

Nutritional stress and body condition in the Great Gray Owl (*Strix nebulosa*) during winter irruptive migrations

Gary R. Graves, Seth D. Newsome, David E. Willard, David A. Grosshuesch, William W. Wurzel, and Marilyn L. Fogel

Abstract: The largest irruptive migration of the Great Gray Owl (*Strix nebulosa* Forster, 1772) recorded since 1831 occurred in Minnesota, USA, during the winter of 2004–2005. We tested the hypothesis that morphometric indicators of nutritional stress covary with stable isotope signatures in a sample of 265 owls killed by vehicle collisions. The ratio of carbon to nitrogen in muscle (C/N_{muscle}) was shown to be a reliable proxy of nutritional stress. $\delta^{13}\text{C}$ values for liver and muscle were significantly higher in owls in poor condition, reflecting the depletion of lipid reserves in fasting individuals. On the other hand, $\delta^{15}\text{N}$ values for liver and muscle were marginally lower or unchanged in owls in poor condition. Stomachs of emaciated owls were less likely to contain prey, implying that many nutritionally stressed individuals were too weak to hunt and were near the tipping point of irreversible fasts. In a broader context, sexual differences in the correlative relationships between stable isotope signatures, C/N, and body condition suggest that the consequences of reversed sexual size dimorphism extend to physiological processes during the nonbreeding season.

Key words: body mass index, Great Gray Owl (*Strix nebulosa*), carbon, nitrogen, C/N, fasting, Minnesota, reversed sexual size dimorphism, stable isotopes, starvation.

Résumé : La plus importante migration irruptive de chouettes lapones (*Strix nebulosa* Forster, 1772) signalée depuis 1831 s'est produite au Minnesota (États-Unis), durant l'hiver de 2004–2005. Nous avons testé l'hypothèse selon laquelle les variations d'indicateurs morphométriques de stress nutritif seraient corrélées à celles de la signature des isotopes stables dans un échantillon de 265 chouettes mortes des suites de collisions avec des véhicules. Il est démontré que le rapport carbone–azote dans les muscles (C/N_{muscle}) est un indicateur substitutif fiable du stress nutritif. Le fait que les valeurs de $\delta^{13}\text{C}$ pour le foie et les muscles étaient significativement plus élevées pour les chouettes en mauvais état témoigne de l'appauvrissement des réserves de lipides dans les individus à jeun. Par contre, les valeurs de $\delta^{15}\text{N}$ pour le foie et les muscles étaient légèrement plus faibles ou inchangées dans les chouettes en mauvais état. La probabilité de trouver des proies dans l'estomac de chouettes émaciées était moins grande, ce qui indique que de nombreux individus soumis à un stress nutritif étaient trop faibles pour chasser et s'approchaient de limite du jeûne irréversible. Dans un contexte plus large, les différences sexuelles des corrélations entre la signature des isotopes stables, C/N et l'état corporel suggèrent que le dimorphisme sexuel inversé de la taille a une incidence sur les processus physiologiques durant la période internuptiale.

Mots-clés : indice de masse corporelle, chouette lapone (*Strix nebulosa*), carbone, azote, C/N, jeûne, Minnesota, dimorphisme sexuel inversé de la taille, isotopes stables, famine.

[Traduit par la Rédaction]

Received 26 January 2012. Accepted 10 April 2012. Published at www.nrcresearchpress.com/cjz on 19 June 2012.

G.R. Graves. Department of Vertebrate Zoology, MRC-116, National Museum of Natural History, Smithsonian Institution, P.O. Box 37012, Washington, DC 20013-7012, USA; Center for Macroecology, Evolution and Climate, University of Copenhagen, DK-2100, Copenhagen Ø, Denmark.

S.D. Newsome. Carnegie Institution of Washington, Geophysical Laboratory, 5251 Broad Branch Road Northwest, Washington, DC 20015, USA; Department of Zoology and Physiology, University of Wyoming, 1000 East University Avenue, Department 3166, Laramie, WY 82071, USA.

D.E. Willard. Zoology Department, Field Museum, 1400 South Lake Shore Drive, Chicago, IL 60605-2496, USA.

D.A. Grosshuesch. Superior National Forest, 2020 West Hwy 61, Grand Marais, MN 55604, USA.

W.W. Wurzel. Carnegie Institution of Washington, Geophysical Laboratory, 5251 Broad Branch Road Northwest, Washington, DC 20015, USA; State University of New York College of Environmental Science and Forestry, SUNY-ESF, 1 Forestry Drive, Syracuse, NY 13210, USA.

M.L. Fogel. Carnegie Institution of Washington, Geophysical Laboratory, 5251 Broad Branch Road Northwest, Washington, DC 20015, USA.

Corresponding author: Gary R. Graves (e-mail: gravesg@si.edu).

Introduction

The most spectacular irruptive migrations observed among Holarctic birds are arguably performed by the Great Gray Owl (*Strix nebulosa* Forster, 1772), a low-density species of the boreal forests of Eurasia and North America (Nero 1980; Mikkola 1983; Cramp 1985). The underlying cause of irruptive movements in this species was shrouded in mystery through the mid-20th century (Bent 1938). This behavior is now thought to be triggered by periodic population fluctuations of microtine rodents, which constitute the bulk of the owl's diet (Nero 1980; Mikkola 1983; Cramp 1985; Bull and Duncan 1993; Newton 2002, 2006; Cheveau et al. 2004). Owls spatially track pulses in microtine populations and may breed in widely separated locations in successive years. Individuals may winter near breeding sites or in locations hundreds of kilometres south of the normal breeding range in response to geographic patterns of vole abundance. Since the first documented irruptive migration in North America in 1831 (Audubon 1838), more than 30 irruptions have been recorded south of the boreal forest in eastern North America (Bull and Duncan 1993; Svingen and Lind 2005). The largest recorded irruptive migration occurred during the winter of 2004–2005, when at least 5200 owls were observed, banded, or found dead in Minnesota (USA), with smaller numbers reported from Wisconsin (USA), Michigan (USA), Ontario (Canada), and Quebec (Canada) (Svingen and Lind 2005). The number reported in Minnesota alone represented roughly 17% of the North American population, recently estimated at 30 000 individuals (Rich et al. 2004).

A spectrum of hypotheses concerning the fates of irruptive populations of Great Gray Owls can be synthesized from natural history observations (Bent 1938; Nero 1980; Nero and Copland 1981; Mikkola 1983; Bull and Duncan 1993; Svingen and Lind 2005; Graves and Niemi 2006). The more optimistic hypotheses suppose that a majority of individuals survive the winter and return in good nutritional condition to their breeding grounds when spring arrives. At the other end of the continuum, irruptive migrations may be one-way trips, with most owls succumbing to starvation, predation, and accidental death. The latter scenario would have significant implications for the population ecology of the Great Gray Owl and the population genetics of the species as well if there is strong natural selection for body size or if migratory irruptions involve only parts of the breeding range. In truth, most irruptive migrations occur in remote areas with low human densities and few roads, making it difficult to mark individuals, track their movements, or monitor survivorship over extended time periods. Consequently, the geographic origin of irruptive migrants, their physiological condition, and the proportion that survive to the following breeding season are unknown.

The large number of specimens salvaged during the 2004–2005 irruption provided an unparalleled opportunity to determine if body condition was linked to stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values of liver and muscle tissue. Stable isotope analysis has emerged as a powerful tool in ecological research because of the predictable relationship between assimilated food and the isotopic composition of biological tissues (DeNiro and Epstein 1978; Estep and Dabrowski 1980; Hobson et al. 1999; Macko et al. 1983). Stable

isotope ratios are widely used by animal ecologists as indicators of body condition (Hobson et al. 1993; Cherel et al. 2005; Mekota et al. 2006) and as tools to reconstruct diet (Chamberlain et al. 2005), trophic niche width (Bearhop et al. 2004; Martínez del Río et al. 2009a), and food-web structure (Estep and Dabrowski 1980; Hobson and Welch 1992). However, much remains to be learned about the physiological processes that influence isotopic discrimination in metabolically active tissues, and in particular, the effects of food and water deprivation on stable isotope ratios (Kelly 2000; Rubenstein and Hobson 2004; Martínez del Río et al. 2009b).

The large series of salvaged specimens also permitted us to investigate the relationship between body condition and reversed sexual size dimorphism (RSD), where males are smaller than females. Great Gray Owls exhibit the most extreme RSD observed among Holarctic owls (Earhart and Johnson 1970; Mueller 1986). Nearly two dozen hypotheses have been proposed for the evolution of RSD in raptorial birds (Earhart and Johnson 1970; Amadon 1975; Andersson and Norberg 1981; Jehl and Murray 1986; Korpimäki 1986; Lundberg 1986; Mueller 1986, 1990; Hakkarainen and Korpimäki 1991; Bildstein 1992; Krüger 2005). These can be roughly classified as (i) ecological hypotheses pertaining to niche partitioning and prey size, (ii) reproductive and physiological hypotheses, and (iii) behavioral hypotheses related to sexual dominance and pair formation. Although the voluminous literature on RSD in raptors has grown markedly in recent decades, there is still no consensus on the selective factors responsible for its evolution and maintenance.

We tested the hypothesis that morphometric indicators of nutritional stress covary with stable carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values of liver and muscle tissue in a sample of 265 Great Gray Owls killed by vehicle collisions. We also tested several related hypotheses. First, did owls experience progressive starvation at the population level during the winter irruption (November through April)? If true, then the proportion of salvaged owls in poor body condition should have increased during the irruptive cycle. We also considered the “fasting endurance” hypothesis for RSD proposed by Lundberg (1986), who suggested that female owls are selected for larger body size and fasting endurance, whereas males are selected for smaller body size and hunting efficiency. If larger body size is advantageous during resource-driven irruptive migrations, then body condition of females should, on average, be measurably better than that of males. Finally, we tested the hypothesis that the ability to capture prey is related to body condition. We predict that the stomachs of emaciated owls would be less likely to contain prey.

Materials and methods

Specimen salvage and preparation

Extraordinary numbers of Great Gray Owls began to appear in northern Minnesota in November 2004. A few breed in Minnesota (Bull and Duncan 1993), but the vast majority were assumed to have immigrated from Ontario and Manitoba. The magnitude of the historic irruption was not apparent until later in the winter when daily counts along some census routes exceeded 100 individuals on at least 13 days between 19 January and 20 March 2005 (Svingen and Lind 2005). This species exhibits little fear of humans and fre-

quently perches on fence posts, road signs, and telephone poles near roadsides (Nero 1980). Indeed, most specimens salvaged during the 2004–2005 migration were recovered as frozen carcasses along roads, presumably killed by collision with vehicles. A minimum of 767 specimens were picked up dead or injured in Minnesota (Svingen and Lind 2005), mostly from northeastern counties (Aitkin, Carlton, Crow Wing, Lake, Itasca, Pine, St. Louis). This total was an order of magnitude larger than the number salvaged during any of the previous 10 irruptive migrations in Minnesota. The extent to which salvaged specimens represent random population samples is unknown.

Salvaged carcasses were dated, labeled with locality data, placed in plastic bags, stored in freezers, and eventually transferred to the Field Museum (FM), Chicago, Illinois, for preparation. Necropsy revealed that most individuals were killed by collisions with vehicles, whereas others showed no signs of physical trauma. We could not determine the date of death, but we assumed that large dark-colored carcasses (length 68 cm; wing span 130 cm) on snow would be quickly scavenged by foxes (*Vulpes vulpes* (L., 1758)), coyotes (*Canis latrans* Say, 1823), gray wolves (*Canis lupus* L., 1758), and ravens (*Corvus corax* L., 1758). Thus, it is likely that most intact frozen carcasses were salvaged within a few days of death.

Specimens were weighed to the nearest gram, prepared as rounded study skins and partial skeletons (generally the trunk and femora), or as complete skeletons. The term “body mass” is used throughout the paper, although our measurements were technically weights (Lidicker 2008). We classified salvaged specimens into two unambiguous age classes based on plumage characters: (1) yearlings in their first winter and (2) adults in their second or later winter. The 2004–2005 irruptive migration was composed almost entirely of adults. Only 2 out of the 265 specimens analyzed in this study were yearlings. We pooled yearlings and adults in the analyses. Although Great Gray Owls often commence breeding during late winter, none of the salvaged owls was in breeding condition (no gonadal enlargement) at the time of death.

Analyses of stomach contents provided additional insight on the potential link between body condition and the ability to capture prey. Stomach contents (1223 prey items) were examined in 585 specimens (258 males, 327 females; Table 1). Whole or partially digested food items (exclusively small mammals) were preserved as skeletons and hair samples. Prey retention rates in the stomachs of Great Gray Owls are unknown, but the presence of hair and bone likely indicates the consumption of prey within 48 h of death (Andrews 1990).

Subcutaneous body fat of owls (Table 1) was classified at necropsy: (1) emaciated (no fat; sunken breast muscle; keel very evident); (2) no fat (breast muscle normal but no fat evident); (3) traces of fat (small amounts of fat around edge of furculum); (4) light fat (a bit of fat filling the bottom of the furculum area; little on other parts of body); (5) moderate fat (substantial solid fat deposits in furculum region; some fat on flanks and pelvic area); (6) heavy fat (fat bulging out of furcular cleft; also significant fat in pelvic region); and (7) extremely fat (subcutaneous fat covering body, sometimes as thick as 6 mm in pelvic area; covering breast and up into neck). All fat classifications were scored by D.E.W. Bulk

samples of liver and pectoral muscle were preserved in 100% ethanol from the same industrial stock. All specimens were deposited in the research collections of the FM.

Body mass index (BMI)

Five axial and two appendicular skeletal characters were measured to the nearest 0.1 mm with digital calipers. Owing to bone breakage, a substantial number of specimens lacked 1–3 skeletal values. Sample sizes for skeletal metrics varied in males ($n = 116$ – 138) and females ($n = 116$ – 125). We restricted the data set to specimens with complete measurements for (i) sternum length (medially, from the manubrium to the caudal edge of the sternum), (ii) keel length (the distance from the carinal apex to the caudal edge of the sternum), (iii) coracoid length, and (iv) femur length. The sternum supports the principal flight muscles (pectoralis and supracoracoideus), which compose as much as 25% of lean body mass in owls (Hartman 1961).

To provide a direct morphological comparison of males and females, we performed a principal components analysis (PCA) on the aforementioned skeletal measurements for the pooled sample of males ($n = 138$) and females ($n = 125$). The first principal component (PC1) extracted from a correlation matrix of untransformed variables was used as a measure of overall body size, unbiased by the nutritional condition of the owl or its stomach contents. All component loadings were highly positive (>0.93) and PC1 explained 89.4% of the total variance in body size. We constructed a BMI by regressing body mass on PC1 scores (from the pooled sample of males and females) with ordinary least squares regression (OLS). The residuals (BMI) represent observed deviations in body mass from values predicted from PCA I (skeletal size). Strongly negative BMI values indicate emaciated individuals in poor condition, whereas strongly positive BMI values indicate owls in good condition with substantial subcutaneous fat deposits.

Stable isotope analyses

We analyzed the nitrogen ($\delta^{15}\text{N}$) and carbon ($\delta^{13}\text{C}$) isotopic composition of liver and pectoral muscle, which exhibit contrasting rates of metabolism and protein synthesis (Hobson and Clark 1992; Bauchinger and McWilliams 2009) and provide data on the physiology of wintering owls at different temporal scales. Given that turnover rates scale allometrically to the -0.25 power of body mass (Carleton and Martínez del Rio 2005), isotope signatures of owl liver likely represent resources incorporated over relatively short time frames (1–2 weeks), whereas those of muscle reflect resources incorporated over moderately longer periods (3–5 weeks).

Liver and pectoral muscle samples were removed from ethanol, repeatedly rinsed in distilled water, and freeze-dried. Lipids were not extracted because we wanted to test the relationship between body condition and C/N of bulk tissues, which has been shown to be a reliable proxy for intramuscular and hepatic lipid stores and nutritional stress (Hayashi 1983; Okumura et al. 2002; Post et al. 2007). Preservation in ethanol can result in significant changes in carbon and nitrogen isotope signatures of bulk tissues, largely through hydrolysis of ^{13}C -depleted lipids and proteins (Sarakinis et al. 2002; Sweeting et al. 2004; Kelly et al. 2006). Experimental tests on a variety of animal tissues indicate that preservation

Table 1. Stomach contents (empty or contained prey) and fat scores of salvaged Great Gray Owls (*Strix nebulosa*) ($n = 585$ specimens) in Minnesota during the 2004–2005 irruptive migration.

Fat scores	Males			Females		
	Empty	Contained prey*	% empty	Empty	Contained prey*	% empty
1	47	7	87	47	10	82
2	15	18	45	13	22	37
3	9	17	35	5	23	18
4	13	40	25	12	41	23
5	8	24	25	21	59	26
6	12	44	21	16	42	28
7	1	3	25	4	12	25

Note: See text for definitions of fat scores.

*Including hair and bone.

effects are rapid and relatively insensitive to ethanol concentration (e.g., 70% vs. 100%). At the time of isotope analysis, all tissue samples had been stored in ethanol taken from the same stock and held under similar conditions for >12 months. Thus, isotope values should be broadly comparable within tissue type.

Approximately 0.5 mg of powdered tissue samples was sealed in clean tin capsules for isotopic analysis. Carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) isotope values were determined using a Carlo Erba elemental analyzer (NC 2500; Carlo Erba, Milan, Italy) interfaced with a Thermo-Finnegan Delta Plus XL mass spectrometer (Carnegie Institution of Washington, Washington, D.C., USA).

Isotopic results are expressed as δ values (Craig 1961): $\delta^{13}\text{C}$ or $\delta^{15}\text{N} = 1000 \times [(R_{\text{sample}}/R_{\text{standard}}) - 1]$, where R_{sample} and R_{standard} are the $^{13}\text{C}/^{12}\text{C}$ or $^{15}\text{N}/^{14}\text{N}$ ratios of the sample and standard, respectively. We used Vienna – Pee Dee Belemnite limestone (V-PDB) for carbon and atmospheric N_2 for nitrogen as standards. The units are expressed as parts per thousand, or per mil (‰). The within-run standard deviation of an acetalinide standard was $\leq 0.2\text{‰}$ for both $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values. We also measured the atomic C/N of liver ($\text{C}/\text{N}_{\text{liver}}$) and muscle ($\text{C}/\text{N}_{\text{muscle}}$) from each specimen.

Statistical analyses

We approached the investigation of body condition in a hierarchical fashion, beginning with simple correlation analysis (Pearson's correlation coefficients; r) to investigate the bivariate relationships among continuous variables. Two-sample Student's t tests (pooled variance) were used to compare mean values of males and females. Chi-square tests were used to evaluate 2×2 frequency tables. Spearman's rank correlation coefficients (r_s) were used to evaluate the strength of the relationship between BMI and categorical body fat scores recorded during specimen preparation. Because ethanol preservation may affect tissues differentially, we did not directly compare mean isotope values of liver and muscle.

We then used a simple general linear model (GLM) to investigate the relationship between BMI and two predictor variables (sex, date of salvage). Two candidate models were examined, one with an interaction term (sex \times date of salvage) and one without. The simpler model presented in the results had a lower Akaike's information criterion (AIC) score (Akaike 1974). All P values are two-tailed ($\alpha = 0.05$).

Results

Size and body condition

Great Gray Owls exhibit significant RSD in body mass and skeletal size. The largest female (1750 g) was threefold larger than the smallest male (582 g) by body mass. Accordingly, males (body mass: 582–1208 g, 895 ± 164 g (mean \pm SD); $n = 138$) were significantly lighter than females (body mass: 664–1750 g, 1143 ± 221 g; $n = 125$; $t = 10.36$, $P < 0.0001$). RSD was also evident in PC1 factor scores extracted from the correlation matrix of skeletal variables (Fig. 1). Skeletal size of males (PC1: -2.28 to 0.57 , -0.81 ± 0.50 (mean \pm SD); $n = 138$) was significantly smaller than in females (PC1: -0.57 to 2.37 , 0.88 ± 0.58 ; $n = 125$; $t = 25.60$, $P < 0.0001$).

Males and females did not differ in categorical measures of subcutaneous fat (seven-point scale) recorded during specimen preparation (Mann–Whitney $U = 8618$, $P = 0.92$; Fig. 2). Fat scores were highly correlated with body mass in males ($n = 138$; $r_s = 0.90$, $P < 0.0001$) and females ($n = 124$; $r_s = 0.89$, $P < 0.0001$) but were uncorrelated with date of salvage in males ($n = 138$; $r_s = 0.04$, $P = 0.56$) and females ($n = 124$; $r_s = 0.03$, $P = 0.76$). Thirty-three percent of males and 28% of females had body masses lower than the minimum values listed for the species in Bull and Duncan (1993), indicating that a substantial fraction of the salvaged specimens were in poor body condition.

BMI scores, which are the residuals from OLS regression of body mass (g) on PC1 scores, were highly correlated with fat scores ($r_s = 0.89$, $P < 0.0001$; Fig. 2). BMI scores of females (-457 to 681 , 35 ± 233 (mean \pm SD); $n = 125$) were significantly higher than those of males (-353 to 320 , -31 ± 165 ; $n = 138$; $t = 2.67$, $P = 0.008$; Fig. 1). BMI scores were uncorrelated with date of salvage in males ($n = 138$; $r = 0.00$, $P = 0.97$) and in females ($n = 125$; $r = 0.09$, $P = 0.33$; Fig. 3). Salvage dates for males and females did not differ significantly ($t = 0.53$, $P > 0.05$).

C/N of bulk tissue

$\text{C}/\text{N}_{\text{liver}}$ values (mean \pm SD) were 4.9 ± 0.6 ($n = 132$) for males and 4.7 ± 0.5 ($n = 116$) for females. $\text{C}/\text{N}_{\text{liver}}$ was uncorrelated with BMI scores and salvage date (Table 2, Fig. 4). $\text{C}/\text{N}_{\text{muscle}}$ values (mean \pm SD) were 4.4 ± 0.5 ($n = 131$) for males and 4.4 ± 0.5 ($n = 116$) for females. $\text{C}/\text{N}_{\text{muscle}}$

Fig. 1. Relationship between body mass index (BMI) and body size (first principal component, PC1) of Great Gray Owls (*Strix nebulosa*) salvaged during the 2004–2005 irruptive migration in Minnesota. Males and females are represented by ● and ○, respectively.

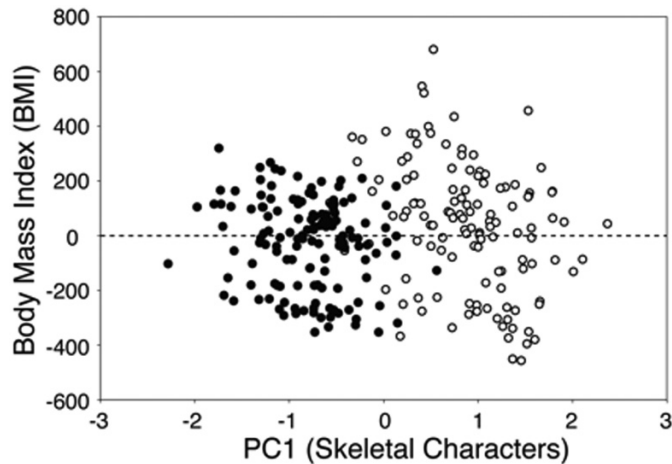
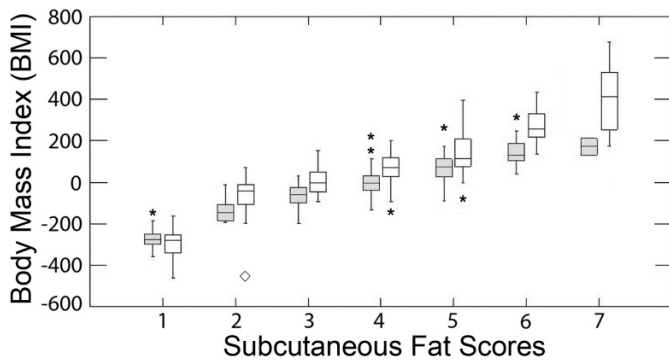


Fig. 2. Box plots depicting the relationship between body mass index (BMI) and body fat scores (1 = emaciated; 7 = extremely fat) in Great Gray Owls (*Strix nebulosa*) salvaged during the 2004–2005 irruptive migration in Minnesota. Males ($n = 140$) are represented by shaded boxes and females ($n = 125$) by unshaded boxes. The bottom and top of the boxes represent the 25th and 75th percentiles, respectively; the horizontal line indicates the median; and the whiskers represent the extreme values. Negative BMI scores represent emaciated individuals.



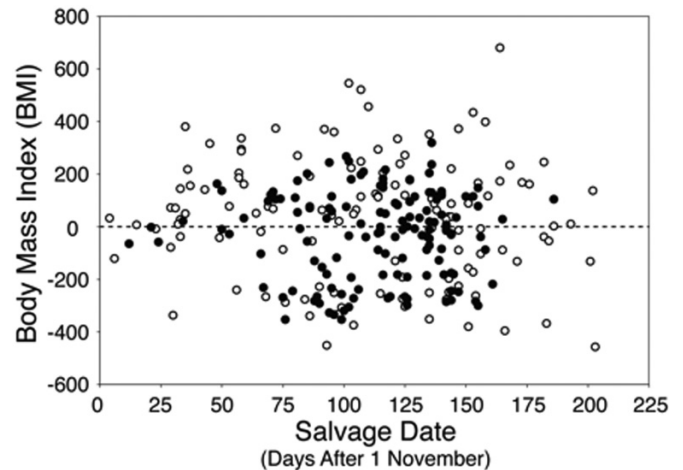
was uncorrelated with salvage date but was positively correlated with BMI scores (Table 2, Fig. 4).

Stable carbon isotopes

$\delta^{13}\text{C}_{\text{liver}}$ values from the pooled sample of males and females ranged from -28.0‰ to -19.2‰ ($-25.8\text{‰} \pm 1.3\text{‰}$, mean \pm SD; $n = 263$). There was no difference between males and females in $\delta^{13}\text{C}_{\text{liver}}$ values ($n = 265$; $t = 1.81$, $P = 0.07$). $\delta^{13}\text{C}_{\text{liver}}$ values were uncorrelated with date of salvage (Table 2). On the other hand, $\delta^{13}\text{C}_{\text{liver}}$ values were negatively correlated with BMI scores in males but not in females (Table 2, Fig. 5). $\delta^{13}\text{C}_{\text{liver}}$ values were negatively correlated with $\text{C}/\text{N}_{\text{liver}}$ in males ($n = 132$; $r = 0.29$, $P = 0.001$) and in females ($n = 116$; $r = 0.21$, $P = 0.02$; Fig. 6).

$\delta^{13}\text{C}_{\text{muscle}}$ values from the pooled sample of males and females ranged from -27.8‰ to -20.6‰ ($-25.5\text{‰} \pm 1.1\text{‰}$, mean \pm SD; $n = 263$). There was no difference between fe-

Fig. 3. Relationship between body mass index (BMI) and salvage date (day 1 equals 1 November 2004) of male (●; $n = 140$) and female (○; $n = 125$) Great Gray Owls (*Strix nebulosa*) obtained during the 2004–2005 irruptive migration in Minnesota.



males and males in $\delta^{13}\text{C}_{\text{muscle}}$ values ($n = 263$; $t = 0.42$, $P = 0.68$). $\delta^{13}\text{C}_{\text{muscle}}$ values were uncorrelated with date of salvage but were highly negatively correlated with BMI scores (Table 2, Fig. 5). $\delta^{13}\text{C}_{\text{muscle}}$ values were negatively correlated with $\text{C}/\text{N}_{\text{muscle}}$ in males ($n = 131$; $r = -0.61$, $P < 0.0001$) and in females ($n = 116$; $r = 0.40$, $P = 0.0001$; Fig. 6).

Stable nitrogen isotopes

$\delta^{15}\text{N}_{\text{liver}}$ values from the pooled sample of males and females ranged from 5.0‰ to 11.6‰ ($8.2\text{‰} \pm 1.0\text{‰}$, mean \pm SD; $n = 263$). There was no difference between males and females in $\delta^{15}\text{N}_{\text{liver}}$ values ($n = 263$; $t = 0.80$, $P = 0.43$). $\delta^{15}\text{N}_{\text{liver}}$ values were uncorrelated with date of salvage (Table 2). However, $\delta^{15}\text{N}_{\text{liver}}$ values were positively correlated with BMI scores in males but not in females (Table 2, Fig. 7). $\delta^{15}\text{N}_{\text{liver}}$ values were uncorrelated with $\text{C}/\text{N}_{\text{liver}}$ in males ($n = 132$; $r < 0.01$, $P = 0.96$) and in females ($n = 116$; $r = -0.03$, $P = 0.79$).

$\delta^{15}\text{N}_{\text{muscle}}$ values for the pooled sample of males and females ranged from 3.1‰ to 9.5‰ ($6.3\text{‰} \pm 0.9\text{‰}$, mean \pm SD; $n = 263$). There was no difference between males and females in $\delta^{15}\text{N}_{\text{muscle}}$ values ($n = 263$; $t = 1.41$, $P = 0.16$). $\delta^{15}\text{N}_{\text{muscle}}$ values were uncorrelated with date of salvage in males (Table 2). $\delta^{15}\text{N}_{\text{muscle}}$ values were positively correlated with BMI scores in males but not in females (Table 2, Fig. 7). $\delta^{15}\text{N}_{\text{muscle}}$ values were positively correlated with $\text{C}/\text{N}_{\text{muscle}}$ in males ($n = 131$; $r = 0.19$, $P = 0.03$) and in females ($n = 116$; $r = 0.22$, $P = 0.02$).

Multivariate predictors of BMI

The GLM model explained only 3% of the variance in BMI (Table 3). As expected from bivariate correlation coefficients (Table 2), sex had a significant effect on BMI scores. However, there was no evidence that the proportion of owls in poor body condition increased over the course of the winter (Table 3, Fig. 3).

Stomach contents and body condition

Mammalian prey items (whole prey, bone, or hair) were found in the stomachs of 362 (62%) out of 585 salvaged

Table 2. Pearson's correlation coefficients for body mass index (BMI), date of salvage, C/N, and carbon and nitrogen isotope values for liver and muscle of Great Gray Owls (*Strix nebulosa*) salvaged during the 2004–2005 irruption.

	BMI				Date of salvage			
	Males	<i>n</i>	Females	<i>n</i>	Males	<i>n</i>	Females	<i>n</i>
C/N _{liver}	-0.05	132	-0.16	116	-0.11	132	0.14	116
C/N _{muscle}	0.56	131	0.54	116	0.15	131	0.08	116
δ ¹³ C _{liver}	-0.48	138	-0.26	125	-0.02	138	0.04	125
δ ¹³ C _{muscle}	-0.63	138	-0.45	125	-0.17	138	-0.05	125
δ ¹⁵ N _{liver}	0.27	138	0.10	125	0.12	138	0.08	125
δ ¹⁵ N _{muscle}	0.27	138	0.17	125	-0.04	138	0.09	125

Note: Correlation coefficients in boldface type are significant at $\alpha < 0.05$, adjusted for the number of simultaneous tests ($P = 0.05/24 = 0.002$).

Fig. 4. Relationship between body mass index (BMI) and C/N of liver and muscle of male (●) and female (○) Great Gray Owls (*Strix nebulosa*) salvaged during the 2004–2005 irruptive migration in Minnesota.

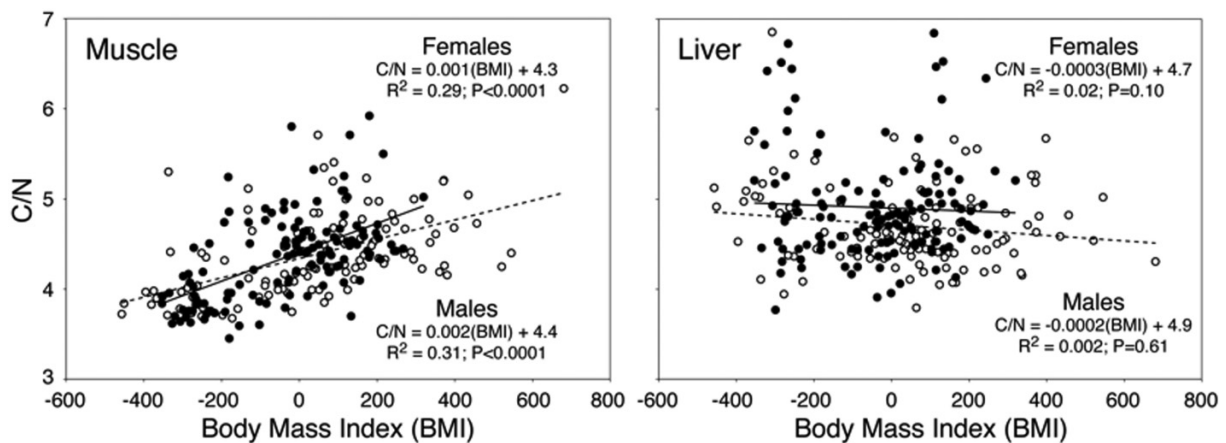
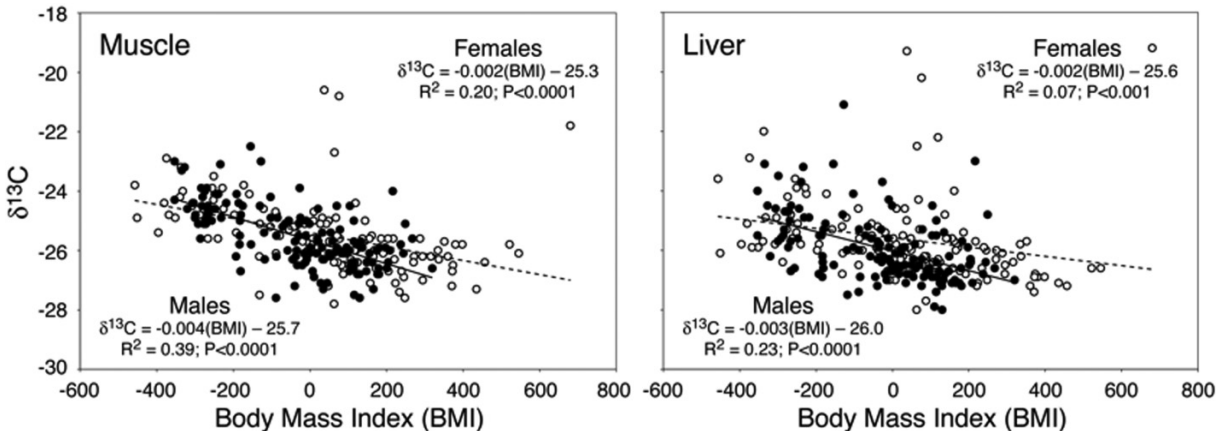


Fig. 5. Relationship between body mass index (BMI) and δ¹³C values of liver and muscle of male (●) and female (○) Great Gray Owls (*Strix nebulosa*) salvaged during the 2004–2005 irruptive migration in Minnesota.



specimens (Table 1). The five most abundant prey species accounted for 96% of the identified items: meadow vole (*Microtus pennsylvanicus* (Ord, 1815)) (78.6%), common shrew (*Sorex cinereus* Kerr, 1792) (5.9%), arctic shrew (*Sorex arcticus* Kerr, 1792) (4.8%), mole shrew (*Blarina brevicauda* (Say, 1823)) (4.2%), and southern red-backed vole (*Clethrionomys gapperi* (Vigors, 1830)) (2.5%). Owls with empty stomachs had significantly lower fat scores than owls with prey in their stomachs ($n = 262$; $t = -8.85$, $P < 0.0001$).

Likewise, individuals with empty stomachs had significantly lower BMI scores ($n = 263$; $t = -9.90$, $P < 0.0001$). However, males were no more likely to have empty stomachs than females ($\chi^2 = 0.26$, $P = 0.61$).

Discussion

Fastings and measures of nutritional condition

Birds and mammals experience three physiological phases

Fig. 6. Relationship between C/N and $\delta^{13}\text{C}$ values of liver and muscle of male (●) and female (○) Great Gray Owls (*Strix nebulosa*) salvaged during the 2004–2005 irruptive migration in Minnesota.

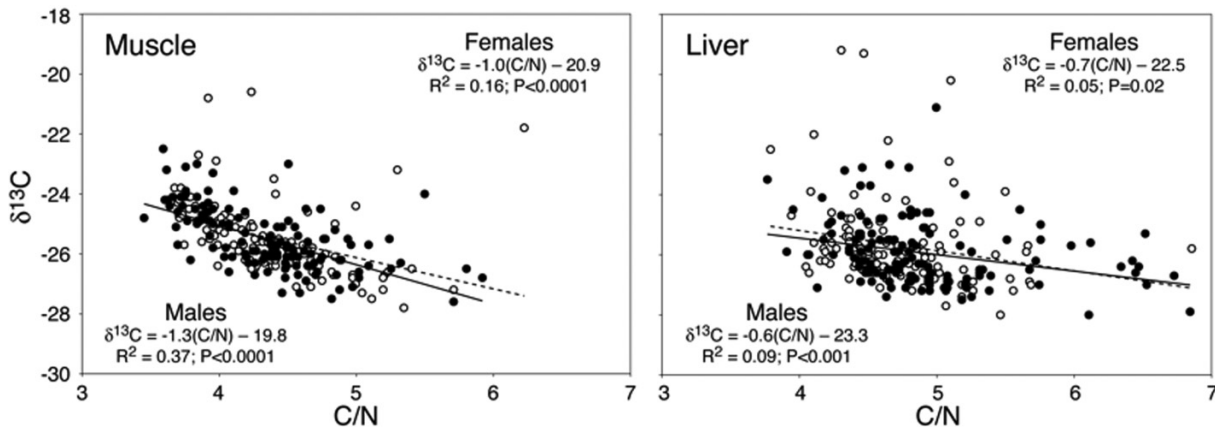


Fig. 7. Relationship between body mass index (BMI) and $\delta^{15}\text{N}$ values of liver and muscle of male (●) and female (○) Great Gray Owls (*Strix nebulosa*) salvaged during the 2004–2005 irruptive migration in Minnesota.

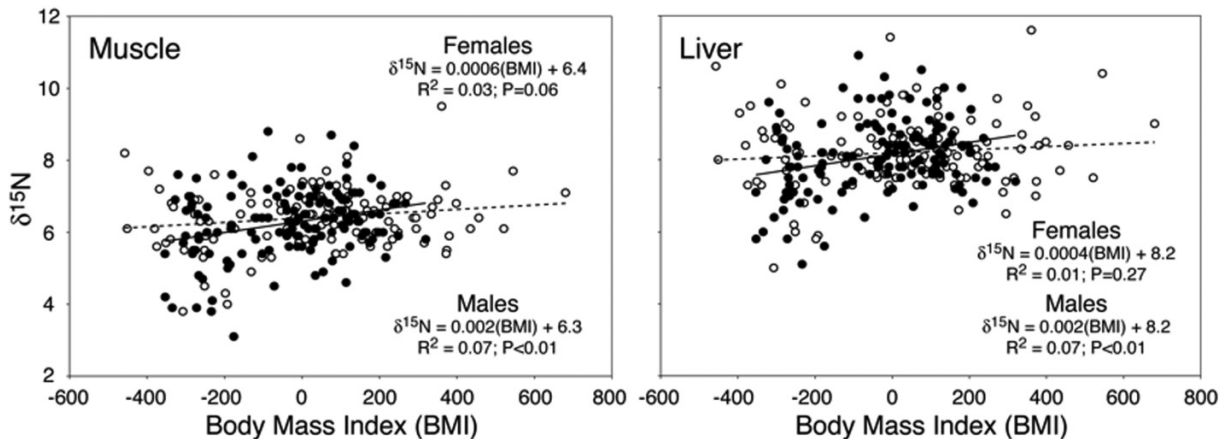


Table 3. Effects of sex and salvage date on body mass index (BMI) of Great Gray Owls (*Strix nebulosa*) salvaged during the 2004–2005 irruptive migration in Minnesota.

Source	Type III sums of squares	df	Mean squares	F	P
Sex	277.985	1	277.985	6.93	0.009
Date of salvage	36.696	1	36.696	0.92	0.34
Error	10 424.545	260	40.094		

as fasting progresses (Castellini and Rea 1992; Wang et al. 2006; Karasov and Martínez del Rio 2007; McCue 2010). After feeding ceases, glycogen stores are depleted over a period of hours and fatty acids are released from adipose tissue as a precursor to phase I. Following this short period of fasting adjustment, gluconeogenesis of amino acids from muscle protein, ketogenesis of ketone bodies, and glycerol from adipose tissue supply the energy needed for brain and organ function. As phase II progresses, the proportion of lipids fueling metabolism increases markedly while protein catabolism declines or remains minimal. The shift from prolonged fasting to what is usually referred to as starvation occurs between phase II and phase III. Protein is metabolized to maintain organ function in phase III after lipid reserves are depleted in phase II. Death ensues when vital organ function can no longer be sustained through protein metabolism. The

ability to reverse a prolonged fast in phase III may vary significantly among taxa.

There have been no experimental studies of fasting in strigid owls, but the physiological responses of birds and mammals to food deprivation (references in McCue 2010), especially those of the distantly related Barn Owl (*Tyto alba* (Scopoli, 1769)) (Handrich et al. 1993; Thouzeau et al. 1997, 1999), provide a context in which the body condition and stable isotope signatures of Great Gray Owls may be interpreted.

Of the 265 salvaged Great Gray Owls analyzed in this study, 28% of females and 33% of males had body masses lower than the minimum values listed for the species by Bull and Duncan (1993). This suggests that prolonged fasting and starvation may occur in a significant fraction of owls during irruptive migrations. However, the proportion of owls in poor

body condition did not increase over the course of the winter. Fat and emaciated individuals were salvaged each month from December through May. The significant number of salvaged specimens with substantial fat deposits indicates that good nutritional condition was no safeguard against accidental death.

Fat scores were positively correlated with BMI scores, which were derived from skeletal measurements and body mass. These in turn were positively correlated with C/N of muscle (Fig. 4), but not in liver, which has a substantially higher metabolism. This suggests that lipid reserves in muscle of emaciated individuals were greatly reduced or exhausted. Overall, our results suggest that C/N of muscle may serve as a general indicator of nutritional condition in owls, with higher C/N indicative of greater lipid reserves (Okumura et al. 2002; Post et al. 2007; Ehrich et al. 2010). This assay is more convenient and less invasive than other methods for quantifying nutritional condition, such as the measurement of bone marrow (Thouzeau et al. 1997) or whole body homogenization followed by lipid extraction (Baduini et al. 2001).

Stable isotope markers

Food manipulation experiments with captive birds and monitoring of fasting wild birds have shown that food restriction produces an increase (Cherel et al. 2005; Williams et al. 2007), a decrease (Hatch et al. 1995), or no effect (Hobson et al. 1993; Kempster et al. 2007) on $\delta^{13}\text{C}$ values of blood or muscle tissues. These analyses included lipid-extracted tissues (Hobson et al. 1993; Kempster et al. 2007) and tissues in which the lipid content was unaltered (Cherel et al. 2005; Williams et al. 2007). Because of experimental constraints and animal welfare protocols, the degree of food deprivation observed in some of these studies was relatively minor compared with the fasting experienced by many of the owls in the present study. Other experiments (reviewed in McCue and Pollock 2008) have reported elevated $\delta^{13}\text{C}$ values in tissues of starving lycosid spiders (Oelbermann and Scheu 2002), migratory locusts (*Locusta migratoria* (L., 1758)) fed on low-quality diets (Webb et al. 1998), and Nile tilapia (*Oreochromis niloticus* (L., 1758)) fed a below-maintenance diet (Gaye-Siessegger et al. 2007).

In general, lipids are depleted in ^{13}C relative to carbohydrates and proteinaceous tissues (Tieszen et al. 1983; Kelly 2000; Teece and Fogel 2007); thus, $\delta^{13}\text{C}$ values of bulk tissues may become more positive after endogenous glycogen, a storage carbohydrate, and lipids are catabolized during extended fasts. Our data exhibit the expected trend in that emaciated, presumably starving, owls had significantly higher $\delta^{13}\text{C}$ values for liver and muscle than individuals with high fat scores. The strongly negative association of $\delta^{13}\text{C}$ values with fat scores was also mirrored in the relationship between $\delta^{13}\text{C}$ values and BMI (Fig. 5) and between $\delta^{13}\text{C}$ values and C/N (Fig. 6).

Nutritional stress and starvation often result in elevated $\delta^{15}\text{N}$ values in the tissues of birds and mammals (Gloutney et al. 1999; Hobson et al. 1993; Cherel et al. 2005; Fuller et al. 2005; Mekota et al. 2006). Enrichment of ^{15}N during fasting occurs when endogenous tissue proteins are catabolized as a source of amino acids, which undergo deamination or transamination to produce ^{15}N -depleted NH_3 . In birds, ^{15}N -

depleted NH_3 is directly excreted or converted to uric acid as a waste product for excretion, leaving a pool of enriched amino acids to be synthesized into proteins (Macko et al. 1986). Some experimental feeding studies, however, have shown an insignificant or mixed relationship between food deprivation and $\delta^{15}\text{N}$ values in growing chicks (Kempster et al. 2007; Williams et al. 2007; Sears et al. 2009). Kempster et al. (2007) suggested there may be a threshold effect in which significant changes in isotopic composition may only be observed during periods of extreme starvation when endogenous protein stores are being catabolized for metabolism (e.g., phase III). The interpretation and generalization of results, however, are complicated by the fact that recent experiments were conducted on rapidly growing juveniles, rather than adults, and that “diet-restricted” individuals were fed enough to permit body mass to marginally increase during the experiment to comply with animal welfare protocols (e.g., Kempster et al. 2007, Williams et al. 2007, Sears et al. 2009). For animals that have completed their growth, the preponderance of evidence suggests that tissues which maintain significant protein synthesis (e.g., liver) will exhibit elevated $\delta^{15}\text{N}$ values if protein is catabolized during an extreme fast (Martínez del Río et al. 2009b).

One notable finding in our study was that $\delta^{15}\text{N}$ values for muscle and liver were uncorrelated or slightly positively correlated with BMI scores. Instead of exhibiting higher $\delta^{15}\text{N}$ values, the pooled sample of emaciated males and females (fat score = 1, $n = 58$) had slightly lower $\delta^{15}\text{N}$ values than owls in good condition (fat scores = 6–7, $n = 55$). This finding runs counter to the expectation of nitrogen enrichment in emaciated or starved individuals documented in several studies (Hobson et al. 1993, 1997; Cherel et al. 2005; Fuller et al. 2005; Mekota et al. 2006). To detect a shift in $\delta^{15}\text{N}$, however, new muscle or liver proteins would need to be synthesized from a pool of amino acids with an enriched ^{15}N content derived from the catabolization of endogenous protein during phase III (Balter et al. 2006).

Our data provide no evidence for a systemic pattern of nitrogen enrichment in emaciated owls. This suggests that $\delta^{15}\text{N}$ enrichment of muscle and liver tissue may not be a hallmark of starvation in this species. However, it is possible that metabolically active tissues (e.g., blood), other than liver and muscle, experienced $\delta^{15}\text{N}$ enrichment in emaciated owls (Martínez del Río et al. 2009b). Also, it is possible that owls recycle nitrogenous constituents during uric acid formation which are then used to synthesize new protein without the telltale signature of $\delta^{15}\text{N}$ enrichment. Experimental studies have shown that the distantly related Barn Owl has the ability to recover after body mass losses of up to 30% during phase II of starvation (Handrich et al. 1993). Although strigid owls may have similar physiological capacities, the fact that nearly a third of the Great Gray Owl specimens analyzed in our study had body masses lower than the minimum values listed for males (825 g) and females (1025 g) by Bull and Duncan (1993) indicates that fasting-mediated mortality may be common during irruptive migrations.

RSD and body condition

Our data provide weak support for the “fasting endurance” hypothesis for the evolution of RSD in owls proposed by Lundberg (1986). Lundberg’s (1986) hypothesis was built on

an extensive body of literature documenting increases in fasting endurance with increasing body mass in birds and mammals (Calder 1974; Boyce 1979; Lindstedt and Boyce 1985). The breeding biology of Great Gray Owls is similar to that of several other boreal strigids in that nesting is often initiated when heavy snow still blankets the landscape (Mikkola 1983; Nero 1980; Bull and Duncan 1993). Females alone incubate the eggs during the month-long incubation period and perform all the brooding for the first 2–3 weeks after hatching (Nero 1980; Mikkola 1983). The smaller male feeds the female and provisions the nestlings. The “fasting endurance” hypothesis posits that large females with greater fat stores may have a selective advantage if prey is delivered at long and unpredictable intervals. Female Great Gray Owls, which are substantially larger in skeletal size and body mass than males, exhibited significantly higher BMI scores. On average, females were 3.1% heavier than expected from skeletal size and males were 3.6% lighter than expected. Sexual differences in the correlation between BMI and carbon and nitrogen isotope signatures suggest that the consequences of RSD extend to the physiological markers of body condition as well (Table 2).

In conclusion, Great Gray Owls engaged in irruptive migrations exhibit wide variation in body conditions, ranging from emaciated individuals in the throes of starvation to apparently healthy individuals with ample fat deposits. Decreases in tissue lipid concentrations during the later stages of phase II fasting may result in higher $\delta^{13}\text{C}$ values but lower elemental C/N. Our results suggest that C/N of muscle may serve as a general indicator of nutritional condition in owls, with higher C/N indicative of greater lipid reserves. Although emaciated owls lacked one of the expected hallmarks of severe starvation, the enrichment of nitrogen isotopes, stomach-content data from a larger sample of salvaged owls (Table 1) indicate that emaciated individuals were in a precarious nutritional state. Approximately 85% of emaciated owls (fat score = 1) had empty stomachs compared with 25% of owls with moderate to high fat scores (4–7). This suggests that the ability to obtain prey was significantly compromised by poor body condition. Furthermore, owls in the final stages of starvation may be unable to digest prey because of gastrointestinal deficiencies (Handrich et al. 1993). Although owls may be able to recover from prolonged fasting (Handrich et al. 1993; Thouzeau et al. 1997, 1999), the low percentage of emaciated Great Gray Owls that had recently fed in this study suggests that many were in irreversible fasts.

Acknowledgements

For salvaging specimens, we thank C. Henderson, J. Hines, M. Hamady, K. Haws, P. Perry, K. Woizeschke, J. Welsh, M. Minchak, S. Wilson, and R. Staffon (all from the Minnesota Department of Natural Resources), J. Lind (from Natural Resources Research Institute), and J. Goggin (from Raptor Research Center). T. Gnoske and M. Hennen of the Field Museum helped with specimen preparation. E. Snyder, C. Mancuso, and B. O'Connor provided laboratory assistance. The manuscript was significantly improved by comments on earlier drafts by John Whiteman and two anonymous reviewers. G.R.G. was supported by the Wetmore Fund (National Museum of Natural History, Smithsonian Institution) and G.R.G. and S.D.N. were supported by the Wal-

cott Fund (National Museum of Natural History, Smithsonian Institution). S.D.N. was also supported by the National Science Foundation (ATM-0502491), Carnegie Institution of Washington, and the W.M. Keck Foundation (072000).

References

- Akaike, H. 1974. A new look at the statistical model identification. *IEEE Trans. Automat. Contr.* **19**(6): 716–723. doi:10.1109/TAC.1974.1100705.
- Amadon, D. 1975. Why are female birds of prey larger than males? *Raptor Res.* **9**: 1–11.
- Andersson, M., and Norberg, R.Å. 1981. Evolution of reversed sexual size dimorphism and role partitioning among predatory birds, with a size scaling of flight performance. *Biol. J. Linn. Soc.* **15**(2): 105–130. doi:10.1111/j.1095-8312.1981.tb00752.x.
- Andrews, P. 1990. Owls, caves, and fossils: predation, preservation, and accumulation of small mammal bones in caves, with an analysis of the Pleistocene cave faunas from Westbury-sub-Mendip, Somerset, UK. University of Chicago Press, Chicago, Ill.
- Audubon, J.J. 1838. *Ornithological biography*. Vol. 4. Adam and Charles Black, Edinburgh, Scotland.
- Baduini, C.L., Lovvorn, J.R., and Hunt, G.L., Jr. 2001. Determining the body condition of short-tailed shearwaters: implications for migratory flight ranges and starvation events. *Mar. Ecol. Prog. Ser.* **222**: 265–277. doi:10.3354/meps222265.
- Balter, V., Simon, L., Fouillet, H., and Lécuyer, C. 2006. Box-modeling of $^{15}\text{N}/^{14}\text{N}$ in mammals. *Oecologia (Berl.)*, **147**(2): 212–222. doi:10.1007/s00442-005-0263-5. PMID:16328553.
- Bauchinger, U., and McWilliams, S. 2009. Carbon turnover in tissues of a passerine bird: allometry, isotopic clocks, and phenotypic flexibility in organ size. *Physiol. Biochem. Zool.* **82**(6): 787–797. doi:10.1086/605548. PMID:19785542.
- Bearhop, S., Adams, C.E., Waldron, S., Fuller, R.A., and MacLeod, H. 2004. Determining trophic niche width: a novel approach using stable isotope analysis. *J. Anim. Ecol.* **73**(5): 1007–1012. doi:10.1111/j.0021-8790.2004.00861.x.
- Bent, A.C. 1938. *Life histories of North American birds of prey*. Part 2. Bull. No. 170. United States National Museum, Washington, D.C.
- Bildstein, K.L. 1992. Causes and consequences of reversed sexual size dimorphism in raptors: the head start hypothesis. *J. Raptor Res.* **26**: 115–123.
- Boyce, M.S. 1979. Seasonality and patterns of natural selection for life histories. *Am. Nat.* **114**(4): 569–583. doi:10.1086/283503.
- Bull, E.L., and Duncan, J.R. 1993. Great Gray Owl (*Strix nebulosa*). In *The birds of North America*. Edited by A. Poole and F. Gill. The Academy of Natural Sciences, Philadelphia, Pa.; The American Ornithologists' Union, Washington, D.C. pp. 1–15.
- Calder, W.A. 1974. Consequences of body size for avian energetics. In *Avian energetics*. Edited by R.A. Paynter. Nuttall Ornithological Club, Cambridge, Mass. pp. 86–151.
- Carleton, S.A., and Martínez del Río, C. 2005. The effect of cold-induced increased metabolic rate on the rate of ^{13}C and ^{15}N incorporation in house sparrows (*Passer domesticus*). *Oecologia (Berl.)*, **144**(2): 226–232. doi:10.1007/s00442-005-0066-8. PMID:15891840.
- Castellini, M.A., and Rea, L.D. 1992. The biochemistry of natural fasting at its limits. *Experientia*, **48**(6): 575–582. doi:10.1007/BF01920242. PMID:1612138.
- Chamberlain, C.P., Waldbauer, J.R., Fox-Dobbs, K., Newsome, S.D., Koch, P.L., Smith, D.R., Church, M.E., Chamberlain, S.D., Sorenson, K.J., and Risebrough, R. 2005. Pleistocene to recent dietary shifts in California condors. *Proc. Natl. Acad. Sci. U.S.A.*

- 102(46): 16707–16711. doi:10.1073/pnas.0508529102. PMID: 16275902.
- Cherel, Y., Hobson, K.A., Bailleul, F., and Groscolas, R. 2005. Nutrition, physiology, and stable isotopes: new information from fasting and molting penguins. *Ecology*, **86**(11): 2881–2888. doi:10.1890/05-0562.
- Cheveau, M., Drapeau, P., Imbeau, L., and Bergeron, Y. 2004. Owl winter irruptions as an indicator of small mammal population cycles in the boreal forest of eastern North America. *Oikos*, **107**(1): 190–198. doi:10.1111/j.0030-1299.2004.13285.x.
- Craig, H. 1961. Isotopic variations in meteoric water. *Science*, **133**(3465): 1702–1703. doi:10.1126/science.133.3465.1702. PMID:17814749.
- Cramp, S., (editor). 1985. *Handbook of the birds of Europe, the Middle East and North Africa: the birds of the Western Palearctic: terns to woodpeckers*. Vol. 4. Oxford University Press, Oxford, U.K.
- DeNiro, M.J., and Epstein, S. 1978. Influence of diet on the distribution of carbon isotopes of animals. *Geochim. Cosmochim. Acta*, **42**(5): 495–506. doi:10.1016/0016-7037(78)90199-0.
- Earhart, C.M., and Johnson, N.K. 1970. Size dimorphism and food habitats of North American owls. *Condor*, **72**(3): 251–264. doi:10.2307/1366002.
- Ehrich, D., Tarroux, A., Stien, J., Lecomte, N., Killengreen, S., Berteaux, D., and Yoccoz, N.G. 2010. Stable isotope analysis: modelling lipid normalization for muscle and eggs from arctic mammals and birds. *Methods Ecol. Evol.* **2**(1): 66–76. doi:10.1111/j.2041-210X.2010.00047.x.
- Estep, M.F., and Dabrowski, H. 1980. Tracing food webs with stable hydrogen isotopes. *Science*, **209**(4464): 1537–1538. doi:10.1126/science.209.4464.1537. PMID:17745967.
- Fuller, B.T., Fuller, J.L., Sage, N.E., Harris, D.A., O'Connell, T.C., and Hedges, R.E.M. 2005. Nitrogen balance and $\delta^{15}\text{N}$: why you're not what you eat during nutritional stress. *Rapid Commun. Mass Spectrom.* **19**(18): 2497–2506. doi:10.1002/rm.2090. PMID: 16106342.
- Gaye-Siessegger, J., Focken, U., Abel, H., and Becker, K. 2007. Starvation and low feeding levels result in an enrichment of ^{13}C in lipids and ^{15}N in protein of Nile tilapia *Oreochromis niloticus* L. *J. Fish Biol.* **71**(1): 90–100. doi:10.1111/j.1095-8649.2007.01469.x.
- Gloutney, M.L., Alisauskas, R.T., Hobson, K.A., and Afton, A.D. 1999. Use of supplemental food by breeding Ross's geese and lesser snow geese: evidence for variable anorexia. *Auk*, **116**: 97–108.
- Graves, G.R., and Niemi, G.J. 2006. Possible predation of Great Gray Owl by a Barred Owl. *J. Raptor Res.* **40**(2): 175. doi:10.3356/0892-1016(2006)40[175:PPOGGO]2.0.CO;2.
- Hakkarainen, H., and Korpimäki, E. 1991. Reversed sexual size dimorphism in Tengmalm's owl: is small male size adaptive? *Oikos*, **61**(3): 337–346. doi:10.2307/3545241.
- Handrich, Y., Nicolas, L., and Le Maho, Y. 1993. Winter starvation in captive Common Barn-Owls: physiological states and reversible limits. *Auk*, **110**: 458–469.
- Hartman, F.A. 1961. Locomotor mechanisms of birds. *Smithsonian Misc. Collect.* **143**: 1–91.
- Hatch, K.A., Sacksteder, K.A., Treichel, I.W., Cook, M.E., and Porter, W.P. 1995. Early detection of catabolic state via changes in $^{13}\text{C}/^{12}\text{C}$ ratios of blood proteins. *Biochem. Biophys. Res. Commun.* **212**(3): 719–726. doi:10.1006/bbrc.1995.2030. PMID: 7626105.
- Hayashi, I. 1983. Seasonal changes in condition factors and in the C:N ratio of the foot of the ormer, *Haliotis tuberculata*. *J. Mar. Biol. Assoc. U.K.* **63**(01): 85–95. doi:10.1017/S0025315400049821.
- Hobson, K.A., and Clark, R.G. 1992. Assessing avian diets using stable isotopes I: turnover of ^{13}C in tissues. *Condor*, **94**(1): 181–188. doi:10.2307/1368807.
- Hobson, K.A., and Welch, H.E. 1992. Determination of trophic relationships within a high Arctic marine food web using $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis. *Mar. Ecol. Prog. Ser.* **84**: 9–18. doi:10.3354/meps084009.
- Hobson, K.A., Alisauskas, R.T., and Clark, R.G. 1993. Stable-nitrogen isotope enrichment in avian tissues due to fasting and nutritional stress: implications for isotopic analyses of diet. *Condor*, **95**(2): 388–394. doi:10.2307/1369361.
- Hobson, K.A., Gibbs, H.L., and Gloutney, M.L. 1997. Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. *Can. J. Zool.* **75**(10): 1720–1723. doi:10.1139/z97-799.
- Hobson, K.A., Atwell, L., and Wassenaar, L.I. 1999. Influence of drinking water and diet on the stable-hydrogen isotope ratios of animal tissues. *Proc. Natl. Acad. Sci. U.S.A.* **96**(14): 8003–8006. doi:10.1073/pnas.96.14.8003. PMID:10393937.
- Jehl, J.R., and Murray, B.G. 1986. The evolution of normal and reverse sexual size dimorphism in shorebirds and other birds. *In* *Current ornithology*. Edited by R.F. Johnston. Plenum Press, New York. pp. 1–86.
- Karasov, W.H., and Martínez del Rio, C. 2007. *Physiological ecology: how animals process energy, nutrients, and toxins*. Princeton University Press, Princeton, N.J.
- Kelly, J.F. 2000. Stable isotopes of carbon and nitrogen in the study of avian and mammalian trophic ecology. *Can. J. Zool.* **78**(1): 1–27. doi:10.1139/z99-165.
- Kelly, B., Dempson, J.B., and Power, M. 2006. The effects of preservation of fish tissue stable isotope signatures. *J. Fish Biol.* **69**(6): 1595–1611. doi:10.1111/j.1095-8649.2006.01226.x.
- Kempster, B., Zanette, L., Longstaffe, F.J., MacDougall-Shackleton, S.A., Wingfield, J.C., and Clinchy, M. 2007. Do stable isotopes reflect nutritional stress? Results from a laboratory experiment on song sparrows. *Oecologia (Berl.)*, **151**(3): 365–371. doi:10.1007/s00442-006-0597-7. PMID:17102993.
- Korpimäki, E. 1986. Reversed size dimorphism in birds of prey, especially in Tengmalm's Owl *Aegolius funereus*: a test of the "starvation hypothesis". *Ornis Scand.* **17**(4): 326–332. doi:10.2307/3676820.
- Krüger, O. 2005. The evolution of reversed sexual size dimorphism in hawks, falcons and owls: a comparative study. *Evol. Ecol.* **19**(5): 467–486. doi:10.1007/s10682-005-0293-9.
- Lidicker, W.Z., Jr 2008. Mass or weight: response to (Murray 2008) and (Chardine 2008). *Auk*, **125**(3): 744. doi:10.1525/auk.2008.2708.
- Lindstedt, S.L., and Boyce, M.S. 1985. Seasonality, fasting endurance, and body size in mammals. *Am. Nat.* **125**(6): 873–878. doi:10.1086/284385.
- Lundberg, A. 1986. Adaptive advantages of reversed sexual size dimorphism in European owls. *Ornis Scand.* **17**(2): 133–140. doi:10.2307/3676862.
- Macko, S.A., Estep, M.L.F., and Lee, W.Y. 1983. Stable hydrogen isotope analysis of foodwebs on laboratory and field populations of marine amphipods. *J. Exp. Mar. Biol. Ecol.* **72**(3): 243–249. doi:10.1016/0022-0981(83)90109-0.
- Martínez del Rio, C., Sabat, P., Anderson-Sprecher, R., and Gonzalez, S.P. 2009a. Dietary and isotopic specialization: the isotopic niche of three *Cinclodes* ovenbirds. *Oecologia (Berl.)*, **161**(1): 149–159. doi:10.1007/s00442-009-1357-2.
- Martínez del Rio, C., Wolf, N., Carleton, S.A., and Gannes, L.Z. 2009b. Isotopic ecology ten years after a call for more laboratory experiments. *Biol. Rev. Camb. Philos. Soc.* **84**(1): 91–111. doi:10.1111/j.1469-185X.2008.00064.x. PMID:19046398.

- McCue, M.D. 2010. Starvation physiology: reviewing the different strategies animals use to survive a common challenge. *Comp. Biochem. Physiol. A Mol. Integr. Physiol.* **156**(1): 1–18. PMID: 20060056.
- McCue, M.D., and Pollock, E.D. 2008. Stable isotopes may provide evidence for starvation in reptiles. *Rapid Commun. Mass Spectrom.* **22**(15): 2307–2314. doi:10.1002/rcm.3615. PMID: 18613003.
- Mekota, A.-M., Grupe, G., Ufer, S., and Cuntz, U. 2006. Serial analysis of stable nitrogen and carbon isotopes in hair: monitoring starvation and recovery phases of patients suffering from anorexia nervosa. *Rapid Commun. Mass Spectrom.* **20**(10): 1604–1610. doi:10.1002/rcm.2477. PMID:16628564.
- Mikkola, H. 1983. *Owls of Europe*. Buto Books, Vermillion, S.D.
- Mueller, H.C. 1986. The evolution of reversed sexual dimorphism in owls: an empirical analysis of possible selective factors. *Wilson Bull.* **98**(3): 387–406.
- Mueller, H.C. 1990. The evolution of reversed sexual dimorphism in size in monogamous species of birds. *Biol. Rev. Camb. Philos. Soc.* **65**(4): 553–585. doi:10.1111/j.1469-185X.1990.tb01238.x.
- Nero, R.W. 1980. *The Great Gray Owl—phantom of the northern forest*. Smithsonian Institution Press, Washington, D.C.
- Nero, R.W., and Copland, H.W.R. 1981. High mortality of Great Gray Owls in Manitoba—winter 1980–81. *Blue Jay*, **39**: 158–165.
- Newton, I. 2002. Population limitation in Holarctic owls. *In Ecology and conservation of owls*. Edited by I. Newton, R. Kavanagh, J. Olsen, and I. Taylor. CSIRO Publishing, Collingwood, Australia. pp. 3–29.
- Newton, I. 2006. Advances in the study of irruptive migration. *Ardea*, **94**: 433–460.
- Oelbermann, K., and Scheu, S. 2002. Stable isotope enrichment ($\delta^{15}\text{N}$ and $\delta^{13}\text{C}$) in a generalist predator (*Pardosa lugubris*, Araneae: Lycosidae): effects of prey quality. *Oecologia (Berl.)*, **130**(3): 337–344. doi:10.1007/s004420100813.
- Okumura, T., Nagasawa, T., Hayashi, I., and Sato, Y. 2002. Effects of starvation on RNA:DNA ratio, glycogen content, and C:N ratio in columellar muscle of the Japanese turban shell *Turbo (Batillus) cornutus* (Gastropoda). *Fish. Sci.* **68**(2): 306–312. doi:10.1046/j.1444-2906.2002.00426.x.
- Post, D.M., Layman, C.A., Arrington, D.A., Takimoto, G., Quattrochi, J., and Montaña, C.G. 2007. Getting to the fat of the matter: models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia (Berl.)*, **152**(1): 179–189. doi:10.1007/s00442-006-0630-x. PMID:17225157.
- Rich, T.D., Beardmore, C.J., Berlanga, H., Blancher, P.J., Bradstreet, M.S.W., Butcher, G.S., Demarest, D.W., Dunn, E.H., Hunter, W.C., Inigo-Elias, E.E., Kennedy, J.A., Martell, A.M., Panjabi, A.O., Pashley, D.N., Rosenberg, K.V., Rustay, C.M., Wendt, J.S., and Will, T.C. 2004. Partners in flight North American landbird conservation plan (version: March 2005). Cornell Lab of Ornithology, Ithaca, N.Y. Available from http://www.partnersin-flight.org/cont_plan/ [accessed 6 July 2008].
- Rubenstein, D.R., and Hobson, K.A. 2004. From birds to butterflies: animal movement patterns and stable isotopes. *Trends Ecol. Evol.* **19**(5): 256–263. doi:10.1016/j.tree.2004.03.017. PMID:16701265.
- Sarakinos, H.C., Johnson, M.L., and Vander Zanden, M.J. 2002. A synthesis of tissue-preservation effects on carbon and nitrogen stable isotope signatures. *Can. J. Zool.* **80**(2): 381–387. doi:10.1139/z02-007.
- Sears, J., Hatch, S.A., and O'Brien, D.M. 2009. Disentangling effects of growth and nutritional status on seabird stable isotope ratios. *Oecologia (Berl.)*, **159**(1): 41–48. doi:10.1007/s00442-008-1199-3. PMID:18975007.
- Svingen, P.H., and Lind, J.W. 2005. The 2004–2005 influx of northern owls. Part II: Great Gray Owl. *Loon*, **77**: 194–208.
- Sweeting, C.J., Polunin, N.V.C., and Jennings, S. 2004. Tissue and fixative dependent shifts of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ in preserved ecological material. *Rapid Commun. Mass Spectrom.* **18**(21): 2587–2592. doi:10.1002/rcm.1661. PMID:15468144.
- Teece, M.A., and Fogel, M.L. 2007. Stable carbon isotope biogeochemistry of monosaccharides in aquatic organisms and terrestrial plants. *Org. Geochem.* **38**(3): 458–473. doi:10.1016/j.orggeochem.2006.06.008.
- Thouzeau, C., Massemin, S., and Handrich, Y. 1997. Bone marrow fat mobilization in relation to lipid and protein catabolism during prolonged fasting in barn owls. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **167**(1): 17–24. doi:10.1007/s003600050043.
- Thouzeau, C., Robin, J.P., Le Maho, Y., and Handrich, Y. 1999. Body reserve dynamics and energetics of barn owls during fasting in the cold. *J. Comp. Physiol. B Biochem. Syst. Environ. Physiol.* **169**(8): 612–620. doi:10.1007/s003600050262.
- Tieszen, L.L., Boutton, T.W., Tesdahl, K.G., and Slade, N.A. 1983. Fractionation and turnover of stable carbon isotopes in animal tissues: implications for $\delta^{13}\text{C}$ analysis of diet. *Oecologia (Berl.)*, **57**(1–2): 32–37. doi:10.1007/BF00379558.
- Wang, T., Hung, C.C.Y., and Randall, D.J. 2006. The comparative physiology of food deprivation: from feast to famine. *Annu. Rev. Physiol.* **68**(1): 223–251. doi:10.1146/annurev.physiol.68.040104.105739. PMID:16460272.
- Webb, S.C., Hedges, R.E.M., and Simpson, S.J. 1998. Diet quality influences the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ of locusts and their biochemical components. *J. Exp. Biol.* **201**(20): 2903–2911. PMID:9739072.
- Williams, C.T., Buck, C.L., Sears, J., and Kitaysky, A.S. 2007. Effects of nutritional restriction on nitrogen and carbon stable isotopes in growing seabirds. *Oecologia (Berl.)*, **153**(1): 11–18. doi:10.1007/s00442-007-0717-z. PMID:17406905.