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Article

Snow buntings preparing for migration increase muscle fiber size and myonuclear domain in parallel with a major gain in fat mass

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In long-distance migrants, preparation for migration is typically associated with increases in fat and body mass, and with an enlargement of pectoralis muscle mass that likely improves flight performance. Although changes in muscle mass or size have been well described in migratory birds, potential changes in muscle ultrastructure during this transition still deserves scrutiny. Using outdoor captive snow buntings (*Plectrophenax nivalis* n = 15) measured during their transition into a spring migratory phenotype as a model system, we studied changes in pectoralis muscle ultrastructure and predicted that muscle fiber diameter could increase in parallel with the gain in body mass. We also expected that larger fibers could either recruit satellite cells to support cellular maintenance and protein turnover, increase myonuclear domain (cytoplasm per nuclei) with a potential increase in protein turnover load per myonucleus, or existing myonuclei could undergo endoreduplication. Buntings increased body mass by 46% within a month, largely due to a > 6-fold increase in body fat. However, this increase in body mass was also associated with a 36% increase in muscle fiber diameter. Both pectoralis muscle mass ($r^2 = 0.57–0.77$) and fiber diameter ($r^2 = 0.32$) correlated with total body mass, without any change in the number of nuclei per fiber. Consequently, variation in myonuclear domain (i.e. the amount of cytoplasm per nucleus), was also positively associated with body mass ($r^2 = 0.51$). Therefore, buntings preparing for migration may experience an increase in muscle contraction force due to larger muscle fibers, but this is also coupled with increases in myonuclear domain, which may force these cells to increase protein production to safeguard satellite cells.

Keywords: cold acclimatization, fattening, migration, muscle ultrastructure, phenotypic flexibility

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Introduction

Avian migration is associated with major physiological changes, especially in species covering long distances between wintering and breeding grounds or in those crossing large geographic barriers. Typical phenotypic adjustments include a hyperphagic stage that is often associated with an enlargement of digestive organs and the accumulation of fat used as fuel (Blem 1976, Dawson et al. 1983, Piersma et al. 1999, Gannes 2002, Landys-Ciannelli et al. 2003, Piersma and van Gils 2011, Munoz-Garcia et al. 2012, Hua et al. 2013, Guglielmo 2018, Le Pogam et al. 2021). Birds preparing for migration also typically increase the size of their flight musculature (Dawson et al. 1983, Marsh 1984, Piersma et al. 1999, Landys-Ciannelli et al. 2003, Piersma and Van Gils 2011), presumably to compensate for a heavier body mass (Dawson et al. 1983, Marsh 1984, Lindström et al. 2000), although this muscle adjustment may not be occurring in all migrants (Bauchinger and Biebach 2005, Bauchinger et al. 2005, Kelsey and Bairlein 2019).

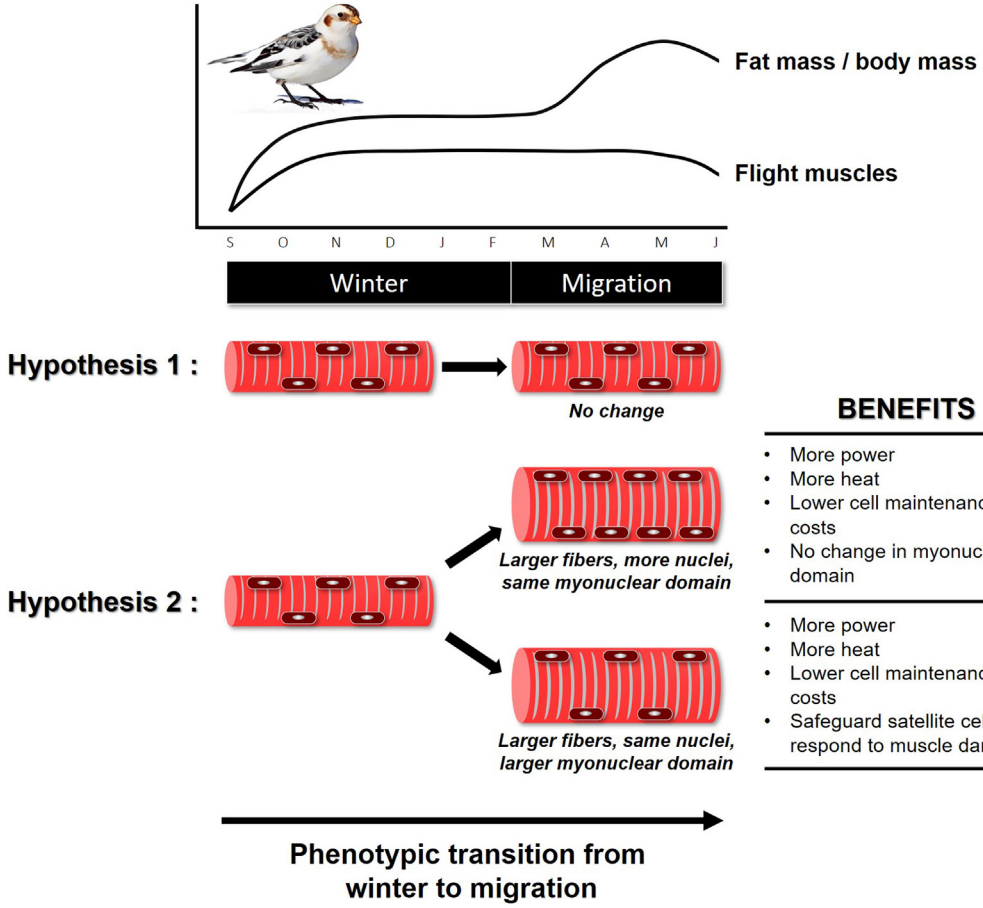
Phenotypic adjustments for migration have been well described at the whole animal and organ level (Newton 2007, Piersma and van Gils 2011). However, ultrastructural changes occurring in flight muscles, such as variation in muscle fiber size, have received much less attention (Marsh 1984, Gaunt et al. 1990, Evans et al. 1992, Velten et al. 2016). Muscle cells are post-mitotic, and changes in muscle mass or size can occur through hypertrophic or hyperplasic growth (Sola et al. 1973, Marsh 1984, Taylor and Wilkinson 1986, Evans et al. 1992, Antonio and Gonyea 1993). As large diameter muscle fibers have low resting metabolic rates, they also have lower maintenance energy demands per unit mass than smaller diameter fibers (Jimenez et al. 2013). Therefore, irrespective of whether a particular species increases the size of its flight muscles prior to departure, large fibers should be beneficial, particularly for migration, since they would reduce the energy required for muscle maintenance. In addition, since larger diameter muscle fibers contain more myofibrils, they would also produce more force during active contraction (Josephson 1975), and by extension likely more heat during shivering (Vézina et al. 2020). Large fibers could thus be advantageous for supporting flight with a heavier body, but also for thermoregulation in species migrating through cold environments (Swanson 1995, Swanson and Dean 1999, Vézina et al. 2017).

Muscles are multinucleated tissue (syncytium) and, while the enlargement of muscle cells improves energy efficiency (Jimenez et al. 2013), fibers with large diameters may also require the recruitment of new nuclei from satellite cells or undergo endoreduplication to prevent increases in myonuclear domain, that is, the volume of cytoplasm per nucleus (Qaisar and Larsson 2014). Larger myonuclear domains could in fact come with a consequent decline in protein turnover capacity per unit cell volume if nuclei maintain the same rate of work after the enlargement of a cell (Brooks et al. 2009), though some work suggests that myonuclei may also have 'spare capacity' (Cramer et al. 2020). As a post-mitotic syncytium, any post-natal growth or repair in muscle happens from a population of stem-like satellite cells that are found in the basement membrane of each muscle fiber and seem to be limited in number and/or proliferative potential during an animal's lifespan (Bruusgaard et al. 2010, Van der Meer et al. 2011, Forcina et al. 2019). Satellite cells can proliferate into existing fibers (Qaisar and Larsson 2014) to maintain a nearly constant nuclear-to-cytoplasmic ratio (Hughes and Schiaffino 1999), but they are also recruited into fibers at increased rates during muscle damage repair (Brooks et al. 2009, Bruusgaard et al. 2010, Forcina et al. 2019).

At the moment, it is unclear how birds use satellite cells during changes in muscle fiber volume. Some studies reported no changes in myonuclear domain during hypertrophic muscle growth, while others have reported an increase (Hikida et al. 1997, McCarthy and Esser 2007, O'Connor and Pavlath 2007, Brooks et al. 2009). In humans, there are evidence that myonuclear domain can be flexible in fast-glycolytic muscle fibers during resistance training (Murach et al. 2018). Thus, whether an adjustment of myonuclear domain is part of muscle growth is still debated (Van der Meer et al. 2011, Murach et al. 2018). One thing is clear however: birds appear to regulate myonuclear domain quite differently than mammals (Jimenez 2020) and currently no data is available on adjustments made by birds during migration. One would hypothesize that recruiting satellite cells into larger fibers twice a year for migration could be a counter-adaptive process in migratory birds as the number of satellite cells may be finite (Brooks et al. 2009, Bruusgaard et al. 2010). Alternatively, fiber hypertrophy could also occur without recruiting satellite cells, to safeguard those cells for muscle repair (Brooks et al. 2009, Bruusgaard et al. 2010), consequently leading to an increase in myonuclear domain and, presumably, to a change in protein turnover capacity per nucleus during migration (Cramer et al. 2020). Myonuclei could also potentially self-renew in the process of endoreduplication, though, there is currently no data on whether bird muscle can undergo endoreduplication (Jimenez 2020). For that reason, our hypotheses were developed around the concept of flexibility of myonuclear domain size.

In this study, we examined migration-related changes in body composition (lean and fat mass), pectoralis muscle mass and muscle fiber ultrastructure during preparation for migration in a passerine known for its migration in cold environments. Snow buntings *Plectrophenax nivalis* are Arctic-breeding, circumpolar cold specialists that winter in open snow-covered landscapes (Montgomerie and Lyon 2011, Snell et al. 2018). In Canada, the eastern population migrates toward its Greenlandic breeding ground early in the spring (Macdonald et al. 2012, McKinnon et al. 2019), and can encounter sub-zero temperatures and snowy conditions during flight and during the weeks following arrival (Meltofte 1983, Snell et al. 2018). Previous studies on outdoor captive snow buntings have shown typical phenotypic adjustments for spring migration, including considerable increases in body mass (up to 31%) and body fat (up to 226%) (Vincent and Bedard 1976, Le Pogam et al. 2021). These birds are also

known to maintain high oxygen carrying capacity and large flight muscles during this period (based on muscle thickness measured by ultrasonography, Le Pogam et al. 2021). In fact, these two traits are both upregulated during the fall (Sept–Nov), maintained through the winter months and spring transition into a migratory phenotype (March, Le Pogam et al. 2020) and up to the beginning of summer (April–May, Le Pogam et al. 2021). Working with birds under outdoor captive conditions, we therefore expected to observe the previously reported increases in fat and body mass while we predicted pectoralis muscle mass would remain stable during that period (Fig. 1, Vincent and Bedard 1976, Le Pogam et al. 2021). For muscle fiber phenotype, however, two scenarios could occur. Buntings could maintain fiber size to pre-migration levels given the previously reported lack of change in flight muscle thickness during fattening (Hypothesis 1 in Fig. 1, Le Pogam et al. 2021), although birds seemingly can increase fiber size with no or unmatched changes in muscle mass (Evans et al. 1992). Alternatively, buntings could increase the size of their muscle fibers in proportion to the gain in body mass to maximize contraction force and energy efficiency through reduced cell maintenance costs (Hypothesis 2 in Fig. 1, Dawson et al. 1983, Marsh 1984, Lindström et al. 2000, Jimenez et al. 2013, Zhang et al. 2018). In this latter case, myonuclear domain could remain constant if fiber growth is associated with an increase in the number of nuclei through satellite cell recruitment. Given the seasonal recurrence of migration and apparent irreversibility of nuclei recruitment (Brooks et al. 2009, Bruusgaard et al. 2010), increases in muscle fiber cells could also occur without changes in nuclei numbers, in which case myonuclear domain would increase with muscle size and body mass.



	BENEFITS	COSTS
Hypothesis 1	• More power • More heat • Lower cell maintenance costs • No change in myonuclear domain	• Loss of satellite cells • Less prepared to respond to muscle damage
Hypothesis 2	• More power • More heat • Lower cell maintenance costs • Safeguard satellite cells to respond to muscle damage	• Larger myonuclear domain • Greater protein turnover

Figure 1. Visual representation of the hypotheses tested in this study. Le Pogam et al. (2021) showed that as snow buntings accumulate fat and gain body mass during the transition from winter to migratory phenotypes, flight muscle thickness remains constant (schematized in the top figure). Hypothesis 1 posits that this lack of change in flight muscle size could be paralleled by a lack of change in muscle fiber size during the transition. In contrast, hypothesis 2 postulates that the transition from winter to migration could be associated with an increase in fiber size, thus providing greater contraction force and lower cell maintenance costs. In this specific case, cell enlargement could involve the recruitment of satellite cells into muscle fibers to increase the number of nuclei and maintain a constant myonuclear domain. Alternatively, as satellite cells are in finite numbers and are needed for muscle damage repair, the enlargement of muscle fibers for migration could also occur while safeguarding satellite cells for later use. This scenario would lead to an increase in myonuclear domain as the birds accumulate fat, gain mass and increase fiber size. The table presents the main benefits and costs of those two possible outcomes. See text for more details.

Material and methods

Captive conditions and whole-animal measurements

This study took place at the avian facility of the Université du Québec à Rimouski, Québec, Canada. The study lasted 56 days from 8 March to 3 May 2019, during the spring migration of snow buntings (Macdonald et al. 2012) and during which captive individuals are actively fattening and developing a migratory phenotype (Vincent and Bedard 1976, Le Pogam et al. 2021). Birds (11 males, 4 females taken from a larger pool of captive buntings) were housed in an outdoor aviary (5.8 m W × 5.3 m D × 2.6 m to 3.6 m H, angled ceiling) exposed to the elements except for direct solar radiation and precipitation. They were fed ad libitum with a commercial seed-mix (Armstrong, Hagersville, ON, Canada) and Mazuri Small Bird Maintenance Mini Diet (no. 562A; Mazuri, Richmond, IN, USA). Birds also had access to water supplemented with electrolytes and vitamins (0.17 g l^{-1}; Electrolytes Plus, 0.44 g l^{-1}; Polytonine A Complex, Vetoquinol N.-A. Inc. Lavaltrie, QC, Canada). The experiment involved 5, biweekly sampling events covering the complete fattening period, where three birds were measured for body composition before being sacrificed for muscle tissue sampling.

During each sampling day, birds were captured with a handheld net and brought into the lab adjacent to the aviary where they were weighed (± 0.01 g). We measured the fat and lean component of body mass non-invasively by quantitative magnetic resonance (QMR, ± 0.01 g; EchoMRI, Houston, TX, USA; Guglielmo et al. 2011, see Le Pogam et al. 2020, 2021 for details). We then euthanatized birds by CO_2 asphyxiation, after which we excised a sample of the left pectoralis muscle (~0.5 g) within 2 min of death, which we immediately fixed inside an avian ringer's solution containing 4% paraformaldehyde. Samples were then shipped to Colgate University for further analysis. Birds carcasses were kept frozen at −30°C until both left and right pectoralis muscles could be dissected, freeze-dried (FreeZone 2.5, Labconco, Kansas City, KS, USA) to less than 1% mass loss per 24 h and fat extracted in a Soxhlet apparatus using petroleum ether to obtain final pectoralis lean dry mass. Unfortunately, the QMR apparatus malfunctioned before the last series of measurements and we consequently could not collect lean and fat mass data for the final three individuals, although fat stores and body mass had already stabilized at their peak levels by that point. One bird was hurt the day preceding its capture and we discovered a bruise on its flight muscles during dissection. No ultrastructure and muscle mass data were used from that bird. All bird handling and collection were approved by the animal care committee of the Université du Québec à Rimouski (CPA-77-19-212), and were conducted under scientific (SC-48) and banding (10889A) permits from Environment and Climate Change Canada.

Muscle sectioning, staining and image analysis

We analyzed the pectoralis major muscle, a muscle thought to be homogeneously composed of fast-twitch oxidative-glycolytic fibers, similar to other migratory passerine species (Lundgren and Kiessling 1988). Fixed muscle sections were placed in a 30% sucrose solution overnight prior to sectioning to cryoprotect samples. Each muscle was mounted, sectioned and stained as described in Vézina et al. (2020). However, only two stains were used in the current study: a 250 mg ml^{-1} solution of wheat germ agglutinin (WGA; Molecular probes, Inc.) to highlight the sarcolemmal membrane and Alexa Fluor 488, and 4′,6-diamidino-2-phenylindole (DAPI; Molecular probes, Inc.) to highlight the nuclei (Vézina et al. 2020). Image analysis to determine myonuclear domain and fiber diameter was performed using Image J also following the protocol from Vézina et al. (2020).

Statistical analyses

We first used ANOVA models to study changes in phenotypic traits; that is, variation in total, lean and fat body mass, pectoralis muscle fresh and lean dry mass, and muscle ultrastructure, through time. For each ANOVA model we therefore included experimental day (i.e. date) as an independent variable. We used Tukey post-hoc tests to compare mean values among sampling days.

We then used linear regression analyses using data across all sampling days to: 1) determine how total body mass covaried with fat and lean mass, 2) determine the influence of total body mass on pectoralis ultrastructure variables and pectoralis mass and, 3) examine relationships between pectoralis muscle mass and ultrastructure variables. When analysing the relationship between fresh muscle mass and body mass, pectoralis mass was subtracted from body mass first to avoid part-whole correlations (Christians 1999), but this was not possible for lean dry muscles since remaining carcasses were kept for another study and were not dried and fat extracted.

We then extended this analysis to determine which of the fat or lean component of body mass better explained variation in pectoralis muscle mass and ultrastructure by including these two uncorrelated ($r^2 = 0.05$, $p = 0.6$) independent variables in multiple regression models (using lean body mass minus pectoralis fresh mass for the analysis on fresh muscle mass). We simplified models to simple linear regressions when only one parameter showed a significant effect.

We confirmed normality and homogeneity of residuals visually for all analyses, which were performed with JMP Pro 14. Presented in the text are means ± SEM.

Results

As expected, snow buntings gained body mass as they accumulated fat during the experiment. Body mass increased by 45.7% from early March to early April ($F_{4,10} = 4.4$, $p = 0.026$, Fig. 2A). This mass increase was accompanied by a 6.5-fold increase in body fat over that same time (2.8 ± 3.3–18.4 ± 3.3 g, $F_{3,8} = 4.8$, $p = 0.034$, Fig. 2B). Consequently, most of the variation in body mass was driven by an increase in fat ($r^2 = 0.92$, $n = 12$, $p < 0.0001$, Fig. 3A, lean mass: $p = 0.9$). There were no detectable changes in lean mass ($p = 0.5$,

Fig. 2C) and fresh pectoralis muscle mass ($p = 0.2$), but there was a trend for a 21.8% increase in lean dry pectoralis mass ($p = 0.077$, Fig. 2D) between early March and early April.

As they gained body mass, birds also increased the size of their pectoralis muscle fibers, with fiber diameter increasing by 36.2% from early March to early April ($F_{4,9} = 4.1$, $p = 0.036$, Fig. 2E). There were, however, no significant changes in the number of nuclei per fiber ($p = 0.4$) over that time. Myonuclear domain tended to vary among sampling days, but showed no clear directional pattern ($p = 0.066$, no significant differences found among sampling days by Tukey test, Fig. 2F).

When analyzing data across all sampling periods, we observed that heavier birds had larger pectoralis muscles (fresh mass: $r^2 = 0.57$, $n = 14$, $p < 0.01$, lean dry mass: $r^2 = 0.77$, $n = 14$, $p < 0.0001$, Fig. 3B). Multiple regression then showed that muscle size was most likely changing in response to the amount of fat individuals carried. Indeed, analysis on fresh pectoralis muscles showed a clear relationship with fat ($F_{1,10} = 12.27$, $p < 0.01$) while lean mass (minus pectoralis fresh mass) showed no significant relationship with muscle mass ($p = 0.1$, simple regression fresh pectoralis mass versus fat mass: $r^2 = 0.44$, $n = 11$, $p = 0.024$). The analysis on lean dry pectoralis muscle showed a contribution of both fat

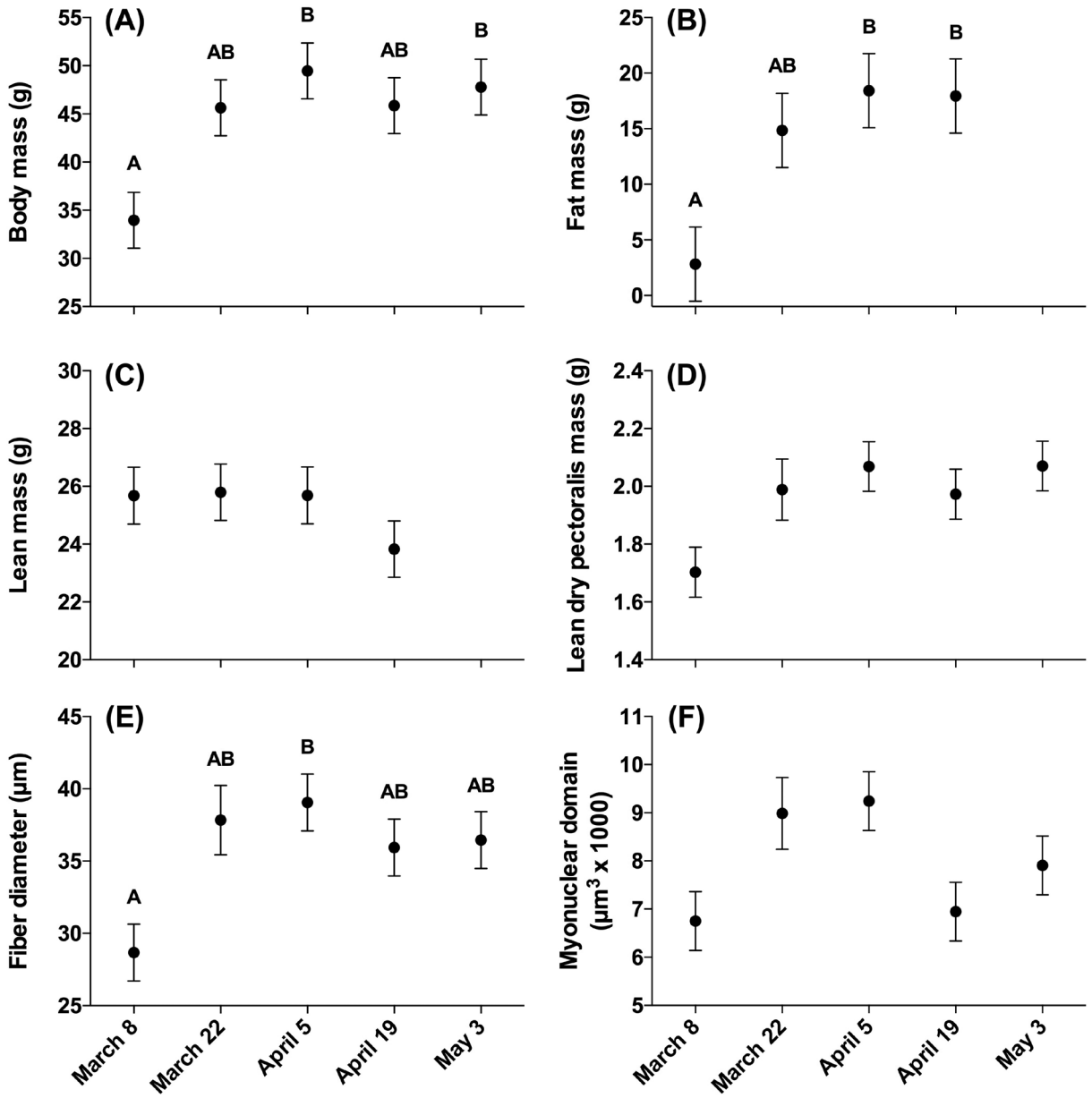


Figure 2. Variation in body (A), fat (B) and lean (C) masses, lean dry pectoralis muscle mass (D), muscle fiber diameter (E) and myonuclear domain (F) in outdoor captive snow buntings undergoing the transition from wintering to migratory phenotypes. Different letters indicate significant differences among mean values.

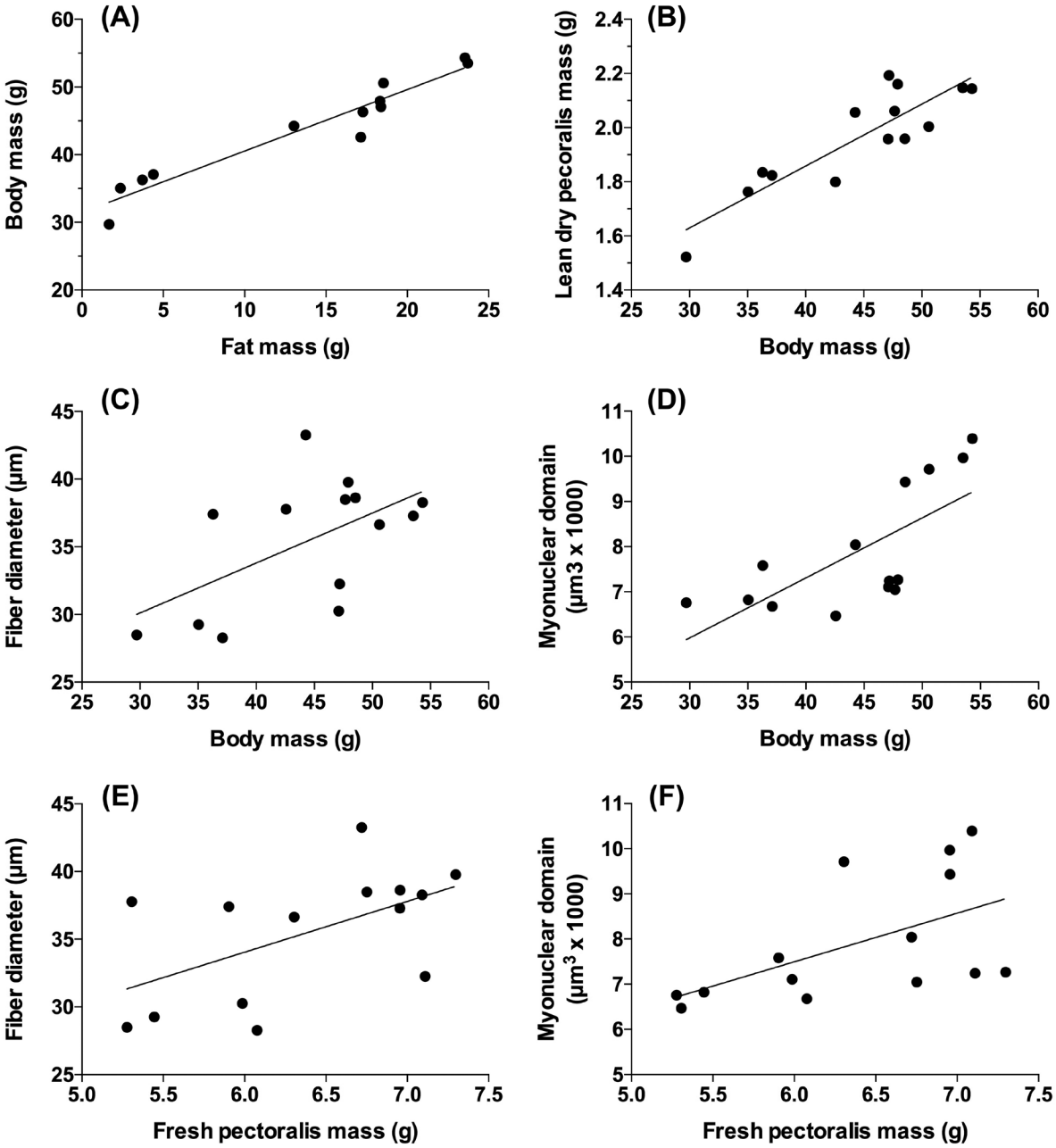


Figure 3. Regression analyses showing interrelationships among body composition and muscle ultrastructure variables in outdoor captive snow buntings undergoing the transition from wintering to migratory phenotypes. The influence of fat mass on body mass is shown in (A) and the influence of body mass on lean dry pectoralis muscle mass is shown in (B). Muscle fiber diameter (C) and myonuclear domain (D) were also related to body mass, and these two variables correlated positively with fresh pectoralis mass (E and F). See text for statistical details.

($F_{1,10}$ = 67.54, $p < 0.0001$) and lean mass ($F_{1,10}$ = 19.03, $p < 0.01$), although in this case lean body mass also included the mass of pectoralis muscles (separate regressions fat mass: $r^2 = 0.67$ n = 11, p = 0.002, lean mass: p = 0.4).

Examining ultrastructural changes in parallel with variation in body mass revealed that the observed increase in muscle fiber diameter was also associated with total body mass ($r^2 = 0.32$, n = 14, p = 0.034, Fig. 3C). The relationship between fat mass and fiber diameter approached significance in a multiple regression model (p = 0.081, lean mass: p = 0.7) and simplifying this model by considering fat alone in a linear regression slightly improved the result (p = 0.062). In contrast, the number of nuclei per fiber did not vary with total body mass or with the fat and lean components of body mass ($p > 0.5$ in all cases). Consequently, as muscle fiber diameter increased in heavier birds without adding more nuclei per cell, myonuclear domain was strongly and positively associated with total body mass ($r^2 = 0.51$, n = 14, $p < 0.01$,

Fig. 3D). Multiple regression showed that this was mostly driven by fat mass ($F_{1,8}=13.2$, $p < 0.01$) with a marginally significant influence of lean mass ($F_{1,8}=5.1$, $p=0.054$, separate regressions fat mass: $r^2=0.45$, $n=11$, $p=0.025$, lean mass: $p=0.3$).

Muscle fiber diameter also correlated positively with pectoralis muscle mass (fresh: $r^2=0.31$, $n=14$, $p=0.039$, Fig. 3E, lean dry: $r^2=0.32$, $n=14$, $p=0.034$) and, as large muscled birds did not have more nuclei per volume in their fibers ($p > 0.2$ in both cases), these birds also had large myonuclear domain (fresh: $r^2=0.31$, $n=14$, $p=0.039$, Fig. 3F, lean dry: $r^2=0.25$, $n=14$, $p=0.065$). Therefore, heavy, fat birds had larger pectoralis muscles and larger fiber diameters. Moreover, each nucleus in these cells also had more cytoplasm to service.

Discussion

Outdoor captive snow buntings accumulated considerable amounts of fat, showing a six-fold increase in body fat during the transition from a wintering to a migratory phenotype and this led to a 46% gain in body mass. Such migratory fuelling in captive birds is common for species with strong endogenous circannual cycles (Piersma et al. 1995, Piersma 2002, Vézina et al. 2011, Karagicheva et al. 2016), including snow buntings (Vincent and Bedard 1976, Le Pogam et al. 2021; this study).

Previous work in the same captive environment showed that buntings increase the thickness of their pectoralis muscles as they acclimate to winter conditions (Le Pogam et al. 2020), and that these muscles remain large throughout the spring migratory transition and most of summer (Le Pogam et al. 2021, Fig. 1). Our analysis showing no significant differences in fresh muscle mass among sampling periods is consistent with this observation, although a trend for a 22% increase in lean dry muscle mass could be observed in our data. Across sampling periods, however, we observed that heavier birds also had heavier pectoralis muscles compared to lighter individuals and that this was likely occurring in response to increased body fat load. Our results therefore suggest that snow buntings do enlarge their flight muscles to a certain degree (44% difference between highest and lowest lean dry mass value, Fig. 3B) to support migration and that this enlargement is occurring in parallel with increases in fat and body mass (Dawson et al. 1983, Marsh 1984, Lindström et al. 2000).

It is unclear at the moment why migratory changes in muscle mass are apparently not translating into thickness variation measurable by ultrasound (Le Pogam et al. 2021). One possibility, as stated by Swanson and Merkord (2013), is that ultrasonography underestimates mass changes in muscles as the technique is limited in its capacity to measure three-dimensional changes in organs on a linear axis. Changes in flight muscle mass as observed here (e.g. non-significant 22% increase in lean dry mass between early March and early April) could thus translate into smaller changes in muscle thickness, making spring increases too subtle for detection by this method (Royer-Boutin et al. 2015). Consequently, both our previous work and this study suggest that snow buntings enlarge their flight muscles in winter to cope with the cold, and maintain that muscle phenotype through migration and summer (Le Pogam et al. 2020, 2021), but that a further increase in pectoralis mass likely occurs in parallel with fat accumulation in preparation for migration. A seasonal, dissection-based, body composition study would be required to test predictions fully and refine this interpretation.

The transition towards a migratory phenotype in snow buntings was associated with a 36% increase in muscle fiber diameter within a month, and birds with large fibers were also those with the heaviest body and pectoralis muscle mass. This upregulation of fiber size therefore supports an increase in pectoralis mass via hypertrophic muscle growth (Marsh 1984, Evans et al. 1992) and suggests that changes in fiber size and muscle mass come as a result of flying with a heavier body (Dawson et al. 1983, Marsh 1984, Lindström et al. 2000, Zhang et al. 2018). As fibers with greater diameter generate more force during contraction while requiring less energy per unit mass to maintain during rest (Jimenez et al. 2013), large muscle fibers should be beneficial for migration in snow buntings. It should be noted here that muscle enlargement for migration has also been associated with considerable metabolic adjustments within cells leading to upregulated lipid transport, delivery and oxidation, in support of prolonged flights (Guglielmo 2010, 2018 for reviews). Assuming larger, more energy efficient, fibers also produce more heat through shivering contraction (Jimenez et al. 2019, Vézina et al. 2020 for cases of large fibers in cold acclimated birds), large fibers would likely be equally beneficial for maintaining cold endurance in this highly cold-specialized species known to migrate in sub-zero temperatures (Meltofte 1983, Snell et al. 2018). In that sense, Le Pogam et al. (2021) recently reported winter level cold endurance throughout spring and most of summer in captive buntings (Swanson 1995, Swanson and Dean 1999, Vézina et al. 2007).

Snow buntings increased muscle fiber diameter and pectoralis muscle mass as they accumulated fat, but muscle fibers did not recruit more nuclei to compensate for this increase. Consequently, myonuclear domain was positively correlated with body and pectoralis muscle mass. The increase in myonuclear domain is considerable (61% when comparing highest and lowest values, Fig. 3D) and suggests that improvements in fiber contraction force may come with a potential upregulation of cell protein turnover capacity (Brooks et al. 2009), which may imply that each nucleus is tapping into its 'spare capacity' for protein upregulation (Cramer et al. 2020). Whether this phenomenon results from an adaptive response for migration, a lack of satellite cells left to recruit or from an environment unsupportive of satellite cell proliferation (Forcina et al. 2019) has yet to be investigated. It remains, however, that this type of phenotypic response would allow for 'safeguarding' or conserving the apparently finite pool of satellite cells associated with each myotube, which could be an important strategy for migratory birds that can face recurrent damage to muscle fibers (Guglielmo et al. 2001).

It should be noted here that Amthor et al. (2009) also reported a lack of satellite cell recruitment in hypertrophic muscle fibers, although this was for a selected line of mice lacking myostatin, with observations conducted exclusively on postnatal muscles. Myonuclear domain could also potentially change at late stages of hypertrophic muscle growth, as suggested by O'Connor and Pavlath (2007). However, our data offer little support for this hypothesis given that lean dry muscle mass appeared stable for the final 6 weeks of the experiment, while myonuclear domain showed no directional changes among weeks. Alternatively, myonuclear domain could potentially remain flexible as suggested by Murach et al. (2018) who found that a lack of myonuclear accretion does not affect contractile function of fast-glycolytic fibers. In fact, whether changes in myonuclear domain are required during hypertrophic growth is still a contested statement (Van der Meer et al. 2011, Forcina et al. 2019) and inconsistent findings are common. For example, Bruusgaard et al. (2010) reported the recruitment of nuclei into existing fibers before observing increases in fiber size during muscle hypertrophy in mice. However, others have stated that changes in myonuclear domain and fiber size can occur within 1–2 weeks and can even take up to 4 weeks (Van der Meer et al. 2011). In contrast, in one of the few papers addressing myonuclear domain in birds, Vézina et al. (2020) demonstrated changes in myonuclear domain within hours of a thermal challenge in black-capped chickadees *Poecile atricapillus*, further challenging the above, mammalian-derived, studies (Jimenez 2020). The inconsistencies with these patterns across the mammalian literature have led to the notion that myonuclear domain size may not be as carefully regulated as once thought (Gundersen and Bruusgaard 2008). In adult avian muscle fibers undergoing hypertrophy and atrophy on a regular yearly cycle for migration, however, satellite cells do not appear to be as actively recruited into existing myotubes as they are in typical mammalian model systems (Bruusgaard et al. 2010). These hypotheses will require more studies.

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Author contributions

François Vézina: Conceptualization (lead); Formal analysis (lead); Funding acquisition (equal); Investigation (lead); Methodology (lead); Project administration (equal); Resources (lead); Writing – original draft (lead); Writing – review and editing (lead). **Ryan O'Connor**: Investigation (equal); Methodology (equal); Writing – review and editing (equal). **Audrey Le Pogam**: Methodology (supporting); Writing – review and editing (equal). **Aliyah De Jesus**: Methodology (equal); Writing – review and editing (equal). **Oliver P. Love**: Funding acquisition (equal); Resources (equal); Validation (equal); Writing – review and editing (equal). **Ana Jimenez**: Conceptualization (equal); Formal analysis (equal); Funding acquisition (equal); Investigation (equal); Methodology (equal); Project administration (equal); Resources (equal); Writing – original draft (equal); Writing – review and editing (equal).

Transparent Peer Review

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Data availability statement

Data available from the Dryad Digital Repository: <http://dx.doi.org/10.5061/dryad.gxd2547kx> (Vézina et al. 2021).

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