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Spare capacity and phenotypic flexibility in the digestive system of a migratory bird: defining the limits of animal design

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Flexible phenotypes enable animals to live in environments that change over space and time, and knowing the limits to and the required time scale for this flexibility provides insights into constraints on energy and nutrient intake, diet diversity and niche width. We quantified the level of immediate and ultimate spare capacity, and thus the extent of phenotypic flexibility, in the digestive system of a migratory bird in response to increased energy demand, and identified the digestive constraints responsible for the limits on sustained energy intake. Immediate spare capacity decreased from approximately 50% for birds acclimated to relatively benign temperatures to less than 20% as birds approached their maximum sustainable energy intake. Ultimate spare capacity enabled an increase in feeding rate of approximately 126% as measured in birds acclimated for weeks at −29°C compared with +21°C. Increased gut size and not tissue-specific differences in nutrient uptake or changes in digestive efficiency or retention time were primarily responsible for this increase in capacity with energy demand, and this change required more than 1–2 days. Thus, the pace of change in digestive organ size may often constrain energy intake and, for birds, retard the pace of their migration.

1. Introduction

Animals living in environments that change over space and time must somehow track the environmental change, and the possession of spare capacity and of flexible phenotypes provides two solutions [1,2]. Phenotypic flexibility in animals refers to reversible modifications to phenotype that occur in response to changes in their environment and associated demands [3–6]. The concept of phenotypic flexibility of physiological traits requires that the capacity of a physiological system is matched to the prevailing demand but can be modulated in response to changes in demand so as to provide some limited excess capacity [7–11]. Considerations of evolutionary economic design suggest that these capacities should be modestly in excess of their corresponding loads ('enough but not too much') [12,13] because of the associated costs of maintaining excess capacity [14–16]. The extent of spare capacity (measured as the ratio of capacity to load) and time scale of phenotypic flexibility of animals are important for predicting animal responses to changing environments, whether natural or anthropogenic [4,6,17–27]. For phenotypically flexible organisms, there can be an immediate spare capacity (i.e. prior to any flexible adjustment, acclimation or acclimatization) and an ultimate spare capacity (i.e. after full acclimation and adjustment), both of which are important for our understanding of constraints on energy intake, diet diversity, niche width and feeding rate, and thus the acquisition of energy and essential nutrients [4,6,20–26].

Although the extent of phenotypic flexibility is fundamentally important for our understanding of animal ecology and evolution [19,28], there are relatively few studies that have directly measured spare capacity in physiological systems (e.g. classic studies by Taylor, Weibel and co-workers [29–31]; Diamond and co-workers [8,10,11,32–34]; and Suarez and co-workers [35,36]), and very few that have measured immediate and ultimate spare capacity of a given

physiological system, and determined the underlying mechanistic basis and the relative time scale over which their phenotypic flexibility occurs (reviewed by [19]). The most intensively studied animal in this regard is the laboratory mouse [8–10,12,25,33,37], and its evolution in a captive environment raises the question of whether the features of its flexibility and capacity appropriately apply to wild animals [19]. The primary objective of our study was to estimate the level of immediate and ultimate spare capacity, and thus the extent of phenotypic flexibility in the digestive system of a migratory bird, the white-throated sparrow (*Zonotrichia albicollis*), in response to increased energy demands and to explain its underlying mechanistic basis.

Phenotypic flexibility of the digestive system in migratory birds is especially impressive in terms of its magnitude, and is a key factor in allowing birds to change feeding rate and diet [5,20,38–41], and thus overcome some of the physiological challenges of long-duration migration [4,9,19,42–45]. We predicted that (i) the immediate spare capacity of the white-throated sparrow would be modest (less than 1.5) when compared with non-flying vertebrates because of the need to economize weight [46–52]. The ultimate capacity of the sparrow can be estimated to be around 2.5 based on results in Kontogiannis [53], although herein we illuminate the required timescale for this change in capacity. In general, changes in gut size or mass in vertebrates are linked to cell turnover rate [54–56] and these changes require at least 2 days to extend digestive capacity [19,34,42,57]. Thus, we also predicted that (ii) sparrows would be unable to adequately adjust to rapid (less than 2 days) changes to colder ambient temperature and the associated increased energy costs of thermoregulation. Our experimental approach involved manipulating ambient temperature over different time scales, which forces endotherms such as sparrows to modify their food and energy intake as they maintain a constant body temperature. We predicted, based on a few other studies (reviewed in [4,42]), that (iii) the primary digestive adjustment of sparrows to increased demand over time will be an increase in organ size (and not tissue-specific biochemical rates), which then increases overall biochemical capacity and so maintains constant gut retention time and digestive efficiency. This is the first published study for any wild vertebrate of both rapid and gradual adjustment of feeding and digestion to high energy demand that simultaneously measures key elements of the digestive system (i.e. food intake, digestive efficiency, gut anatomy, retention time of digesta, rates of nutrient absorption). This allows us to reveal the mechanism(s) of digestive system adjustment primarily responsible for the demonstrated phenotypic flexibility.

2. Material and methods

(a) Bird capture and maintenance

The 40 white-throated sparrows (*Zonotrichia albicolli*) used in this study were captured using mistnets during late October in Madison, Wisconsin, USA (43°8′ N, 89°20′ W). Birds were immediately weighed and banded after capture, and then housed individually in stainless steel cages (60 × 45 × 33 cm) under constant light cycle (12 L : 12 D; lights on at 07.00 h) and temperature (+21°C ± 1°C). Each day at 14.00–16.00 h, birds were presented with excess food and water, ensuring ad libitum conditions. All birds were fed a semisynthetic diet (62% starch, 13% protein, 8% fat, 5% cellulose) that was similar in macronutrient composition to seeds, included mixtures of essential amino acids [58] and vitamins and minerals (AIN-76 and N-Salt mix, ICN Biomedicals, Inc.), and had been used successfully for maintaining sparrows in the laboratory for months [59]. Daily food intake was estimated as the difference between the amount of dry food offered and that remaining after 1 day, corrected for spillage.

(b) Temperature schedule and experimental design

We randomly assigned 16 birds to a cold treatment (−20°C ± 1°C) and the other 24 birds to a warm treatment (+21°C). All 40 birds continued on the same daily light schedule (12 L : 12 D). For warm birds, ambient temperature was kept constant at +21°C. All cold birds were maintained in temperature-controlled animal rooms (±1°C from +21 to −20°C) at the University of Wisconsin Biotron facility. The temperature schedule for all cold birds ensured that they were acclimated for at least 12 days at either −5°C or −20°C before being tested. For cold birds, the ambient temperature was +1°C for 16 days (21 October–5 November) and then −5°C for the next 16 days (6–21 November). On 21 November, ambient temperature was gradually decreased by −2°C per day until reaching −20°C on 28 November where it remained until the end of the experiment on 13 December. For birds at less than 0°C, a small hotplate was placed in each cage to keep water in a glass petri dish from freezing. Food was less than 0.1% water so it remained unfrozen and palatable when ambient temperature was less than 0°C.

Eight cold- (−5°C) and warm-acclimated (+21°C) birds were tested at −5°C on 18–21 November with two birds in each group tested on a given day. Eight cold- (−20°C) and warm-acclimated (+21°C) birds were tested at −20°C on 9–12 December with two birds in each group tested on a given day. On each pretest day, two warm-acclimated birds were brought to the cold room at 07.00 h and placed in separate cages with excess food and water. Two cold-acclimated birds were randomly selected as test birds for comparison. At 07.00 h on the test day, the four test birds were moved to special observation cages within the cold room (see detailed methods in the electronic supplementary material), and were provided food and water ad libitum. Food intake, retention time and extraction efficiency of two cold-acclimated and two warm-acclimated birds were measured during a 4–5 h test period that began at 13.30 h when the birds were gavaged with radiolabelled nutrients and markers (see detailed methods in the electronic supplementary material). At 07.00 h on the post-test day, we moved the four test birds to a room-temperature laboratory, euthanized them, and then measured digestive organ mass and length and total body composition (lean, fat), as well as nutrient uptake rates of L-leucine in perfused, isolated small intestine (see detailed methods in the electronic supplementary material) of each bird sequentially over roughly 3 h. This test-day procedure was repeated for four consecutive days at each of the test temperatures (−5°C, −20°C) so that a total of eight birds per treatment group were tested. The remaining warm-acclimated birds ($n = 8$) were tested at +21°C on 16–17 December using the same protocol described above for pretest, test and post-test days.

(c) Statistical analysis

We used one-way analysis of variance (ANOVA) to compare body mass, food intake, extraction efficiency, retention time, summed uptake, gut morphometrics and body composition of birds after at least 12 days of acclimation at +21°C, −5°C or −20°C. We used *t*-tests when comparing these dependent variables between birds acclimated to different temperatures but tested at the same temperature. We used univariate repeated-measures analysis of variance (RMANOVA) to compare body mass and food intake over the 3 days of the experiment for birds acclimated at +21°C, −5°C or −20°C. We also used RMANOVA to compare nutrient

uptake rates and mass of 1 cm intestinal sleeves between proximal and distal sections of the small intestine, and between temperature treatment groups. When we detected significant temperature treatment effects, we used post hoc Bonferroni multiple comparisons tests to compare a given dependent variable across the +21°C, −5°C or −20°C treatment groups. Percentage data were arcsine-square-root-transformed prior to analysis. Results are given as mean ± s.e. unless otherwise noted. All statistical analyses were performed using SYSTAT (v. 12.0).

3. Results

(a) Effects of acclimation temperature on body mass and food intake

Birds acclimated for at least 12 days at one of the three temperatures (−20°C, −5°C or +21°C) maintained similar body mass but ate significantly more food at colder temperatures (electronic supplementary material, table S1). Body mass of these acclimated birds was also similar 3 days before the test day, on the test day and on the post-test day (RMANOVA, temperature effect: $F_{2,22} = 0.01$, $p = 0.99$; time effect: $F_{4,44} = 0.61$, $p = 0.66$; temperature × time effect: $F_{4,44} = 1.15$, $p = 0.35$).

Birds acclimated at +21°C and then immediately moved to −5°C increased their food intake over the 2 days at −5°C while food intake of birds acclimated and tested at −5°C remained relatively constant and above that of birds immediately moved to −5°C (figure 1*a*; RMANOVA, temperature effect: $F_{1,14} = 20.07$, $p = 0.001$; time effect: $F_{2,28} = 1.70$, $p = 0.20$; temperature × time effect: $F_{2,28} = 5.29$, $p = 0.01$). Despite the significant increase in food intake of birds moved from +21°C to −5°C, body mass of these birds decreased within 1 day in the cold while that of birds acclimated and tested at −5°C remained constant (figure 1*b*; RMANOVA, temperature effect: $F_{1,14} = 13.9$, $p = 0.002$; time effect: $F_{2,28} = 21.5$, $p < 0.0001$; temperature × time effect: $F_{2,28} = 15.6$, $p < 0.0001$).

Birds acclimated at +21°C and then immediately moved to −20°C also increased their food intake at −20°C while food intake of birds acclimated and tested at −20°C remained relatively constant and well above that of birds immediately moved to −20°C (figure 1*a*; RMANOVA, temperature effect: $F_{1,14} = 34.91$, $p < 0.0001$; time effect: $F_{2,28} = 1.59$, $p = 0.22$; temperature × time effect: $F_{2,28} = 13.10$, $p < 0.0001$). Despite the significant increase in food intake of birds moved from +21°C to −20°C, body mass of these birds decreased within 1 day in the cold while that of birds acclimated and tested at −20°C remained constant (figure 1*b*; RMANOVA, temperature effect: $F_{1,14} = 3.8$, $p = 0.07$; time effect: $F_{2,28} = 36.7$, $p < 0.0001$; temperature × time effect: $F_{2,28} = 12.9$, $p < 0.0001$).

(b) Effects of acclimation temperature on retention time and extraction efficiency

Retention time of PEG was not statistically different ($F_{2,21} = 0.9$, $p = 0.44$) for birds acclimated at −20°C, −5°C or +21°C (electronic supplementary material, table S2). Retention time of birds acclimated at +21°C and then tested at either −5°C or −20°C was similar to that for birds acclimated and tested at −5°C or −20°C (electronic supplementary material, table S2). Although acclimation temperature had a significant effect on food intake and body mass, we found no significant effect of acclimation temperature on extraction efficiency of [^{14}C]starch for sparrows acclimated at +21°C, −5°C or −20°C ($F_{2,19} = 3.01$, $p = 0.07$). Extraction efficiency of [^{14}C]starch was 48–57% for sparrows acclimated at each temperature as well as for birds acclimated at +21°C and then tested after only 1 day at either −5°C or −20°C (electronic supplementary material, table S2).

(c) Effects of acclimation temperature on body composition and gut morphometrics

Birds acclimated for at least 12 days at one of the three temperatures (−20°C, −5°C or +21°C) had similar percentage body fat (26.5 ± 8.2, 34.7 ± 1.6, 34.3 ± 3.5, respectively; $F_{2,7} = 1.16$, $p = 0.37$). Birds acclimated at +21°C and then moved to −5°C or −20°C had significantly lower percentage body fat (14.1 ± 2.6 and 11.5 ± 4.0, respectively) than birds acclimated at −5°C or −20°C ($t_8 = 5.93$, $p < 0.0001$). All birds had similar lean mass (overall mean: 4.3 ± 0.1 g dry protein) regardless of treatment group (acclimated birds: $F_{2,7} = 0.67$, $p = 0.54$; moved birds: $t_8 = 1.76$, $p = 0.12$).

Birds acclimated for at least 12 days at −20°C had heavier livers, and longer and heavier small and large intestines compared with birds acclimated at +21°C (figure 1*c*; electronic supplementary material, table S3). Gizzard and pancreas mass were similar for birds acclimated at the three temperatures. In general, digestive organs of birds acclimated at least 12 days at −5°C were intermediate in mass and length compared with birds acclimated at +21°C and −20°C (electronic supplementary material, table S3).

Comparison of digestive organ size in birds acclimated at +21°C and then moved to colder temperatures (either −5°C or −20°C) for only 2 days provides an indication of the pace of modulation in digestive organ size in sparrows exposed to cold temperatures. All digestive organs of birds acclimated at +21°C were similar to those of birds acclimated at +21°C and then moved for 2 days to −20°C (*t*-tests, $p > 0.59$ for all organs; electronic supplementary material, table S3). Birds acclimated at +21°C and then moved for 2 days to −20°C had lighter livers and shorter and lighter small and large intestines compared with birds acclimated at −20°C (electronic supplementary material, table S3, last two columns). Thus, more than 2 days but less than 12 days were required for modulation of digestive organs such as liver and intestine in white-throated sparrows.

(d) Effects of acclimation temperature on *in vitro* intestinal uptake of nutrients

Although birds acclimated at −20°C had heavier and longer small intestines than birds acclimated at +21°C, the mass of 1 cm sleeves of small intestine from birds in these two treatment groups was similar to that of birds acclimated at +21°C and tested at −20°C (figure 2*a*; RMANOVA, temperature effect: $F_{2,18} = 0.667$, $p = 0.53$; intestinal position effect: $F_{1,18} = 38.66$, $p < 0.0001$; temperature × position effect: $F_{2,18} = 0.166$, $p = 0.85$). Uptake of L-leucine was normalized to milligram wet intestine, although these data can also be expressed per centimetre length of intestine using conversion factors in figure 2*a* and the electronic supplementary material, table S3. Specific uptake rates of leucine (figure 2*b*) were on average lower for birds acclimated and tested at −20°C compared with the other two groups (temperature effect: $F_{2,17} = 5.71$, $p = 0.01$) primarily because leucine uptake

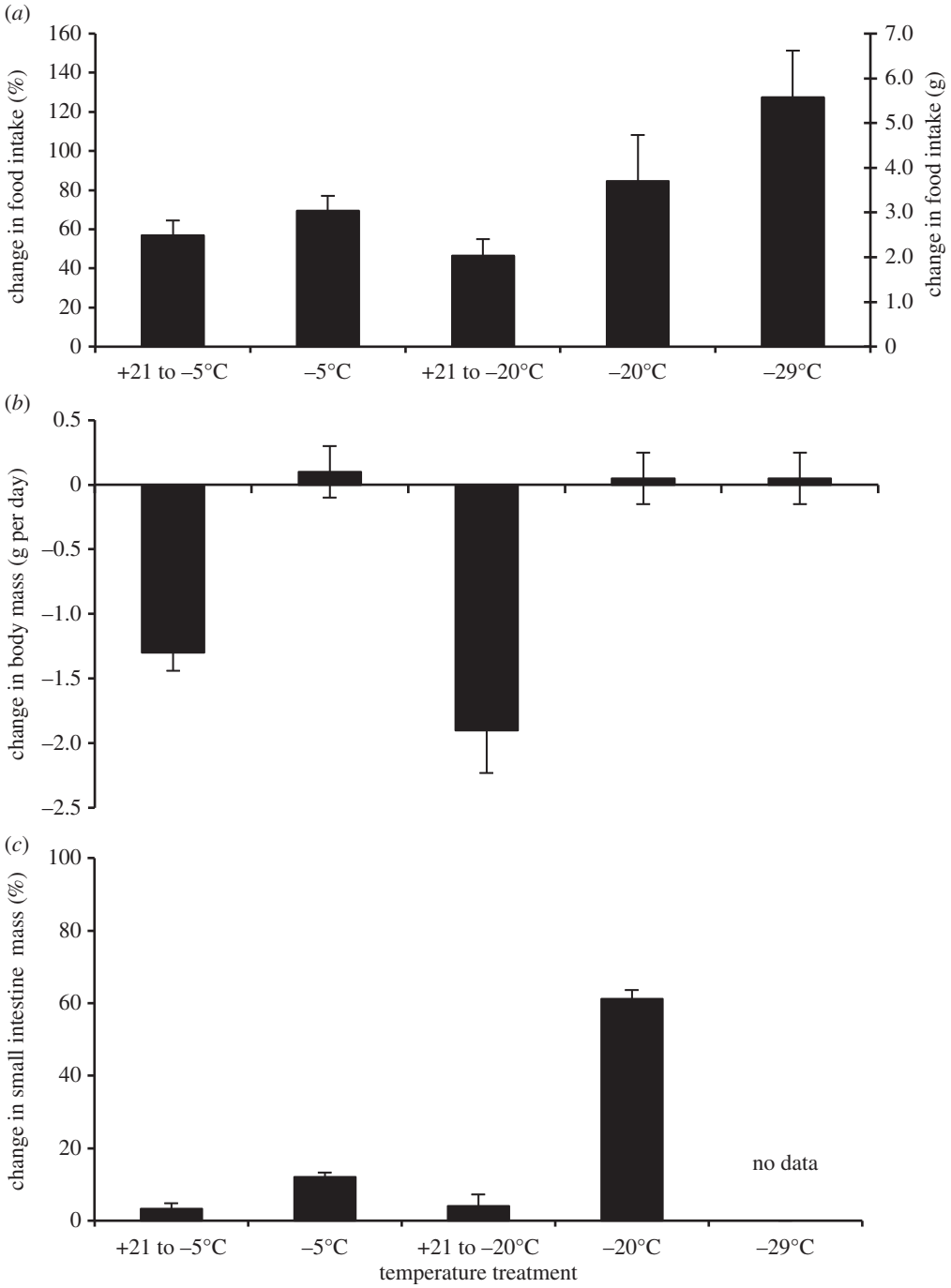

Figure 1. Changes in (*a*) food intake, (*b*) body mass and (*c*) small intestine mass for all treatment groups relative to that of birds acclimated for at least 12 days at +21°C. (*a*) Birds moved from +21°C to −5°C or −20°C increased their food intake by 45–57% (immediate spare capacity), whereas birds acclimated for at least 12 days at these same cold temperatures increased their food intake by 69–83% (ultimate spare capacity). Food intake of white-throated sparrows acclimated to −29°C [53] was 126% higher (ultimate spare capacity) than for sparrows in our study acclimated to +21°C. (*b*) Despite their immediate spare digestive capacity, birds moved from +21°C to −5°C or −20°C were unable to maintain constant body mass. (*c*) The primary digestive adjustment of white-throated sparrows to increased energy demands was an increase in size of the digestive tract (e.g. small intestine), although the bird's capacity to increase gut size requires time.

increased along the small intestine in birds acclimated at +21°C and tested at either +21°C or −5°C, but not in birds acclimated and tested at −20°C (intestinal position effect: $F_{1,17} = 26.08$, $p < 0.0001$; intestinal position × temperature effect: $F_{2,17} = 5.15$, $p = 0.02$).

We estimated summed uptake rate of the entire small intestine for leucine by multiplying uptake rates per milligram for the proximal and distal region (figure 2*b*) by mass of the intestine per centimetre in each region (figure 2*a*) and then by the length of the two regions of the small intestine (electronic supplementary material, table S3). Summed uptake in birds acclimated and tested at −20°C was higher than in birds acclimated at +21°C and tested at either +21°C or −20°C (figure 2*c*; one-way ANOVA: $F_{2,21} = 3.33$, $p = 0.05$).

4. Discussion

We quantified both immediate and ultimate spare capacity in a migratory bird, and identified the digestive constraints responsible for the limits on sustained energy intake. Immediate spare capacity enabled an increase in feeding rate of roughly 50% in birds acclimated to relatively benign temperatures (+21°C) and then switched immediately to

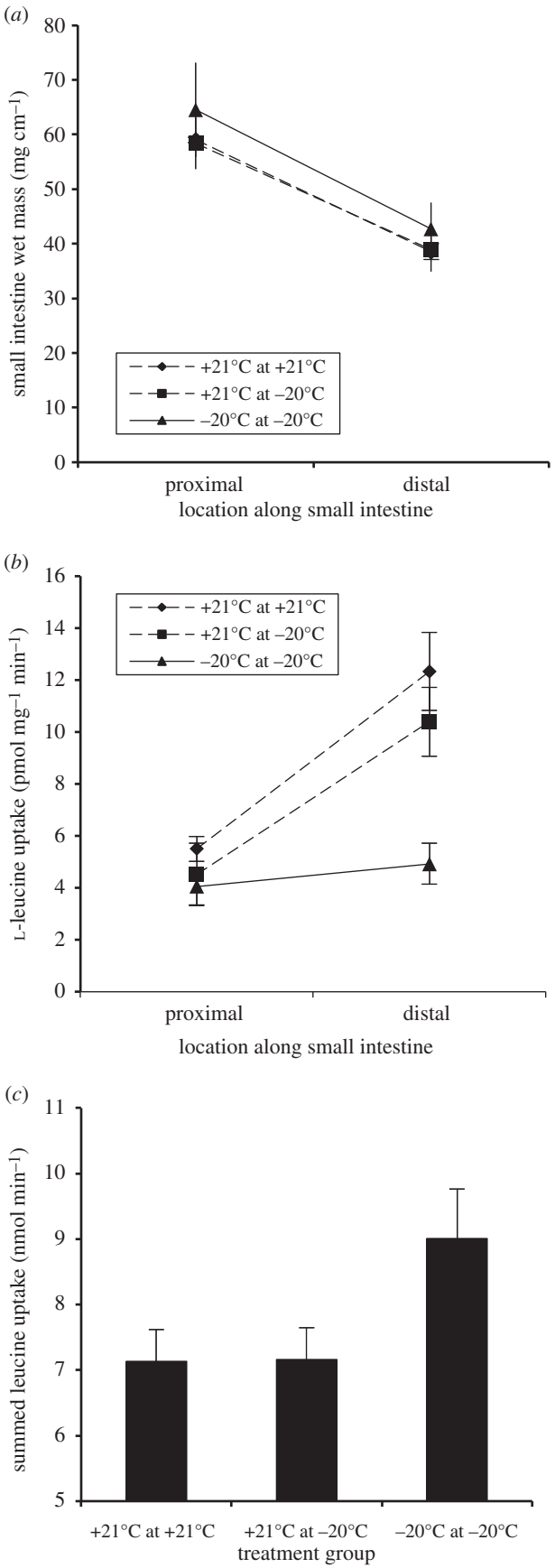

Figure 2. Effects of temperature acclimation (+21°C versus −20°C) and a rapid switch from +21 to −20°C on white-throated sparrow (*a*) wet mass (g) of 1 cm sleeves of intestine, (*b*) specific uptake rate of leucine (pmol per mg per min) in the proximal and distal small intestine, and (*c*) summed uptake of leucine (nmol per min). Birds ($n = 8$ per group) were acclimated and tested at +21°C, acclimated and tested at −20°C, or acclimated at +21°C and then tested after only 2 days at −20°C.

colder temperatures (−20°C). This immediate spare capacity declined to less than 20% when birds were acclimated to colder temperatures (−20°C) and as they approached their maximum sustainable limits (less than the −30°C determined by Kontogiannis [53]). Thus, as predicted by theory, immediate spare capacity decreased in extent with energy demand (electronic supplementary material, figure S1). However, sparrows lost body mass when rapidly (less than 1–2 day acclimation) switched to colder temperatures, thus demonstrating that the immediate spare capacity was inadequate and that compensatory digestive adjustments required more time. Ultimate spare capacity enabled an increase in feeding rate of around 126% as measured in birds acclimated for weeks at −29°C compared with +21°C (electronic supplementary material, figure S1). Our suite of measurements of key features of the digestive system suggested that increases in intestinal size and mass, and not tissue-specific differences in nutrient uptake, or changes in digestive efficiency or retention time, were primarily responsible for the increase in capacity with energy demand when given adequate acclimation time. Below we discuss these results in more detail and emphasize their ecological implications.

(a) Extent of phenotypic flexibility: estimating immediate and ultimate spare capacity

We estimated immediate spare capacity by rapidly changing ambient temperature, and measuring food intake and key features of the digestive system. A rapid time course is necessary because within a few days adjustments occur in the digestive system so that immediate spare capacity is no longer measured [42]. White-throated sparrows acclimated for weeks at −20°C required 83% more food than birds at +21°C, as indicated by their greater feeding rates while maintaining body mass. When birds were switched rapidly from +21°C to −5°C or −20°C they increased feeding rate only 45–55% and lost body mass (figure 1). We assume that such estimates of immediate spare capacity are maximal because these birds must be highly motivated to eat, yet they did not eat enough to maintain body mass. Six other studies have quickly challenged animals to increase rate of feeding and digestion through cold stimulus, forced activity or reduction in feeding time [46,48,50–52]. In all species studied to date (Djungarian hamster, *Phodopus sungorus*; yellow-rumped warbler, *Setophaga coronata*; broad-tailed hummingbird, *Selasphorus platycercus*; house mouse, *Mus musculus*; prairie vole, *Microtus ochrogaster*), including white-throated sparrows in our study, immediate spare capacity was quite modest at 9–50%, although that measured for one individual bat (*Glossophaga longirostris*) was unusually high (73%) [19,49].

If given enough acclimation time at colder temperatures, sparrows can satisfy the elevated energy demands associated with living in the cold, as evidenced by their ability to maintain body mass after at least two weeks of acclimation at −5°C and −20°C (our study), although white-throated sparrows could not survive at temperatures below an average −29°C even when allowed at least 50 days acclimation [53]. We estimated the ultimate spare capacity of sparrows at 126% (electronic supplementary material, figure S1), which is the increase in feeding rate for sparrows acclimated at −29°C [53], their low critical temperature, relative to our sparrows acclimated at +21°C. Kontogiannis [53] found that forced nocturnal activity (simulating migratory restlessness during migration) also increased

daily energy expenditure (and hence food intake) such that exercised birds could not survive at temperatures below −15°C. This suggests that the limits to maximum sustained energy for sparrows are not related to heat dissipation or heat generation, and (as we discuss below) are limited more centrally by digestive constraints. Ultimate spare capacity was 95–130% in four of the five species for which there were also measures of immediate spare capacity (no estimate for the bat [19], although the House mouse had exceptionally high ultimate spare capacity of 488% [48]), perhaps because of artificial selection on many fronts [60]. Our estimates of ultimate spare capacity (2.3 increase) are within the range of 2–3 estimated for other biological systems studied to date [4,9,11,34] (recently reviewed in [4]).

(b) Digestive adjustments to increases in energy demand: defining the limitation

Ours is the first study to demonstrate how multiple key elements of the vertebrate digestive system (i.e. digestive efficiency, retention time of digesta, gut anatomy, rates of nutrient absorption) respond to rapid as well as gradual changes in food intake associated with temperature change. We have shown that birds acclimated to cold temperatures eat more and adjust their digestive traits to maintain constant digestive efficiency and retention time. We detected no change in nutrient uptake rates per unit of intestine, but remarkable increases in size and mass of small and large intestine, which resulted in greater summed nutrient uptake at the whole-animal level. The primary digestive adjustments were in size and mass of liver, small intestine and large intestine (but not gizzard or pancreas). This is in contrast to shorebirds that changed gizzard mass in response to consuming more hard-shelled molluscs [4,61], and humans that changed rate of pancreatic enzyme secretion in response to duodenal perfusion of essential amino acids [62]. The semisynthetic diets that we fed to white-throated sparrows required no grinding and were designed to be easily digestible, and this may explain the lack of change in gizzard size. In general, the lack of change in digestive efficiency and the disproportionate change in only the parts of the digestive system associated with assimilation are not consistent with the symmorphosis hypothesis, which posits that quantitative changes in functional demand will be satisfied by quantitative changes in all parts of a sequential system [30,63] (also see [4]). Given that total lean mass of sparrows remained constant across treatments, and yet key parts of their digestive system substantially increased in mass and size with increased food intake, other non-digestive organs must have decreased in size and mass, as shown for other migratory birds [64]. Our results also suggest that the maximum sustained energy intake of sparrows was centrally limited primarily by the overall size of the gut.

(c) Digestive adjustments to increased energy demand takes time

Our results highlight the interplay between food intake, gut size, retention time of digesta and digestive efficiency, and how this determines the pace of digestive change. When birds were given time to acclimate to cold temperatures, they adjusted their food intake to compensate for the increase in energy expenditure associated with higher thermoregulatory costs. Simple digestion optimization models predict that such increased food intake should decrease retention time, and so decrease digestive efficiency. However, our previous studies with warblers [51] and waxwings [65,66], and this study of sparrows, show that digestive efficiency is maintained constant despite significant changes in food intake and retention time. Birds are able to maintain constant digestive efficiency in such situations primarily because of phenotypic flexibility in gut size [5,19,42].

The pace of digestive change in general, or the pace at which gut size increases in response to demand, more specifically, determines when digestive constraints limit energy allocation [19]. Given the design of our study, we estimate that birds require more than 2 days but less than 12 days for such adjustments of gut size in response to these increased energy demands. Turnover time of intestinal enterocytes is 2–3 days for small birds [55,67], compared with 8–12 days for larger birds [67,68], and digestive organs of most birds increase in size within 1–6 days in response to changes in food intake (reviewed in [5]). Although extent of phenotypic flexibility in digestive organs of birds is especially remarkable [4,42] and is related to tissue turnover rates [55], a variety of vertebrates increase gut size as energy demand increases [19,27,54]. When increases in energy demand outpace the rate of digestive organ flexibility, then digestive features can constrain energy intake [19]. For example, when a variety of vertebrates were deprived of food for several days their guts atrophied, and when food was restored their feeding rate was often constrained for up to several days until their guts were rebuilt and full function restored [55,69–75]. Likewise, when songbirds are hyperphagic in response to changes in daylight or forced exercise, there is an associated increase in surface area and volume of the gut that also requires several days [6,20,76]. In sum, adjustments in gut size of a variety of vertebrates require at least a few days, and perhaps as much as one week, depending on body size and type of digestive change [19,42,54]. For actively migrating birds that fast while flying and must stop to feed and refuel, this pace of digestive change may often be too slow, and so digestive constraints may directly retard the pace of migration [42,77,78].

(d) Ecological implications of phenotypic flexibility

Predicting how organisms will fare given certain climate change requires understanding the current physiological limits of organisms [79–81], especially those limits that determine their distribution and abundance, including, for example, temperature tolerance [82,83] and those that determine maximum sustainable metabolic rate [11]. Climatic niche modelling uses these physiological tolerances to estimate how changes in climate will affect the ecology of species assuming no change in tolerances (e.g. [84–88]) and predicts spatial tracking of climate to stay within physiological limits [89–91]. Phenotypic flexibility plays an underappreciated role in how animals may respond to climate change [92], and seems especially important for migratory birds [93,94] and migratory bats [95]. If immediate spare capacity consistently decreases with increasing energy demand, as we have shown, then the extent to which phenotypic flexibility can accommodate environmental change will be more limited at the colder (higher elevation, higher latitudes) areas along species range boundaries. Temperature manipulations are especially relevant

because the distribution and abundance of vertebrates are often delimited by environmental temperature [80,84,91,96].

Digestive limitations deserve more attention from ecologists because they shape the functional response of predators [97–99], explain variation in predator energy budgets [26], and influence daily foraging patterns [100,101] and optimal foraging decisions [45] in a variety of animals. The digestive system includes some of the most metabolically and energetically costly organs in animals [102]. Thus, the limits on maximum sustained metabolic rate may be primarily set by the high costs of disproportionately increasing the mass of energy-supplying organs such as the digestive system [11,27], which makes it likely that phenotypic flexibility and digestive constraints regularly influence an animal's ecology.

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Electronic Supplementary Materials (McWilliams & Karasov 2014 Proc R Soc b):

A. Detailed methods for measuring retention time, extraction efficiency, nutrient uptake rates, gut morphometrics, and body composition

(1) Retention time and extraction efficiency

Special observation cages were used to reduce behavioral stress associated with our presence while birds were observed and their individual excreta collected (see [1] for full description). Food intake was similar from 0700-1300 hrs on the pretest day (when in their regular cages) and the test day (when in the observation cages) suggesting that this acclimation period was adequate.

Retention time of digesta was measured using the inert marker [1,2-^{3}H] polyethylene glycol (PEG; DuPont BEB Research Products, Wilmington, DE) with MW 4000. Extraction efficiency of starch was measured using the inert marker method [2]. We used 74 kBq tritiated PEG as the inert marker together with 18.5 kBq [uniformly labeled ^{14}C] starch (*Nicotiana tobacum* L. from American Radiolabeled Chemicals, Inc., St. Louis, MO) and 21 ul of distilled water as a carrier solution for each gavage. Each bird had food available to it for at least 6 hrs before being gavaged with the radiolabelled solution at 1330 hr. After the gavage, all birds were placed back in their cages where they readily consumed their food and water; thus, a small volume of marker and nutrient was inserted into the flow of food in the bird's digestive system. Excreta were collected singly for the first 30 min and thereafter every 15 min for 4-5 h.

Mouth-to-anus total mean retention time was calculated as the sum of the products of the proportion of inert marker excreted during each time interval multiplied

by the elapsed time since ingestion of marker [3]. Extraction efficiency was calculated as: 100 - 100[(M_f/N_f)X(N_e/M_e)], where M_f is the radioactivity of the inert marker (PEG) in food, N_f is radioactivity of the nutrient ([^{14}C]starch) in food, N_e is radioactivity of nutrient in excreta, and M_e is radioactivity of inert marker in excreta.

(2) Nutrient uptake rates, gut morphometrics, and body composition

Nutrient uptake rates were measured in warm-acclimated birds tested at their acclimation temperature of +21 C (n=9) or after two days at -20 C (n=8), and in eight cold-acclimated birds tested at their acclimation temperature of -20 C. All birds were weighed and then anesthetized using methoxyflurane. The gut was exposed and then cut just proximal to the gizzard and at the rudimentary caeca, cleaned of extraneous tissue, and then placed in cold (0^{o}C) avian Ringer (solution composition in mM was 161 CaCl, 4.7 KCL, 2.5 $CaCl_2$, 1.2 KH_2PO_4, 1.2 $MgSO_4$, and 20 $NaHCO_3$). The gizzard was excised, opened, rinsed of contents, blotted dry, and then weighed. The liver and pancreas were excised, blotted dry, and then weighed. The small and large intestine were perfused with cold avian Ringer. One end of the intestine was then held against a ruler while the other end was gently pulled until the intestine was taut. After release, the length of small and large intestine was measured. The small and large intestine were quickly blotted dry, weighed, and the small intestine was then placed back in cold avian Ringer.

We measured intestinal uptake of L-leucine, a representative amino acid that has been used in other studies, as described in [4, 5]. One-centimeter everted sleeves of intestine were mounted on metal rods and kept in cold avian Ringer solution until an uptake measurement was made (always within 1 h of anesthetization with

methoxyflurane). The solution was oxygenated with 95% O_2-5% CO_2 to yield pH 7.3-7.4 at 37°C and osmolarity was 350 mOsm. After a 5-min preincubation in Ringer solution at 37°C, tissues were incubated for 2 min in Ringer at 37°C over a stir bar at 1,200 rpm [6]. We measured uptake of 0.01 mM L-[2,3-^{3}H]leucine into the tissue across the brush-border membrane using [^{14}C(U)]PEG to correct for adherent fluid. Uptakes of 0.01 mM L-leucine were measured in single one-centimeter sleeves from the proximal and distal halves of the small intestine.

Fifteen birds that were not used for measurements of nutrient uptake were killed and the following organs were immediately removed, cleaned of digesta, blotted dry, and then measured: gizzard mass, liver mass, small intestine length and mass, large intestine length and mass, pancreas mass. For all 40 sparrows used in this study, all organs except the small intestine and pancreas were placed back inside the bird and then the carcass was stored frozen. Later, the carcass minus the intestine and pancreas was thawed, plucked, freeze-dried, and ground in a small coffee-grinder. We refluxed 1 g dried (at 50°C) subsamples with petroleum ether for 6 h [7] in a Goldfisch apparatus to measure whole-body fat content. Lean mass (g dry) was defined as total body mass (g dry) without feathers and intestine and pancreas, minus fat content (g dry).

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B. Tables

Table 1: Body mass and food intake of white-throated sparrows acclimated for at least 12 days to one of three temperatures (-20 C, -5 C, or +21 C). The 8 sparrows maintained at +21 C that were included in this comparison were acclimated only at this temperature and never used in the acute cold temperature experiments.

Acclimation temperature	n	Body mass (g) on test day	Mean food intake[1] (g dry per day)	Food intake during[1] 4-hr test period (g dry)
-20 C	8	26.9 ± 0.9	8.05 ± 1.05 A	2.26 ± 0.38 A
-5 C	8	25.1 ± 0.4	7.39 ± 0.35 B	1.93 ± 0.17 AB
+21 C	8	26.0 ± 0.7	4.36 ± 0.72 C	1.31 ± 0.24 B
	$F_{2,21}$	1.52	6.66	3.10
	P-value	0.24	0.006	0.05

[1]means with different letters are significantly different (P<0.05) according to Bonferroni pairwise comparisons from the one-way ANOVA.

Table 2: Retention time (min) of digesta and extraction efficiency (%) of [^{14}C]starch in white-throated sparrows acclimated for at least 12 days to one of three temperatures (-20 C, -5 C, or +21 C) and then tested at the same temperature as acclimated or, for two groups of sparrows acclimated at +21 C, tested one day after being moved to -5 C or -20 C.

	Retention time (min) of digesta			Extraction efficiency (%) of starch		
Acclimation	Test temperature			Test temperature		
temperature	-20 C	-5 C	+21 C	-20 C	-5 C	+21 C
-20 C	93.8 ± 8.1	--	--	48.3 ± 4.1	--	--
-5 C	--	105.9 ± 8.9	--	--	57.4 ± 2.2	--
+21 C	108.7 ± 8.0	97.5 ± 9.1	108.7 ± 10.1	53.7 ± 2.2	50.7 ± 3.1	49.2 ± 1.8
t-value[1]	1.15	0.67		1.11	1.78	
P-value[1]	0.27	0.52		0.30	0.10	

[1]t-test compares retention time of digesta or extraction efficiency of [^{14}C]starch for birds acclimated to cold temperature (either -5 C or -20 C) versus birds acclimated to warm temperature (+21 C) and then tested after only one day at the same cold temperature (either -5 C (n=8) or -20 C (n=7))

Table 3: Digestive organ mass and length (± SE) in white-throated sparrows exposed to different temperatures. Three groups of eight sparrows were acclimated for at least 12 days to one of three temperatures (-20 C, -5 C, or +21 C) whereas two other groups of eight sparrows were acclimated for at least 12 days at +21 C and then moved to either -5 C or -20 C for two days.

	Acclimation temperature				Acclimation temperature was +21 C and then two days at colder temperature.			
Digestive Organ	+21 C	-5 C	-20 C	P-value[1]	-5 C	P-value[2]	-20 C	P-value[2]
Gizzard (mg)	429 ± 18	434 ± 25	452 ± 27	0.78	434 ± 27	0.99	444 ± 25	0.83
Liver (mg)	700 ± 41A	765 ± 21A	942 ± 83B	0.01	683 ± 39	0.09	708 ± 106	0.05
Small intestine:								
length (mm)	154 ± 6A	163 ± 3AB	178 ± 6B	0.02	153 ± 3	0.03	152 ± 3	0.01
mass (mg)	982 ± 89A	1069 ± 54A	1583 ± 131B	0.01	1015 ± 61	0.52	1022 ± 110	0.02
Large intestine:								
length (mm)	9.8 ± 0.3A	11.8 ± 0.8B	12.6 ± 0.6B	0.001	11.4 ± 0. 5	0.63	9.7 ± 0.9	0.05
mass (mg)	69 ± 4A	55 ± 4A	99 ± 9B	0.001	63 ± 7	0.34	70 ± 8	0.05
Pancreas (mg)	109 ± 12	96 ± 4	114 ± 9	0.37	106 ± 5	0.35	101 ± 6	0.26

[1]ANOVA comparing digestive organ mass or length for sparrows acclimated for at least 12 days to one of three temperatures (df = 2, 21). Letters denote significant differences between means within a row based on post hoc Bonferroni multiple comparisons test.

[2]t-test comparing digestive organ size of sparrows acclimated to cold temperature (either -5 C or -20 C) with digestive organ size of sparrows acclimated to warm temperature (+21 C) and then tested after only two days at either -5 C (n=8) or -20 C (n=7).

C. Figure

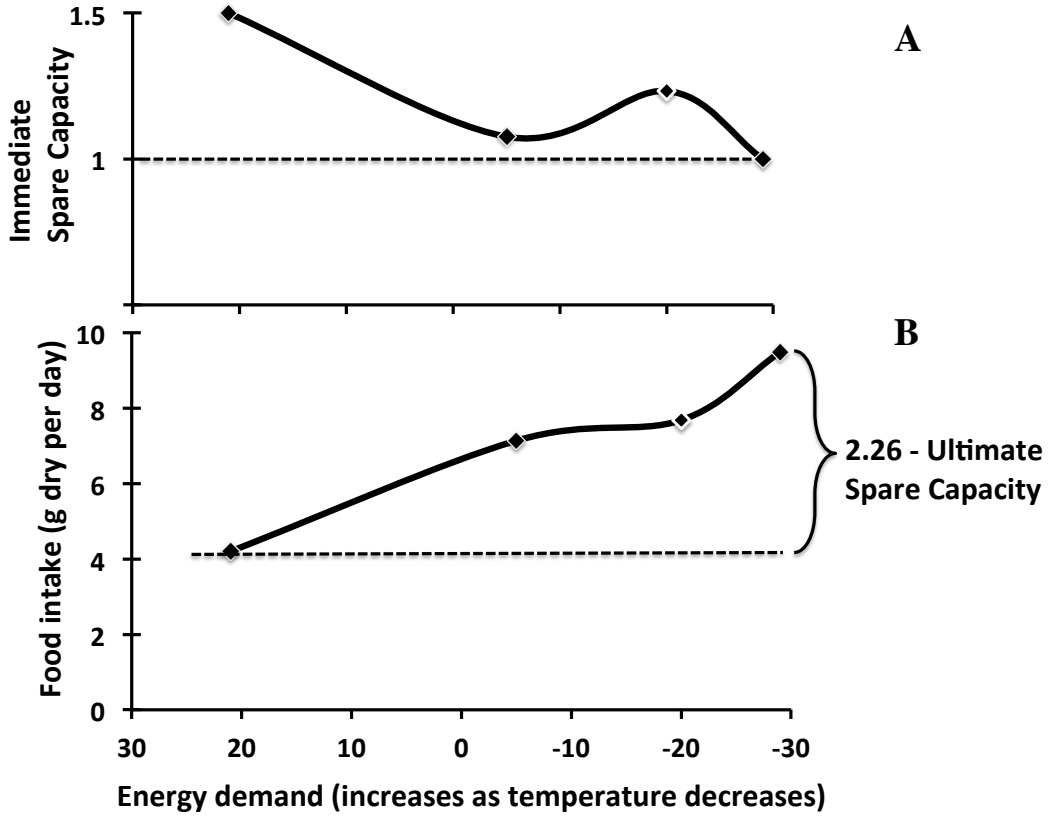

Figure S1. Estimating immediate and ultimate spare capacity for white-throated sparrows exposed to increasing energy demands imposed by cold temperatures. (A) Immediate spare capacity for birds acclimated at +21 C: the ratio of food intake for birds acclimated at +21 C and then tested at -20 C relative to food intake for birds acclimated and tested at +21 C; for birds acclimated at -5 C: the ratio of food intake for birds acclimated at -20 C relative to food intake for birds acclimated at -5 C; for birds acclimated at -20 C: the ratio of food intake for birds acclimated at -29 C relative to food intake for birds acclimated at -20 C. (B) Ultimate spare capacity for white-throated sparrows (2.26) is the ratio of food intake for birds acclimated and tested at -29 C relative to food intake of birds acclimated and tested at +21 C. The lower temperature limit of -29 C is for inactive white-throated sparrows; exercising sparrows were unable to survive below -15 C because their food intake was already maximal and similar to inactive sparrows at -29 C. Note

that this estimate of ultimate spare capacity does not include any remaining immediate spare capacity although this is expected to be negligible as sparrows approach their maximum sustained energy budget.