsies had a similar impact. Our work suggests that carefully collected muscle biopsies can be safely sampled from at least one species of wild seabird.

Wing beat frequency correlated positively with body mass and negatively with fibre diameter. Average air speed was positively predicted by mass and the number of nuclei per fibre, and negatively by maximum distance flown from the colony. Food-supplemented kittiwakes had lower average air speed than control individuals. Our study focused on a single species, and, as such, we are only attempting to draw intraspecific conclusions. Studying muscle condition in the context of flight performance is important in understanding how muscle ultrastructure affects an individual's daily activities, namely the ability to forage, escape predators and reproduce. For declining species, it is especially important to understand how muscle ultrastructure is linked to behaviour. Kittiwakes are a cold-water species (Hatch, 2013), and heat stress may trigger physiological changes as seen in heat-shocked house sparrows *Passer domesticus* (Jimenez and Williams, 2014b), potentially selecting for individuals that have a muscle ultrastructure allowing them to quickly adapt to changes in the environment. Future analyses could examine changes in muscle ultrastructure in seabirds as a result of heat stress, as in house sparrows (Jimenez and Williams, 2014b), or the link between flight performance and satellite cells, which are responsible for regeneration of muscles, as another way of examining muscle health, with possible links to senescence (Schmalbruch and Hellhammer, 1977).

Acknowledgements
Dr Z. M. Benowitz-Fredericks provided muscle biopsy training to K.M.L. and S.W., and assisted in the field. S. M. Collins, L. M. Lacey, and A. Mouillier assisted with data collection. Dr J. Meyers and Dr W.-C. Liu allowed us to use their cryostats.This project was completed as part of K.M.L.'s Honour's thesis and we thank the McGill Bieler School of Environment, especially K. Roulet, for organizing the Honours programme.

Competing interests
The authors declare no competing or financial interests.

Author contributions
Conceptualization: K.M.L., S.W., A.G.J., K.H.E.; Methodology: K.M.L., S.W., K.B., A.P., A.G.J., K.H.E.; Software: K.M.L., S.W., A.P.; Validation: K.M.L., S.W., A.P.; Formal analysis: K.M.L., S.W., K.B., A.P., A.G.J., K.H.E.; Investigation: K.H.E.; Resources: K.H.E.; Data curation: K.M.L.; Writing - original draft: K.M.L.; Writing - review & editing: K.M.L., S.W., K.B., A.P., A.G.J., S.A.H., K.H.E.; Visualization: K.M.L., S.W.; Supervision: S.W., A.G.J., K.H.E.; Project administration: S.A.H., K.H.E.; Funding acquisition: A.G.J., S.A.H., K.H.E.

Funding
This project was supported by the Natural Sciences and Engineering Research Council of Canada (Discovery Grant) and by Canada Research Chairs in Arctic Ecology (to K.H.E.), Institute for Seabird Research and Conservation (SAH), and the Northern Scientific Training Program (Polar Knowledge Canada, K.H.E., K.M.L.).

Data availability
GPS-accelerometer data are available from Movebank: Black-legged kittiwake *Rissa tridactyla* Middleton Island Movebank ID: 1326534946: https://www.movebank.org/cms/webapp?gwt_fragment=page=studies,path=study1326534946.

Supplementary information
Supplementary information available online at
https://jeb.biologists.org/lookup/doi/10.1242/jeb.234104.supplemental

References
Ainley, D. G., Ford, R. G., Brown, E. D., Suryan, R. M. and Irons, D. B. (2003). Prey resources, competition, and geographic structure of kittiwake colonies in Prince William Sound. *Ecology* **84**, 709-723. doi:10.1890/0012-9658(2003)084[0709:PRCAGS]2.0.CO;2

Baker, M. C. (1981). A muscle biopsy procedure for use in electrophoretic studies of birds. *The Auk* **98**, 392-393.

Bittner, G. D. and Traut, D. L. (1978). Growth of crustacean muscles and muscle fibers. *J. Comp. Physiol.* **124**, 277-285. doi:10.1007/BF00657059

Bowlin, M. S. and Wikelski, M. (2008). Pointed wings, low wingloading and calm air reduce migratory flight costs in songbirds. *PLoS ONE* **3**, e2154-e2154. doi:10.1371/journal.pone.0002154

Brooks, N. E., Schuenke, M. D. and Hikida, R. S. (2009). Ageing influences myonuclear domain size differently in fast and slow skeletal muscle of rats. *Acta Physiol.* **197**, 55-63. doi:10.1111/j.1748-1716.2009.01983.x

Brown, K., Jimenez, A. G., Whelan, S., Lalla, K., Hatch, S. A. and Elliott, K. H. (2019). Muscle fiber structure in an aging long-lived seabird, the black-legged kittiwake (*Rissa tridactyla*). *J. Morphol.* **280**, 1061-1070. doi:10.1002/jmor.21001

Bruusgaard, J. C., Johansen, I. B., Egner, I. M., Rana, Z. A. and Gundersen, K. (2010). Myonuclei acquired by overload exercise precede hypertrophy and are not lost on detraining. *Proc. Natl. Acad. Sci. USA* **107**, 15111-15116. doi:10.1073/pnas.0913935107

Burke, R. E., Levine, D. N., Tsairis, P. and Zajac, F. E. (1973). Physiological types and histochemical profiles in motor units of the cat gastrocnemius. *J. Physiol.* **234**, 723-748. doi:10.1113/jphysiol.1973.sp010369

Burleigh, I. G. (1977). Observations on the number of nuclei within the fibres of some red and white muscles. *J. Cell Sci.* **23**, 269.

Caldow, R. W. G. and Furness, R. W. (1993). A histochemical comparison of fibre types in the M. pectoralis and M. supracoracoideus of the great skua *Catharacta skua* and the herring gull *Larus argentatus* with reference to kleptoparasitic capabilities. *J. Zool.* **229**, 91-103. doi:10.1111/j.1469-7998.1993.tb02623.x

Chiarandini, D. J. and Stefani, E. (1983). Calcium action potentials in rat fast-twitch and slow-twitch muscle fibres. *J. Physiol.* **335**, 29-40. doi:10.1113/jphysiol.1983.sp014516
Chivers, L. S., Hatch, S. A. and Elliott, K. H. (2016). Accelerometry reveals an impact of short-term tagging on seabird activity budgets. *The Condor* **118**, 159-168. doi:10.1650/CONDOR-15-66.1
Collins, P. M., Green, J. A., Elliott, K. H., Shaw, P. J. A., Chivers, L., Hatch, S. A. and Halsey, L. G. (2020). Coping with the commute: behavioural responses to wind conditions in a foraging seabird. *J. Avian Biol.* **51**, 1-11. doi:10.1111/jav.02057
Coulson, J. (2011). *The Kittiwake*. London: T & AD Poysner.
Dietz, M. W., Piersma, T. and Dekinga, A. (1999). Body-building without power training: endogenously regulated pectoral muscle hypertrophy in confined shorebirds. *J. Exp. Biol.* **202**, 2831.
Dodge, S., Bohrer, G., Weinzierl, R., Davidson, S. C., Kays, R., Douglas, D., Cruz, S., Han, J., Brandes, D. and Wikelski, M. (2013). The environmental-data automated track annotation (Env-DATA) system: linking animal tracks with environmental data. *Movement Ecology* **1**, 1-14.
Elliott, K. H., Chivers, L. S., Bessey, L., Gaston, A. J., Hatch, S. A., Kato, A., Osborne, O., Ropert-Coudert, Y., Speakman, J. R. and Hare, J. F. (2014). Windscapes shape seabird instantaneous energy costs but adult behavior buffers impact on offspring. *Movement Ecol.* **2**, 17. doi:10.1186/s40462-014-0017-2
Evans, K. O., Burger, L. W., , Jr, Faircloth, B. C., Palmer, W. E. and Carroll, J. P. (2009). Effects of tissue collection methods on morphometrics and survival of captive neonatal Northern Bobwhite. *J. Wildlife Management* **73**, 1241-1244. doi:10.2193/2008-011
Fraser, K. C., Davies, K. T. A., Davy, C. M., Ford, A. T., Flockhart, D. T. T. and Martins, E. G. (2018). Tracking the conservation promise of movement ecology. *Front. Ecol. Evol.* **6**, 150. doi:10.3389/fevo.2018.00150
Frederick, P. C. (1986). Parental desertion of nestlings by white Ibis (Eudocimus albus) in response to muscle biopsy. *J. Field Ornithol.* **57**, 168-170.
Gese, E. M., Terletzky, P. A. and Cavalcanti, S. M. C. (2016). Identification of kill sites from GPS clusters for jaguars (*Panthera onca*) in the southern Pantanal, Brazil. *Wildl. Res.* **43**, 130-139. doi:10.1071/WR15196
Gill, V. A. and Hatch, S. A. (2002). Components of productivity in black-legged kittiwakes *Rissa tridactyla*: response to supplemental feeding. *J. Avian Biol.* **33**, 113-126. doi:10.1034/j.1600-048X.2002.330201.x
Gleeson, T. T. and Harrison, J. M. (1988). Muscle composition and its relation to sprint running in the lizard Dipsosaurus dorsalis. *Am. J. Physiol.* **255**, 470-477. doi:10.1152/ajpregu.1988.255.3.R470
Hatch, S. A. (2013). Kittiwake diets and chick production signal a 2008 regime shift in the Northeast Pacific. *Mar. Ecol. Prog. Ser.* **477**, 271-284. doi:10.3354/meps10161
Hatch, S. A., Roberts, B. D. and Fadely, B. S. (1993). Adult survival of Black-legged Kittiwakes Rissa tridactyla in a Pacific colony. *Ibis* **135**, 247-254. doi:10.1111/j.1474-919X.1993.tb02841.x
Hill, A. V. (1938). The heat of shortening and the dynamic constants of muscle. *Proc. R. Soc. Lond. B Biol. Sci.* **126**, 136-195. doi:10.1098/rspb.1938.0050
Jacobsen, L. B., Jensen, N. O., Willemoes, M., Hansen, L., Desholm, M., Fox, A. D., Tøttrup, A. P. and Thorup, K. (2017). Annual spatiotemporal migration schedules in three larger insectivorous birds: European nightjar, common swift and common cuckoo. *Anim. Biotelemetry* **5**, 4. doi:10.1186/s40317-017-0119-x
Jimenez, A. G. and Williams, J. B. (2014a). Differences in muscle fiber size and associated energetic costs in phylogenetically paired tropical and temperate birds. *Physiol. Biochem. Zool.* **87**, 752-761. doi:10.1086/677922
Jimenez, A. G. and Williams, J. B. (2014b). Rapid changes in cell physiology as a result of acute thermal stress House sparrows, Passer domesticus. *J. Therm. Biol.* **46**, 31-39. doi:10.1016/j.jtherbio.2014.10.001
Jimenez, A. G., Locke, B. R. and Kinsey, S. T. (2008). The influence of oxygen and high-energy phosphate diffusion on metabolic scaling in three species of tail-flipping crustaceans. *J. Exp. Biol.* **211**, 3214. doi:10.1242/jeb.020677
Jimenez, A. G., Dasika, S. K., Locke, B. R. and Kinsey, S. T. (2011). An evaluation of muscle maintenance costs during fiber hypertrophy in the lobster *Homarus americanus*: are larger muscle fibers cheaper to maintain? *J. Exp. Biol.* **214**, 3688-3697. doi:10.1242/jeb.060301
Jimenez, A. G., Dillaman, R. M. and Kinsey, S. T. (2013). Large fibre size in skeletal muscle is metabolically advantageous. *Nat. Commun.* **4**, 2150. doi:10.1038/ncomms3150
Jimenez, A. G., O'Connor, E. S., Brown, K. J. and Briggs, C. W. (2019a). Seasonal muscle ultrastructure plasticity and resistance of muscle structural changes during temperature increases in resident black-capped chickadees and rock pigeons. *J. Exp. Biol.* **222**, jeb201855. doi:10.1242/jeb.201855
Jimenez, A. G., O'Connor, E. S. and Elliott, K. H. (2019b). Muscle myonuclear domain, but not oxidative stress, decreases with age in a long-lived seabird with high activity costs. *J. Exp. Biol.* **222**, jeb211185. doi:10.1242/jeb.211185
Johnston, I. A., Fernández, D. A., Calvo, J., Vieira, V. L. A., North, A. W., Abercromby, M. and Garland, T. (2003). Reduction in muscle fibre number during the adaptive radiation of notothenioid fishes: a phylogenetic perspective. *J. Exp. Biol.* **206**, 2595. doi:10.1242/jeb.00474
Johnston, I. A., Abercromby, M., Vieira, V. L. A., Sigursteindóttir, R. J., Kristjánsson, B. K., Sibthorpe, D. and Skúlason, S. (2004). Rapid evolution of muscle fibre number in post-glacial populations of Arctic charr *Salvelinus alpinus*. *J. Exp. Biol.* **207**, 4343. doi:10.1242/jeb.01292
Johnston, I. A., Abercromby, M. and Andersen, Ø. (2006). Muscle fibre number varies with haemoglobin phenotype in Atlantic cod as predicted by the optimal fibre number hypothesis. *Biol. Lett.* **2**, 590-592. doi:10.1098/rsbl.2006.0500
Kinsey, S. T., Locke, B. R. and Dillaman, R. M. (2011). Molecules in motion: influences of diffusion on metabolic structure and function in skeletal muscle. *J. Exp. Biol.* **214**, 263. doi:10.1242/jeb.047985
Kitaysky, A. S., Wingfield, J. C. and Piatt, J. F. (2001). Corticosterone facilitates begging and affects resource allocation in the black-legged kittiwake. *Behav. Ecol.* **12**, 619-625. doi:10.1093/beheco/12.5.619
Methenitis, S. K., Zaras, N. D., Spengos, K. M., Stasinaki, A.-N. E., Karampatsos, G. P., Georgiadis, G. V. and Terzis, G. D. (2016). Role of muscle morphology in jumping, sprinting, and throwing performance in participants with different power training duration experience. *J. Strength Cond Res.* **30**, 807-817. doi:10.1519/JSC.0000000000001147
Nathan, R., Getz, W. M., Revilla, E., Holyoak, M., Kadmon, R., Saltz, D. and Smouse, P. E. (2008). A movement ecology paradigm for unifying organismal movement research. *Proc. Natl. Acad. Sci. USA* **105**, 19052-19059. doi:10.1073/pnas.0800375105
Patterson, A., Gilchrist, H. G., Chivers, L., Hatch, S. and Elliott, K. (2019). A comparison of techniques for classifying behavior from accelerometers for two species of seabird. *Ecol. Evol.* **9**, 3030-3045. doi:10.1002/ece3.4740
Pennycuick, C. J. (2008). Modelling the flying bird. In *Theoretical Ecology Series* (ed. C. J. Pennycuick), pp. 1-480. Academic Press.
Piersma, T., Gudmundsson, G. A. and Lilliendahl, K. (1999). Rapid changes in the size of different functional organ and muscle groups during refueling in a long-distance migrating shorebird. *Physiol. Biochem. Zool.* **72**, 405-415. doi:10.1086/316680
Qaisar, R. and Larsson, L. (2014). What determines myonuclear domain size? *Indian J. Physiol. Pharmacol.* **58**, 1-12.
Ricklefs, R. E. (2008). The evolution of senescence from a comparative perspective. *Funct. Ecol.* **22**, 379-392. doi:10.1111/j.1365-2435.2008.01420.x
Rosenblatt, J. D. and Parry, D. J. (1992). Gamma irradiation prevents compensatory hypertrophy of overloaded mouse extensor digitorum longus muscle. *J. Appl. Physiol.* **73**, 2538-2543. doi:10.1152/jappl.1992.73.6.2538
Rosenblatt, J. D., Yong, D. and Parry, D. J. (1994). Satellite cell activity is required for hypertrophy of overloaded adult rat muscle. *Muscle Nerve* **17**, 608-613. doi:10.1002/mus.880170607
Rosser, B. W. C. and George, J. C. (1986). The avian pectoralis: histochemical characterization and distribution of muscle fiber types. *Can. J. Zool.* **64**, 1174-1185. doi:10.1139/z86-176
Sato, K., Watanuki, Y., Takahashi, A., Miller, P. J. O., Tanaka, H., Kawabe, R., Ponganis, P. J., Handrich, Y., Akamatsu, T., Watanabe, Y. et al. (2007). Stroke frequency, but not swimming speed, is related to body size in free-ranging seabirds, pinnipeds and cetaceans. *Proc. R. Soc. B* **274**, 471-477. doi:10.1098/rspb.2006.0005
Sato, K., Daunt, F., Watanuki, Y., Takahashi, A. and Wanless, S. (2008). A new method to quantify prey acquisition in diving seabirds using wing stroke frequency. *J. Exp. Biol.* **211**, 58-65. doi:10.1242/jeb.009811
Schmalbruch, H. and Hellhammer, U. (1977). The number of nuclei in adult rat muscles with special reference to satellite cells. *Anat. Rec.* **189**, 169-175. doi:10.1002/ar.1091890204
Schmidt-Wellenburg, C. A., Biebach, H., Daan, S. and Visser, G. H. (2007). Energy expenditure and wing beat frequency in relation to body mass in free flying Barn Swallows (*Hirundo rustica*). *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **177**, 327-337. doi:10.1007/s00360-006-0132-5
Talesara, G. L. and Goldspink, G. (1978). A combined histochemical and biochemical study of myofibrillar ATPase in pectoral, leg and cardiac muscle of several species of bird. *Histochem. J.* **10**, 695-709. doi:10.1007/BF01003119
Tennekes, H. (2009). *The Simple Science of Flight, Revised and Expanded Edition: From Insects to Jumbo Jets*. Cambridge, United States: MIT Press.
Terzis, G., Spengos, K., Kavouras, S., Manta, P. and Georgiadis, G. (2010). Muscle fibre type composition and body composition in hammer throwers. *J. Sports Sci. Med.* **9**, 104-109.
Trappe, T. A., Standley, R. A., Liu, S. Z., Jemiolo, B., Trappe, S. W. and Harber, M. P. (2013). Local anesthetic effects on gene transcription in human skeletal muscle biopsies. *Muscle Nerve* **48**, 591-593. doi:10.1002/mus.23860
Vézina, F., Cornelius Ruhs, E., O'Connor, E. S., Le Pogam, A., Régimbald, L., Love, O. P. and Jimenez, A. G. (2019). Consequences of being phenotypically mismatched with the environment: rapid muscle ultrastructural changes in cold-shocked black-capped chickadees (Poecile atricapillus). *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **318**, R274-R283. doi:10.1152/ajpregu.00203.2019
Weber, J.-M. (2009). The physiology of long-distance migration: extending the limits of endurance metabolism. *J. Exp. Biol.* **212**, 593. doi:10.1242/jeb.015024
Westneat, D. F. (1986). The effects of muscle biopsy on survival and condition in white-throated sparrows. *Wilson Bull.* **98**, 280-285.
Westneat, D. F., Payne, R. B. and Doehlert, S. M. (1986). Effects of muscle biopsy on survival and breeding success in indigo buntings. *Condor* **88**, 220-227. doi:10.2307/1368919

Wilson, A. M., Hubel, T. Y., Wilshin, S. D., Lowe, J. C., Lorenc, M., Dewhirst, O. P., Bartlam-Brooks, H. L. A., Diack, R., Bennitt, E., Golabek, K. A. et al. (2018). Biomechanics of predator–prey arms race in lion, zebra, cheetah and impala. *Nature* **554**, 183. doi:10.1038/nature25479

Zhang, J. X., Gray, J., Lalonde, D. H. and Carr, N. (2017). Digital necrosis after lidocaine and epinephrine injection in the flexor tendon sheath without phentolamine rescue. *J. Hand Surg. [Am.]* **42**, e119-e123. doi:10.1016/j.jhsa.2016.10.015