Seasonal Variation of Resting Metabolic Rate and Body Mass in Free-Living Weasels *Mustela nivalis*

Paulina A. Szafrańska¹*, Karol Zub¹, Marek Konarzewski¹, Marek Konarzewski²
¹Mammal Research Institute, Polish Academy of Sciences, Białowieża 17-230, Poland; ²University of Białystok, Białystok 15-097, Poland

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ABSTRACT

Metabolic rates and body mass of mammals vary seasonally along with ambient temperatures and food availability. At the population level, seasonal changes in metabolic rate and mass can be due to selective mortality or emigration of individuals whose metabolic rate or mass differs from the average for the population. Alternatively, the metabolic rates of individuals can change seasonally, such that the population average increases or decreases due to shifts in the physiology of the overall population. The latter implies that individuals respond in a similar manner to changing seasonal conditions. We studied seasonal changes in body mass (BM) and resting metabolic rate (RMR) in free-ranging male weasels (*Mustela nivalis*) to test the consistency of these traits in individuals caught in different seasons of the year. At the population level, BM was remarkably stable across the seasons ($F_{1,124} = 0.25$, $P = 0.9$). In contrast, BM-corrected RMR varied significantly between seasons and was the lowest in winter ($F_{1,115} = 9.13$, $P < 0.0001$). We demonstrated that individual weasels were consistent in how their BM and RMR deviated from the seasonal means for the population (intraclass correlation, $\tau = 0.78$ and $0.33$, respectively). This variation among individuals explained ~76% and 27% of the total variation of BM and basal metabolic rate, respectively. Hence, the relatively constant BM at the population level across seasons is due to a relative constancy of BM in individuals. Our study is one of relatively few research projects that demonstrate that seasonal changes in RMR observed in the wild population are in part due to a consistency in individual responses to changing environmental conditions.

Introduction

Seasonal adjustments of metabolic rate and body mass have long been recognized as hallmarks of physiological responses of mammals to changes in important environmental factors such as ambient temperature and food availability (MacMillen and Garland 1989; Veloso and Bozinovic 1993; Lovegrove 2003; Cruz-Neto and Bozinovic 2004; Mathias et al. 2004; Rezende et al. 2004; Silva et al. 2004; Perissinotti et al. 2009). The interest in studying these responses has recently been rejuvenated by the need for better understanding of the phenotypic flexibility of physiological traits in the context of adaptation to climate change (Tieleman and Williams 2002; McKechnie 2008; Raichlen et al. 2010). Several studies have demonstrated a reduction of body mass (BM) in overwintering mammals (Iverson and Turner 1974; Aars and Ims 2002), as well as in BM-corrected metabolic rates (Harrington et al. 2003; Arnold et al. 2004, 2006; Fuglesteg et al. 2006; Kumagai et al. 2006); mainly basal (resting) metabolic rate (BMR or RMR, the lowest measured metabolic rate of a resting animal in its thermoneutral zone). On the other hand, numerous authors have reported winter increases in BM and RMR (Sparling et al. 2006; Zhang and Wang 2007; Sheriff et al. 2009) or a lack of appreciable seasonal variation (Mauget et al. 1996; Harrington et al. 2003; Wang et al. 2006).

Such inconsistent patterns could be explained by species-specific differences in seasonal adaptations. For example, in some species an increase of BM at the beginning of winter reflects an increased fat deposition (Fuglei and Øritsland 1999; Fuglesteg et al. 2006), whereas a decrease in RMR following winter is interpreted as a shift in energy allocation toward reproduction (Sparling et al. 2006; Rønning et al. 2008). However, it is important to note that the interpretation of patterns of seasonal variation is based on an assumption of a consistency in individual responses to changing seasonal conditions. While this assumption is met in studies carried out under laboratory or seminatural conditions (e.g., Mauget et al. 1996; Fuglei and Øritsland 1999; Arnold et al. 2004, 2006; Bozinovic et al. 2004; Fuglesteg et al. 2006; Kumagai et al. 2006; Sparling et al. 2006; Kito et al. 2009), there are practically no studies (except one; Iverson and Turner 1974) focused on that problem in the wild. There are also relatively few studies that investigate seasonal changes in RMR or BMR in wild populations (Boratyński and Koteja 2009; Larivée et al. 2010; Careau et al. 2013). Information on longitudinal consistency is essential for the proper interpretation of the variation of studied traits at the population level, the reason being that between-season changes in the population means that the traits in question can be due to (1) selective mortality/emigration of individuals characterized by a...
specific value of the trait, (2) a concerted shift of trait values at the individual level, which represents the expression of phenotypic plasticity in the trait expression of individuals (Gienapp et al. 2008), or (3) a mix of 1 and 2.

In an earlier study (Szafranska et al. 2007) we demonstrated significant within-season repeatability of BM and RMR in free-living male weasels Mustela nivalis. We quantified this consistency using an intraclass correlation coefficient (Lessells and Boag 1987). Here we applied the same approach to examine the individual consistency of both traits between seasons. More specifically, we examined whether individual weasels were consistent in how their BM and RMR deviated from the seasonal means for the population. By doing so we aimed to reveal sources affecting between-season variation of those traits at the population level as specified by points 1–3.

We have already demonstrated that an extreme (range, 40–120 g) individual variation of BM and high RMR makes weasels a useful model for studies of small-mammal energetics in natural settings (Szafranska et al. 2007; Zub et al. 2009, 2011). Weasels live in a polygyrous system (Moors 1980; King 1989) in which body size determines the reproductive success of males (Blackenhorn 2000; Badayaev 2002). Weasels are also characterized by an exceptionally high mass-corrected RMR (Casey and Casey 1979). As a result, weasel BM and energy expenditure rates are sensitive to changes of environmental conditions, mainly food quality and temperature. In turn, these factors affect their spatial distribution and determine their survival (Zub et al. 2011). To our knowledge, our study is one of the first attempts to analyze the interseason consistency of traits related to energy utilization in a wild population of a small mammal.

Material and Methods

Study Area and Handling of Animals

Our study was carried out in Bialowieza Forest (NE Poland, 23°52'E, 52°42'N). This part of Europe is under the influence of a continental climate, characterized by very distinct seasonality. Summers are hot (mean temperature in July during the past 30 yr = 19.2°C), and winters are cold (mean temperature for January = −3.2°C) and snowy (mean snow cover = 10 cm; Bialowieza meteorological station). Weasels were trapped in wooden live box traps according to the procedure of Je˛drzejewski et al. (1995). We restricted our study to males to avoid the confounding effect of pregnancy or lactation. Animals were immediately transported to the laboratory in the Mammal Research Institute, Polish Academy of Sciences. Here they were held in separate cages with water ad lib. and fed one or two mice per day, which met their energy requirements (Zub et al. 2011). Metabolic rate was measured the following night, and animals were released at the location of trapping after a maximum of 4 d in captivity. All procedures were approved by the nature conservancy authorities (permits DOPweg-4201-04-6/03/jr, DOPog-4201-04-43/05/aj, LKE 2003/04, and LKE 2004/06).

Measurements of RMR and BM

For RMR measurements, we used a positive-pressure, open-circuit respirometry system. Outside atmospheric air was pushed through a column of Drierite (W. A. Hammond Drierite) to remove water vapor and then forced through a copper coil submerged along with the metabolic chamber in a water bath to equalize and control the temperature. The airstream was then divided into reference and measurement streams, each fed to a separate mass-flow controller (Sierra Instruments, Monterey, CA, or ERG-1000, Warsaw, Poland). The measurement stream was forced through the metabolic chamber (volume 2,300 cm³) at a mean rate of 900 mL min⁻¹. The airstreams were then directed to a computer-controlled Sable Systems TR-1 setup (Las Vegas, NV). The analyzed stream of air was redried (Drierite), subsampled at 200 mL min⁻¹, and then passed through the sensor of an FC-10b oxygen analyzer. All metabolic trials were carried out at night, starting from 1800 hours in winter and 2000 hours in summer, during weasels’ resting phase (Je˛drzejewski et al. 2000).

Before the trials weasels were fed only in the morning, so they were not fasted for longer than 12 h before measurement. We were concerned that longer fasting would compromise the animals’ welfare and/or increase their restlessness (Price 1971). We cannot therefore consider our measurements as taken in a truly postabsorptive state; thus, we refer to them as RMR rather than BMR. Measurements were taken during the last 2 h of the 3–6-h trial period at 30°C, a temperature within the thermoneutral zone (Casey and Casey 1979). Each animal was weighed to the nearest 0.1 g prior to the metabolic trial. This BM was usually slightly higher than the BM determined immediately after capture. However, both measures were highly correlated (r = 0.87, P < 0.0001), so we elected to use BM before RMR measurements for all analyses of BM repeatability.

Digital signals from the analyzer were stored using Winwedge 3.0 software (Taltech, Philadelphia) and subsequently analyzed with Sable Systems’ Datacan V software. CO₂ was not scrubbed from the analyzed airstream; therefore, we calculated oxygen consumption by means of equation (5) of Hill (1972), assuming respiratory quotient = 0.8 (Koteja 1996). We carried out all analyses for the lowest 2, 4, and 6 min for which oxygen concentration did not change more than 0.01%. We present the results for the lowest 4 min, which yielded the highest values of repeatability.

Data Collection and Analysis

For population-level analyses of seasonal changes in BM and RMR, we used measurements taken from 82 weasels captured in our study project between 2004 and 2008. During this period we collected 511 measurements of BM and 171 measurements of RMR; we used those data to track seasonal changes at the population level. We assigned them to four seasons, reflecting changes in both weather and weasel biology: winter (December–February), when temperatures are the lowest and animals are not very active (Je˛drzejewski et al. 2000; Zub et al. 2011);
Seasonal Variation in Resting Metabolic Rate

Figure 1. Relationship between mean individual body mass and the difference between individual body mass in a given season and population mean in a given season.

Table 1: Summary of ANOVA/ANCOVA statistics for body mass (BM) and resting metabolic rate (RMR) of male weasels

<table>
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<td>F</td>
<td>df</td>
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<tr>
<td>Season</td>
<td>.25</td>
<td>3, 124</td>
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<tr>
<td>Individual (season)</td>
<td>30.17</td>
<td>121, 369</td>
</tr>
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<td>BM</td>
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Results

As indicated by ANOVA, population-wide seasonal changes of BM were not statistically significant (table 1; fig. 2). In contrast, RMR varied significantly between seasons, with highest and lowest levels attained during summer/autumn and winter, respectively (table 1; fig. 1).

The consistency of individual BM explained ~76% of its total variance. Individual consistency of RMR was much lower, albeit still statistically significant, and accounted for 27% of the total variation. BM was significant as a covariate in RMR analysis (table 1), with no appreciable interaction with seasonal effect (F_{3,135} = 0.21, P = 0.2).

To further explore individual consistency (repeatability) of BM in relation to seasonal variation, we calculated the intraclass correlation coefficient for the subset of 33 weasels, for which we had data from at least two different seasons. The correlation was statistically significant (τ = 0.78, P = 0.0001; fig. 4). Likewise, cross season repeatability of RMR calculated for the subset of 22 individual weasels was statistically significant (τ = 0.33, P = 0.01; fig. 5).

Discussion

In our previous study (Szafranska et al. 2007) we demonstrated high within-season individual repeatability of BM and RMR in male weasels. Here, we found that the position of individuals always below the population mean, while a male with 150-g BM was always above the population mean independent of the season. To avoid the problem of overestimating, we divided the resulting difference by the mean individual BM to account for the season-independent mass differences between small and large weasels. We then estimated the between-season consistency (repeatability) of BM as the coefficient of intraclass correlation by means of the "mixed" SAS procedure using the vcorr option.

We used the same tool to estimate the intraclass correlation (i.e., between-season consistency) of RMR. In this model we used season as a fixed factor and BM as a covariate, which accounted for the effect of BM on RMR.

We did not log transform BM prior to analyses as it was normally distributed. Although RMR was normally distributed, we log transformed both BM and RMR prior to the analyses including both traits because they are allometrically related (McNab 2002).
in relation to the population mean was also repeatable between seasons (figs. 4, 5) despite considerable changes in key environmental factors, such as the ambient temperature and food availability (Zub et al. 2008), characteristic of our study site.

At the population level, seasonal changes of BM and RMR have been shown in numerous mammalian species (Geębczyński et al. 1972; Wunder et al. 1977; Merritt and Zegers 1991, 2002; Aalto et al. 1993; McDevitt and Andrews 1995; Corp et al. 1997; Volutra 1997; Zhang and Wang 2007; Sheriff et al. 2009; Boratyński and Koteja 2009). The cross species analysis showed that the small mammals below 500 g decrease in BM even as much as 50% during winter (Lovegrove 2005). However, the Dehnel effect, which underlines this change in many of those species (Lovegrove 2005), was not found in weasels. The decrease in winter BM on the population level is well documented (Merritt and Zegers 1991, 2002; McDevitt and Andrews 1995; Volutra 1997). It is explained by low food availability followed by a halt in growth and reduced energy requirements. In some species no seasonal changes are observed (i.e., southern red-backed vole Myodes gapperi [Merritt and Zegers 2002], southern flying squirrel Glaucomys volans [Merritt and Zegers 2002], plateau pikas Ochotona curzoniae [Wang et al. 2006]). However, these studies reported the changes in population means and discussed potential reasons for this phenomenon without giving consideration to the empirical changes on an individual level across seasons. Studies focusing on individual variation were almost exclusively carried out in laboratory or seminatural settings. Increase in BM was observed mainly in larger mammals and was caused primarily by fat storage (e.g., snowshoe hares [Sheriff et al. 2009], Mongolian gerbils [Zhang and Wang 2007], arctic foxes [Fuglei and Ørtisland 1999], gray seals [Sparling et al. 2006], badgers [Harlow 1981]).

For small mammals, Iverson and Turner (1974) focused on a cross seasonal individual reduction of BM in a free-living population and interpreted it as a means of decreasing energy demands, postulated by theoretical assumptions (Ergon et al. 2004). In another study, on tundra voles, Aars and Ims (2002) found mass loss by large individuals and mass gain by small individuals. The authors explained this phenomenon as reaching optimal size for winter conditions. Some studies showed that changes in population mean could be caused by higher mortality of the smallest individuals (red squirrels Tamiasciurus hudsonicus [Laviereé et al. 2010], bank vole Myodes glareolus [Boratyński and Koteja 2009]). The remarkable consistency of weasels’ BM suggests that it is tightly controlled, probably in line with their extreme mode of specialization in burrow hunting for rodents (King 1989; Jędrzejewski et al. 1992). Tight regulation of BM may also be indicative of the long-term individual metabolic fine-tuning to the prevailing environmental conditions. Indeed, observations of radio-collared weasels from the same population suggest very high site fidelity, as observed animals remain in the same type of habitat over long periods (Zub et al. 2008). Heavier individuals are able to compensate for higher food requirements by sticking to habitats with larger prey species than those available to smaller male weasels (Zub et al. 2011).

In contrast, our results indicate a surprising between-seasonal consistency of BM in free-ranging male weasels (figs. 1, 3) across the unusually wide range of weasels’ BM (69–124 g). Individual consistency explained almost 76% of the total variation of BM of male weasels. All males in our study were adults, so taking into account their short expected life span of less than 1 yr (King 1989), we conclude that male weasels maintain stable BM over their whole lifetime.

Our results indicate that weasels did not reduce their food requirements by lowering BM. Thus, the only way they can adjust energy demands to harsh winter conditions is through changes in mass-corrected metabolic rates. RMR constitutes up to 50% of weasels’ daily energy budget (Zub et al. 2011). Here, we found significant between-season differences in RMR (fig.
3). We recently estimated that a reduction of RMR in winter allows weasels to reduce time spent outside shelter to less than 2 h a day (Zub et al. 2011). However, theoretical considerations suggest that larger (heavier) individuals are generally more likely to suffer higher winter mortality due to difficulties associated with finding sufficient food and/or because the additional foraging time would result in an increased exposure to predation (Ergon et al. 2004). In an earlier study (Zub et al. 2011) we demonstrated that although weasels’ winter mortality is high, it is independent of BM. Larger individuals were also able to compensate for higher food requirements by acquiring territories with larger prey species (root vole on meadows) compared with those available to smaller individuals (bank vole in the forest; Zub et al. 2011). Such separation of foraging niches effectively offsets the expected negative association between BM and winter survival predicted from theoretical considerations of energy balance.

The decrease in RMR in winter was observed on a population level in many species in the wild as well as in animals artificially exposed to cold conditions. It was only theoretically discussed as a response to low food quality or quantity or a shift in energy allocation toward reproduction (e.g., Gębczyński 1972; Aalto et al. 1993; McDevitt and Andrews 1995; McDevitt and Speakman 1996; Merritt and Zegers 2002; Arnold et al. 2006; Sparling et al. 2006; Rønning et al. 2008; Sheriff et al. 2009). What is interesting is that such decrease also occurred in seminatural conditions in individuals fed ad lib. (Corp et al. 1997; Fuglei et al. 1999; Arnold et al. 2004; Fuglesteg et al. 2006). Some mammals behave similarly to almost all birds species (for review see McKechnie 2008; Swanson 2010) and increase metabolic rate to support the metabolic demands caused by increased exposure to cold on a population level (Wunder et al. 1977; Meritt and Zegers 2002). Such inconsistent results could probably be explained by differences in species-specific seasonal adaptations. However, only a detailed study on an individual
level could answer the questions about real reasons for pop-
ulation patterns.

Our results indicate that almost one-third of the total pop-
ulation-wide variation of BM-corrected RMR can be attributed
to the individual level. This, along with a considerable consis-
tency (repeatability) of individual RMR, suggests that pop-
luation-wide reduction in BM-corrected winter RMR is in part
due to concerted changes at the individual level rather than
selective mortality and/or emigration of individuals character-
ized by a specific values of BM and RMR. The above conclusion
is derived from the major findings of our study, that the be-
tween-season repeatability of BM-corrected individual RMR
significantly contributes to the observed population patterns of
seasonal changes of metabolic expenditures (fig. 5). Repeat-
ability, which we used as a measure of trait consistency over
time, reflects here the effect of both genetic and environmental
(phenotypic) components (Falconer and Mackay 1996; Dohm
2002). In the case of this study, the repeatability of individual
position with respect to the population mean reflects the lack
of plasticity of BM and to some extent its genetic determination
(Dohm 2002). Likewise, it is probable that the magnitude of
seasonal changes in RMR at the individual level is underlined
by an additive genetic component (Broggi et al. 2009).

Despite an ongoing interest in ecophysiological studies on
BM and metabolic rates, estimates of repeatability are primarily
available for laboratory model rodents or wild species main-
tained under seminatural or laboratory conditions (e.g., Hayes
et al. 1992, 1998; Horák et al. 2002; Labocha et al. 2004). In a
meta-analysis, Nespolo and Franco (2007) found no systematic
differences in the level of repeatability of metabolic rates esti-
-imated in the field and laboratory studies and concluded that
repeatability of metabolic rates is generally fairly high. However,
none of the studies analyzed by Nespolo and Franco (2007)
explicitly tackled between-season repeatability of BM and RMR.
This is important because environmental variation is lower
under laboratory-controlled conditions (Riska et al. 1989),
which is likely to inflate repeatability estimates (Bech et al.
1999). There are only a few articles reporting long-term re-
peatability of BM and RMR in wild mammalian populations
(Szafranska et al. 2007; Boratyński and Koteja 2009; Larivée
et al. 2010; Careau et al. 2013). Larivée et al. (2010) demonstrated
that long-term RMR of overwintering North American squir-
rels was repeatable only in females. Studies of wild chipmunks
demonstrated lack of individual repeatability in mass-corrected
RMR across seasons (Careau et al. 2013). Furthermore, Durate
et al. (2010) recently reported the lack of repeatability of RMR
measured pre- and postreproduction in laboratory mice. These
studies suggest low within-individual consistency of RMR as-
so, a significant consistency (repeatability) of between-season
RMR (corrected for BM) is likely to be less prominent in other
species, as they are likely to adjust their energy budgets through
changes in both BM and energy expenditures. Nevertheless,
our study adds to a few existing long-term studies suggesting
that both BM and BMR/RMR can be a target of natural selec-
tion in free-ranging mammalian populations (see also Hayes
and O’Connor 1999; Boratyński and Koteja 2009; Larivée et al.
2010). Furthermore, our results suggest that the range of met-
abolic responses to prevailing environmental conditions is re-
peatable and characteristic for a given individual. Together with
heritability of BM and RMR per se on the same population of
weasels (Zub et al. 2012), our results suggest several genetic
mechanisms underlying the level of BMR/RMR. Here we dem-
onstrated that changes of physiological states, such as those
incurred by reproduction or overwintering, can be traceable in
the wild. Their consistency merits further much-needed studies
on individual variation of metabolic traits under natural
conditions.

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Literature Cited

1993. Metabolic rates of the bank voles (Clethrionomys glar-
culus) in Europe along a latitudinal gradient from Lapland
Aars J. and R.A. Ims. 2002. Intrinsic and climatic determinants
of population demography: the winter dynamics of tundra
Arnold W., T. Ruf, and R. Kuntz. 2006. Seasonal adjustment of
energy budget in a large wild mammal, the Przewalski
Arnold W., T. Ruf, S. Reimoser, F. Tataruch, K. Onderscheka,
and F. Schober. 2004. Nocturnal hypometabolism as an over-
wintering strategy of red deer (Cervus elephas). Am J Physiol
of basal metabolism in breeding female kittiwakes (Rissa tri-
Badayaev A.V. 2002. Growing apart: an ontogenic perspective
on the evolution of sexual size dimorphism. Trends Ecol Evol
17:369–378.


