

Measuring growth patterns in the field: effects of sampling regime and methods on standardized estimates

J.G.A. Martin and F. Pelletier

Abstract: Although mixed effects models are widely used in ecology and evolution, their application to standardized traits that change within season or across ontogeny remains limited. Mixed models offer a robust way to standardize individual quantitative traits to a common condition such as body mass at a certain point in time (within a year or across ontogeny), or parturition date for a given climatic condition. Currently, however, most researchers use simple linear models to accomplish this task. We use both empirical and simulated data to underline the application of mixed models for standardizing trait values to a common environment for each individual. We show that mixed model standardizations provide more accurate estimates of mass parameters than linear models for all sampling regimes and especially for individuals with few repeated measures. Our simulations and analyses on empirical data both confirm that mixed models provide a better way to standardize trait values for individuals with repeated measurements compared with classical least squares regression. Linear regression should therefore be avoided to adjust or standardize individual measurements

Résumé : Bien qu'on emploie fréquemment les modèles à effets mixtes en écologie et en évolution, leur utilisation avec les traits standardisés qui changent selon la saison ou au cours de l'ontogénie reste limitée. Les modèles mixtes représentent une façon robuste pour standardiser les traits quantitatifs individuels en fonction d'une condition commune telle que la masse corporelle à un moment donné dans le temps (au cours de l'année ou durant l'ontogénèse) ou la date de mise bas dans une condition climatique donnée. Actuellement, la plupart des chercheurs utilisent des modèles linéaires simples pour accomplir cette tâche. Nous utilisons à la fois des données empiriques et des données simulées pour souligner l'utilisation des modèles mixtes pour standardiser les valeurs des traits en fonction d'un environnement commun pour chaque individu. Nous montrons que les standardisations faites par les modèles mixtes fournissent des estimations plus précises des paramètres de masse que les modèles linéaires pour tous les régimes d'échantillonnage et spécialement pour les individus pour lesquels il existe peu de mesures répétées. Nos simulations et nos analyses de données empiriques confirment toutes les deux que les modèles mixtes représentent une meilleure façon de standardiser les valeurs des traits chez les individus pour lesquels il existe des mesures répétées, par comparaison aux régressions classiques des moindres carrés. On devrait donc éviter d'utiliser la régression linéaire pour ajuster ou standardiser les mesures individuelles.

[Traduit par la Rédaction]

Introduction

Quantitative measurements of biological traits, such as body mass or size, are critical in many studies of the ecology and evolution of wild vertebrates. Generally, it is impracticable to measure all individuals at the same time or in the same condition (such as age). Consequently, all traits with a growing pattern over a season or during ontogeny cannot be used in analyses without accounting for that temporal variation. Standardizing trait values for a specific age or time period is consequently inevitable for a wide range of taxa (e.g., fishes (Freitas et al. 2006), reptiles (Blouin-Demers et al. 2005), mammals (Côté et al. 1998)). Typi-

cally, two different approaches are used to standardize morphological measurements. If a single measure per animal is available, a least squares model for the entire sample can be used to standardize individual trait values to a particular date or age (Clutton-Brock et al. 1982; Zedrosser et al. 2007). When repeated measurements of the same individual are available over time, researchers often use a classical least squares model for each individual to predict the individual value of the trait at a given point in time (Festa-Bianchet et al. 1996). Alternatively, when individuals have few repeated measures, the mean residuals deviation from the population trend for each individual can also be used (Beauplet and Guinet 2007).

Received 12 July 2010. Accepted 9 February 2011. Published at www.nrcresearchpress.com/cjz on 26 May 2011.

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Mixed effects models allow one to use repeated measurements on the same individual, by including one or more random terms such as identity of an animal, to control for interindividual variation on parameter estimates (Pinheiro and Bates 2000). These models are commonly used in ecological and evolutionary studies as an analytical tool to control for “pseudoreplication” (sensu Hurlbert 1984) or to estimate individual variation in phenotypic plasticity (Kruuk 2004; Nussey et al. 2007; Bolker et al. 2009). However, fewer studies have used these models to standardize individual trait values (McRoberts et al. 1998; Bridges et al. 2002; Schaalje et al. 2002; Pelletier et al. 2007; Feder et al. 2008). In this article, we aim to emphasize that mixed effects models provide a more accurate approach to standardizing trait values (for example, to get age-specific body mass for each individual) compared with fitting separate least squares regressions for each individual. The advantage of linear mixed models is the use of BLUPs (best linear unbiased predictors), which are a weighted average that combine information from individuals with information from the population (Pinheiro and Bates 2000). The BLUP borrows information from other individuals in the population and shrinks the predictions toward the overall population mean. The amount of shrinkage is greater when between subject variance is small, within subject variance is high, or when the number of observations for an individual (n_i) is small (Pinheiro and Bates 2000). The shrinkage property is appealing when there is little information for a particular individual (small n_i), when individuals are similar in their growth patterns, or when measurement error is large relative to among-individual variation. The BLUPs benefit from increased precision, resulting in values that should have a smaller mean-squared error than multiple regression estimates.

To illustrate the use of mixed models to standardize traits at a given point in time, we used both an empirical and a simulation approach. First, we used body-mass data from a marked population of bighorn sheep (*Ovis canadensis* Shaw, 1804) at Ram Mountain, Alberta. Then, we simulated seasonal mass variation (intercept and slope) for 40 000 individuals under varying conditions. The mixed model method can be applied to any other quantitative measurements (including horn or bone length (Bonenfant et al. 2009), body size (McRoberts et al. 1998), parturition date (Nussey et al. 2005) or exploration (Martin and Réale 2008)) for which a predictable change is required to standardize individual trait values at a specific condition (e.g., date, age, or environmental condition). To evaluate the effect of sampling regime and methods on trait estimation, we estimate slope (rate of mass gain) and intercept (mass at day 0) for each individual with both linear mixed models (hereafter LMM) and least squares regressions (hereafter LM) and compared the accuracy (i.e., closeness of a measure to its true value; sensu Sokal and Rohlf 1995) of both methods for different numbers of repeated measures per individual. Furthermore, covariates that vary between but not within individual could be included in LMM but could not be fitted using a LM approach. To explore impact of such fixed effects on LMM accuracy, we simulated an age effect and we compared the accuracy of LMM models fitted with and without age as a covariate.

Methods

LM and LMM models

When standardizing traits using a multiple least squares regression approach (LM), one needs to fit a regression for each individual, noted s , following the model:

$$Y_{sj} = \mu_s + \beta_s X_{sj} + e_{sj}$$

where μ_s and β_s are the least squares estimates of intercept and slope for the individual s , X is the environmental covariate for which the trait should be standardized, and e is a residual that is assumed to be uncorrelated across observations and normally distributed with a mean of zero and variance σ_e^2 . This model assumes that σ_e^2 is known. However, because it is not known, an estimate is used in its place. If there are n individuals for which traits should be standardized, then n regressions should be fitted.

Using a linear mixed model (LMM) approach, one needs to fit a model over all individuals but specifying individual identity (individual intercept) and an interaction between individual and the covariate (individual slope) as random effects. Such model can be described by the following equation:

$$Y_{ij} = \mu + \delta_i + (\beta + \Delta_i)X_{ij} + \varepsilon_{ij}$$

where μ and β are the population average intercept and slope, respectively, whereas δ_i and Δ_i are the deviations from those population averages for an individual i . δ_i and Δ_i have means of zero and a covariance structure defined by a matrix, Ψ . Ψ is a 2×2 matrix with σ_δ^2 , the intergroup variance in intercepts, and σ_Δ^2 , the intergroup variance in slopes, on the diagonal and $\sigma_{(\delta,\Delta)}$, the covariance between intercept and slope as the off-diagonal element. For clarity, we refer to the estimators of these elements as V_I , V_S , and $\text{cov}(I,S)$, respectively. ε is a residual that is assumed to be uncorrelated across observations and normally distributed with a mean of zero and variance V_R . The model assumes V_R to be known, but an estimate is used in its place.

Empirical data

At Ram Mountain, Alberta, bighorn sheep have been baited with salt and caught repeatedly in a corral trap from May to September since 1971 (Festa-Bianchet et al. 1996). At first capture, as lamb or yearlings, each sheep was marked with plastic ear tags or visual collars. Consequently, >95% of individuals were of known age and recognizable each year. Body-mass measurements (kg) were recorded at each capture using a Detecto spring scale to the nearest 0.125 kg. More details can be found in Festa-Bianchet et al. (1996). Capture success varies with sex–age class, but approximately 85% of the sheep are caught at least once and most (>75%) are caught from two to nine times each summer. For adult sheep, mass gain during summer is asymptotic and the growth curve can be linearized using the square root of date with 25 May as day 1 (Festa-Bianchet et al. 1996). Trapping and handling protocols for bighorn sheep were approved by the Université de Sherbrooke Animal Care Committee (protocol FP 2008-01), which adheres to the guidelines of the Canadian Council on Animal Care.

We randomly selected 50 different adult females for which

Table 1. Comparison of individual estimates of intercepts (mass at day 0) and slopes (growth rate) for 50 female bighorn sheep (*Ovis canadensis*) that were weighed five times within one summer at Ram Mountain, Canada.

	LM			LMM			Mean Δ MSE (95%CI)
	R^2	P	β (SE)	R^2	P	β (SE)	
Intercepts							
$N = 5$	0.99	<0.001	1.05 (0.01)	—	—	—	—
$N = 4$	0.95	<0.001	1.05 (0.03)	0.97	<0.001	0.97 (0.02)	-0.42 (-0.76 to -0.12)
$N = 3$	0.83	<0.001	1.04 (0.06)	0.92	<0.001	0.95 (0.04)	-1.38 (-3.44 to -0.14)
$N = 2$	0.36	<0.001	0.98 (0.22)	0.82	<0.001	0.91 (0.06)	-19.54 (-61.02 to -1.96)
Slopes							
$N = 5$	0.99	<0.001	1.29 (0.02)	—	—	—	—
$N = 4$	0.87	<0.001	1.27 (0.07)	0.92	<0.001	0.88 (0.04)	-2.15 (-3.73 to -0.34)
$N = 3$	0.62	<0.001	1.20 (0.13)	0.70	<0.001	0.72 (0.06)	-7.82 (-14.84 to -1.75)
$N = 2$	0.16	<0.001	1.06 (0.47)	0.34	<0.001	0.50 (0.09)	-146.8 (-846.7 to -12.15)

Note: Intercept and slope estimates were calculated with a linear model (LM) or a linear mixed model (LMM). We compared the estimates obtained using a LM with 5, 4, 3, and 2 values per individual against the estimates obtained using a LMM including all 5 values. R^2 , P , β , and SE values reported for models with 2, 3, and 4 values per individual are mean estimates over 100 simulations. Mean Δ MSE is the mean difference in MSE of LMM and LM estimates over all the simulations for mass adjusted 15 September. Negative Δ MSE value indicate that LMM are more accurate. 95%CI of Δ MSE were estimated as 2.5% and 97.5% quantiles of the distribution of Δ MSE over all the simulations.

we have collected 5 body-mass measures within the same field season. We estimated individual slope (the daily rate of mass gain) and intercept (mass at day 0) for each ewe using individual LM with mass as a dependent variable and square root of date of measurement as an independent variable. We also estimated each individual slope and intercept using a LMM. We fitted body mass as a function of the square root of the date as a fixed factor and female identity, as well as an interaction between identity and square root of date, as random effects. Correlation between estimates obtained with both methods based on 5 measurements was nearly perfect ($N = 50$, $r = 0.999$) for both intercept and slope estimates. Because we did not have true values of slopes and intercepts, we considered estimates obtained with 5 measurements as “reference values” to estimate accuracy of estimates obtained with a smaller number of measurements per individual.

To explore how sampling regime may affect estimates obtained from LM and LMM, we randomly selected 4 mass measurements for each of the 50 ewes that we considered in the previous analysis. We estimated the slope and intercept for each individual in this new set of data using LM and LMM models. We used the slope (mass gain rate) and intercept (mass at day 0) obtained by each of the models to standardize mass on 5 June (day 12) and 15 September (day 114). Using linear regression, we then compared slopes, intercepts, and standardized mass calculated by both methods to “reference values”. We then estimated the mean-squared error (MSE) for each method to predict individual intercept, slope, and mass at day 114. MSE is calculated as the mean of the squared difference between predicted and true values. A smaller MSE value indicates a higher accuracy. A negative difference in MSE between LM and LMM (Δ MSE = $\text{MSE}_{\text{LMM}} - \text{MSE}_{\text{LM}}$) is interpreted as a better accuracy by the LMM method than the LM. To decrease the potential bias in estimates owing to random sampling of the subsample data set, we repeated these steps 100 times and reported mean estimates of slopes, R^2 , and P values over all the simulations (see Table 1). Finally, we repeated the whole procedure by randomly selecting first 3 and then 2 mass

measurements of mass per ewe to see how the estimates changed according to the individual sampling regime.

Simulated data

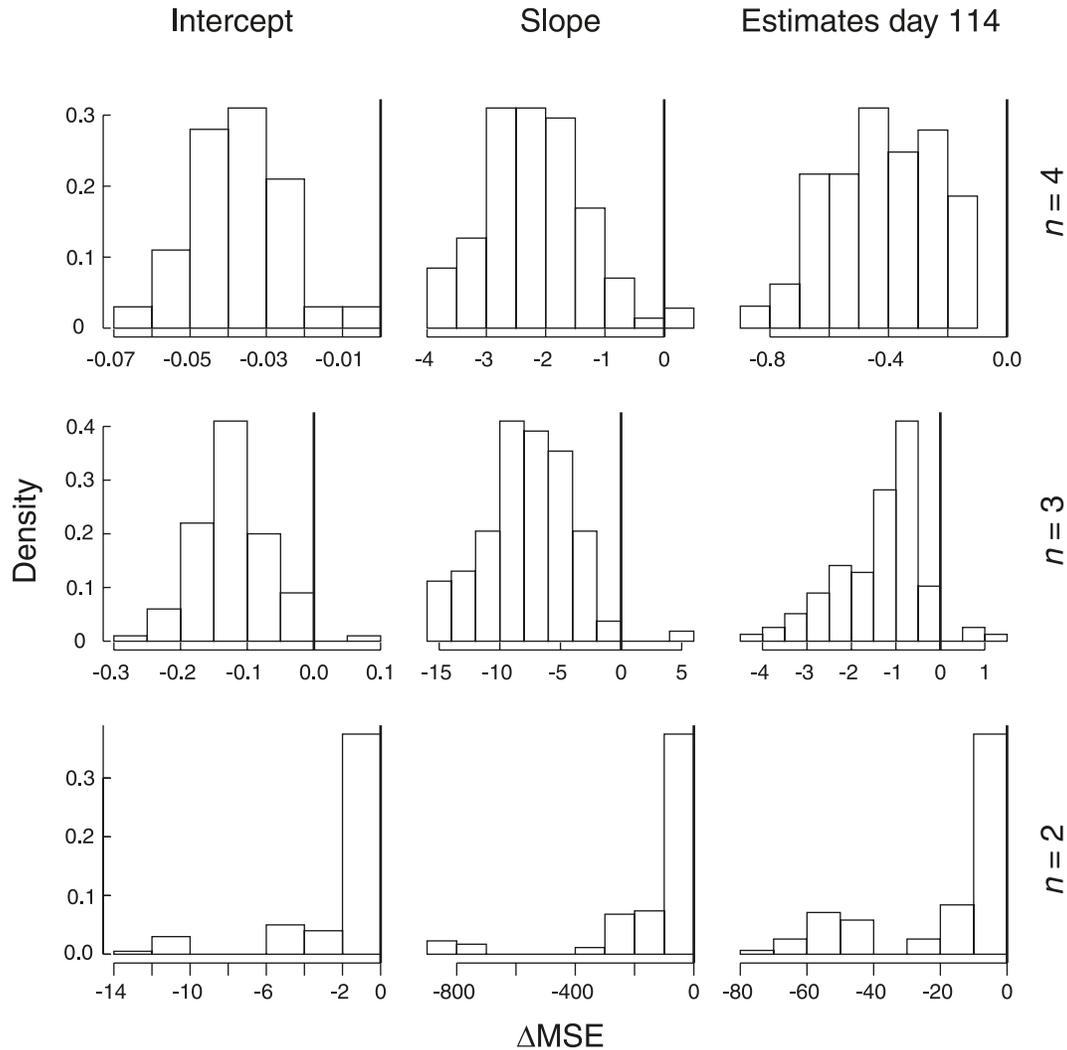
Following the pattern of variation in sheep mass, we simulated summer mass variation as a linear relation with the square root of date considering 24 May as day 0. We considered two age categories (juvenile and adult) and simulated data assuming that adults were twice as big at day 0 and gained mass twice as fast compared with juvenile individuals. We used 50 kg and 0.8 kg-day^{-0.5} as the mean intercept (μ) and slope (β), respectively, for juveniles. We considered that individuals were measured from 24 May (day 0) to 1 October (day 130). For 20 individuals, we assigned age randomly (age_{*i*}, coded as 1 for juveniles and 2 for adults) and we drew random intercepts (δ_i) and slopes (Δ_i) from normal distributions with means of zero and variance components V_I , V_S , and $\text{cov}(I,S)$. Based on these parameters for individuals, we simulated between 2 and 5 mass measures (Y) for each individual following:

$$Y_{ij} = \mu_{\text{age } i} + \delta_i + (\beta_{\text{age } i} + \Delta_i)X_{ij} + \varepsilon_{ij}$$

where $\mu_{\text{age } i} = 50 \cdot \text{age}_i$ and $\beta_{\text{age } i} = 0.8 \cdot \text{age}_i$ are the age-specific mean intercept and slope, respectively. ε is the within-subject error drawn from a normal distribution with mean zero and variance V_R . X is the date (square root transformed) of measures drawn from a uniform distribution between 0 and 130.

Using this data set ($N = 60$ observations from 20 individuals with 2–5 repeated measures), we estimated intercept (mass at day 0) and slope (the daily rate of mass gain) using LMs with mass as a dependent variable and square root of date of measurement as an independent variable. We also estimated each individual slope and intercept using a LMM for all individuals. We fitted body mass as a function of the square root of the date as a fixed factor and individual identity (random intercept), as well as an interaction between identity and square root of date (random slope), as random effects. We then estimated the Δ MSE between the two meth-

Fig. 1. Difference in mean square error (Δ MSE) between linear mixed model (LMM) and linear regression (LM) estimates as a function of the number of observations per individuals based on subsampling of the data set of bighorn sheep (*Ovis canadensis*). Black vertical bars represent the equivalence between LMM and LM estimates (Δ MSE = 0). Note that negative values represent a better fit of LMM than LM. Δ MSE were estimated for 100 simulated models for each sampling size.



ods to predict individual intercept, slope, and mass at day 114. To explore how sampling regime may affect estimates obtained from LM and LMM, we estimate Δ MSE using only individuals with 5, 4, 3, or 2 measures. The whole procedure was then repeated 500 times (i.e., 10 000 simulated individuals). To assess the differences in LMM vs. LM performance to predicted true values, we plotted Δ MSE distribution over all the 500 simulations and estimated R^2 of the regression of predicted values on estimated values for both methods over all the 10 000 simulated individuals.

To evaluate the impact of including other covariates in the mixed model, we fitted another mixed model of mass in which we added age and age \times date interaction as fixed effects. We then compared MSE of LMMs both with and without those covariates over all the 500 simulations.

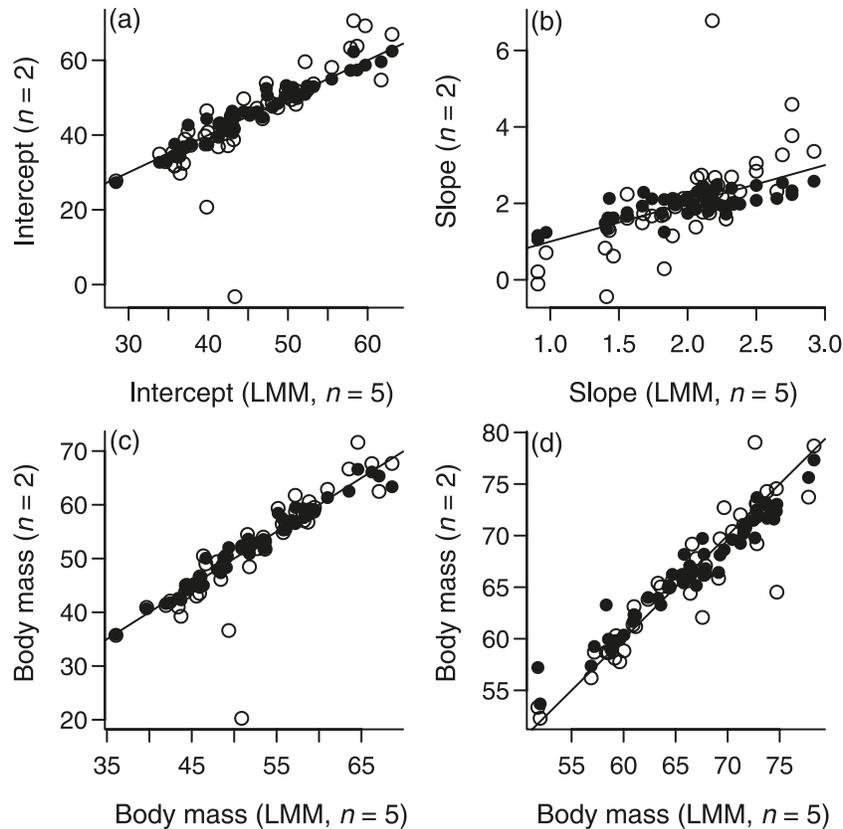
To explore a wide range of situations, simulations were done for four different covariance structures: (1) large inter-individual variance in both intercept and slope ($V_I = 45$, $V_S = 2$, and $V_R = 5$; the variance partition coefficient (VPC; Goldstein et al. 2002; Martin et al. 2011) ranges between 0.90

and 0.98 for dates between day 0 and day 114) corresponding to the actual structure of the sheep data at Ram Mountain; (2) large intercept variance and small slope variance ($V_I = 45$, $V_S = 0.1$, and $V_R = 5$; VPC range = 0.90–0.91); (3) small intercept variance and large slope variance ($V_I = 5$, $V_S = 2$, and $V_R = 45$; VPC range = 0.10–0.83); (4) small variance in both intercept and slope ($V_I = 5$, $V_S = 0.1$, and $V_R = 45$; VPC range = 0.10–0.26). In all cases, we simulated the covariance between intercept and slope ($\text{cov}(I,S)$) as zero. V_I and V_R are expressed in kg^2 but V_S is in $\text{kg}^2 \cdot \text{day}^{-1}$ and thus are not directly comparable. The VPC is an environment-specific repeatability calculated as

$$\text{VPC} = \frac{[V_I + X\text{cov}(I,S) + X^2V_S]}{[V_I + X\text{cov}(I,S) + X^2V_S + V_R]}$$

When $V_S = 0$, VPC is equivalent to the standard estimate of repeatability (Martin et al. 2011). VPC could be estimated for a given value of X (square root of date in our simulations), and thus corresponds to the repeatability of the traits for a specific

Fig. 2. Comparison of parameters and body-mass estimations of female bighorn sheep (*Ovis canadensis*) obtained with a mixed model using 5 mass measurements per individual and linear regression (LM; ○) or linear mixed models (LMMs; ●) using only two values per individual. (a) Intercepts (LM: $R^2 = 0.62$, $P < 0.001$; LMM: $R^2 = 0.94$, $P < 0.001$). (b) Slopes (LM: $R^2 = 0.50$, $P < 0.001$; LMM: $R^2 = 0.56$, $P < 0.001$). (c) Body mass adjusted to 5 June (LM: $R^2 = 0.71$, $P < 0.001$; LMM: $R^2 = 0.92$, $P < 0.001$). (d) Body mass adjusted to 15 September (LM: $R^2 = 0.86$, $P < 0.001$; LMM: $R^2 = 0.95$, $P < 0.001$). The line represents a hypothetical correlation of 1. Results from only one simulation are represented. The mass data were obtained from repeated captures of 50 female bighorn sheep at Ram Mountain, Canada.



date being equivalent to the standard repeatability when $V_S = 0$. All models were fitted using R version 2.9.1 (R Development Core Team 2009) and LMMs were fitted with the “lmer” function in “lme4”, a package used to fit mixed models in R (Bates et al. 2008). The “lmer” function allows one to fit a generalized linear mixed model with additive random effects using a restricted maximum likelihood optimization, and it assumes that the covariance matrix is positive definite and that the residual variance is homogeneous. All mixed models were assessed with an unstructured covariance matrix.

Results

Empirical data

As expected, estimates obtained from LMM were more accurate than estimates from LM for all sampling regime, and these differences increased with decreasing individual sampling regime (Table 1, Fig. 1). Accuracy of estimates decreased with the decreasing number of values and was lowest when individuals have only two values (Table 1). Estimates of intercept and slope with LM were more influenced by outliers than those obtained with LMM (Figs. 2a–2d).

Simulated data

For simulations with large interindividual variance in both intercept and slope, LMM provided more accurate estimates

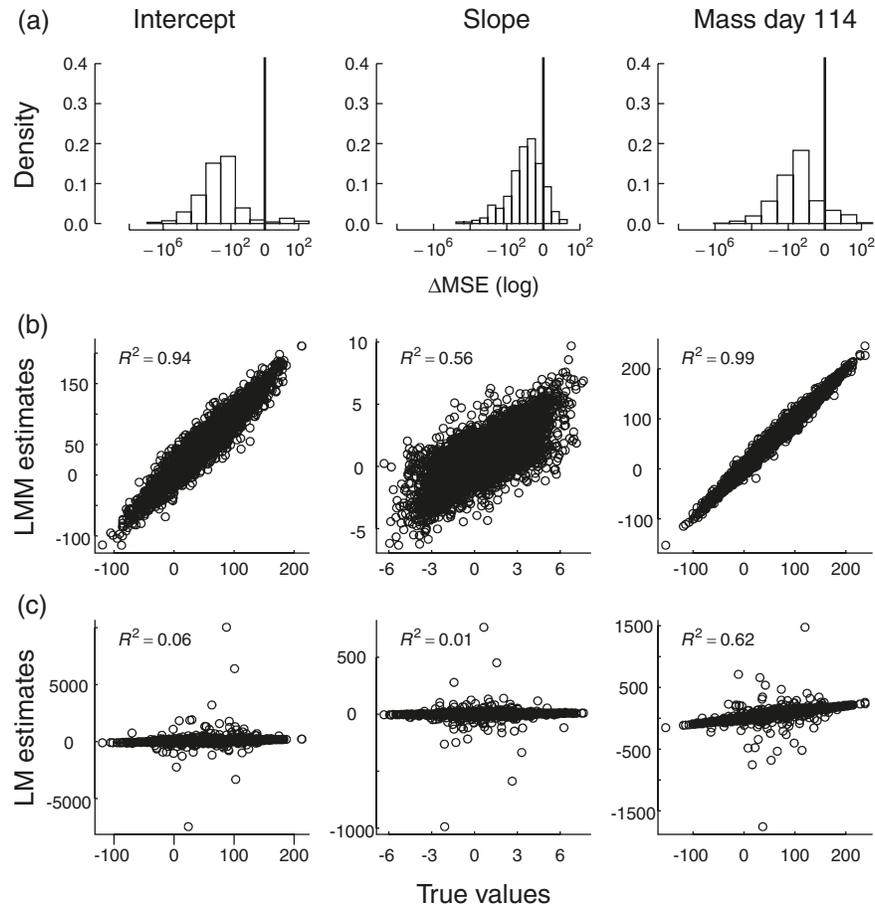
than LM over all the simulations (negative ΔMSE for intercept, slope, and estimated mass; Fig. 3a). Estimates obtained from LMM were always within ranges of true values, whereas LM estimates were more dispersed resulting in more and larger outliers (Figs. 3b, 3c). LM and LMM perform similarly for individuals with 5 measures (Fig. 4); however, estimates from LMM were more accurate for all other sampling regimes and these differences increased with decreasing individual sampling regime (Fig. 4). Including age and an interaction between age and date in the LMM did not increase accuracy of individual estimates (mean difference in MSE between model without and with age included (with 2.5 and 97.5 quantiles)—intercept: -0.03 (-1.20 to 0.83); slope: -2.03 (-88.7 to 57.1); adjusted mass to September: -0.22 (-17.09 to 13.59)).

In the three other simulations (large V_I and small V_S ; small V_I and large V_S ; small V_I and small V_S), ΔMSE between LMM and LM were negative in all situations indicating that estimates obtained with LMM were more accurate than those obtained with LM. The distribution of ΔMSE between models with and without age as a covariate always included zero in their 95% range.

Discussion

In this article, we underline a simple and robust approach

Fig. 3. Difference between standardization from a linear mixed model (LMM) versus linear regression (LM) of body mass from simulated data. (a) Difference in mean square error (Δ MSE) between LMM and LM. Black vertical bars represent equivalence between LMM and LM estimates (Δ MSE = 0). Note that negative values represent better fit of LMM than LM. Plots represent the relation between true (simulated) values and (b) estimates from LMM, and between true (simulated) values and (c) estimates from LM. All plots are based on 500 simulations corresponding to 8000 individuals with 2–5 observations with a large intercept and slope variance.



to standardizing trait values using mixed models that has been rarely used in ecology for this purpose. Our simulations and analyses on empirical data both indicate that mixed models provide individual estimate with higher accuracy than least square regression. This confirms that mixed models provide a better way to standardize trait values for individuals with repeated measurements compared with classical least squares regression. In a simple linear case, when there are 5 or more data points per individual, both linear regression and linear mixed model perform similarly. However, when data sets contain individuals with fewer observations (2–3) per individual, as is often the case in field studies, a linear mixed model should be used for standardizing trait values.

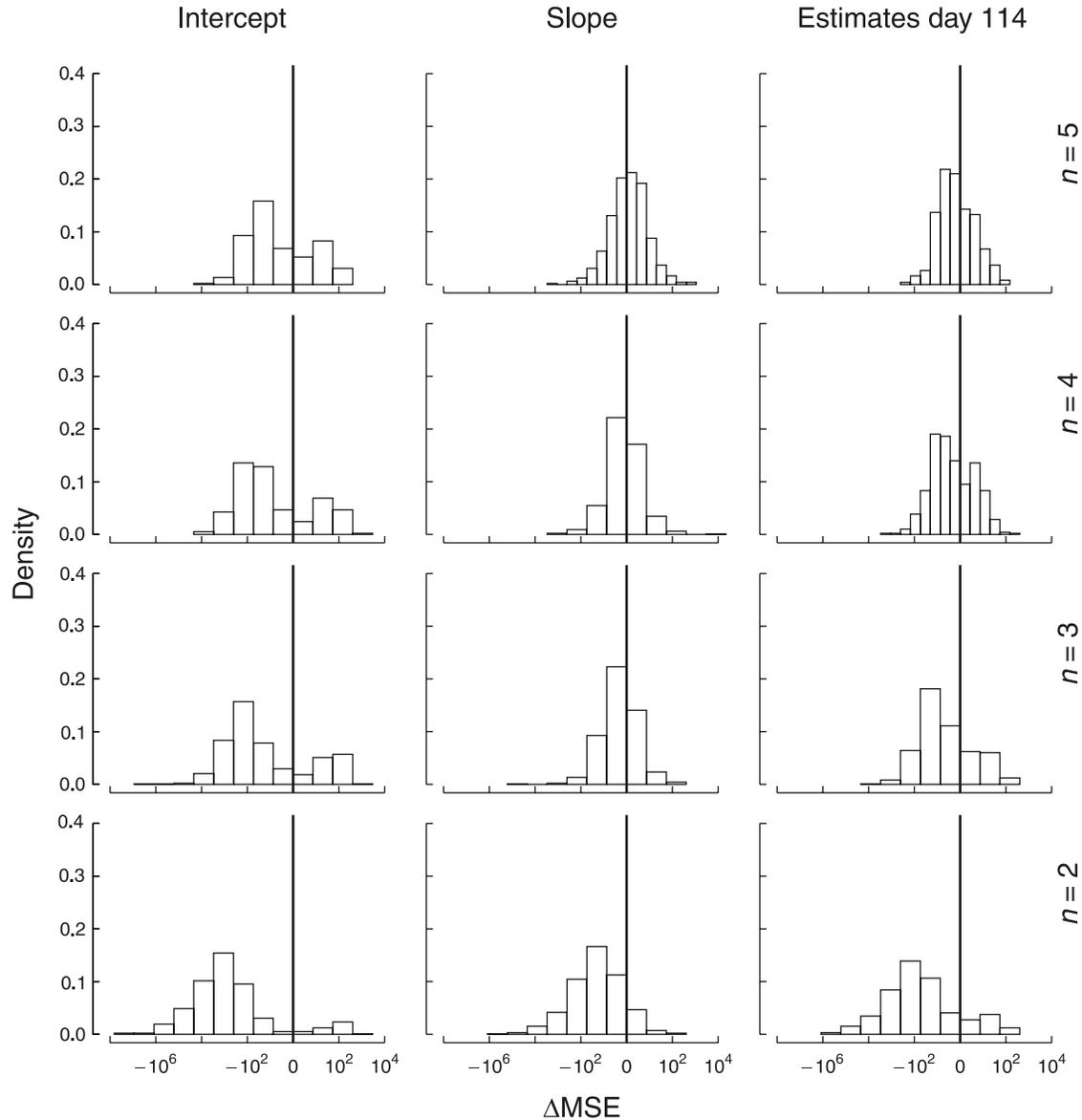
Our simulations were conducted assuming no correlation between intercept and slope, which represents the worse case scenario in terms of estimation accuracy of variance components (Martin et al. 2011). Thus, our results are very conservative. When individual intercept and slope are correlated, components of variance and thus BLUPs are more accurate. However, the presence of such correlation does not affect estimation with LM method. Thus, if the intercept–slope correlation is non-null, LMM will also outperform LM for trait standardization.

For the analyses of both empirical and simulated data, we

have applied linear mixed models with a homogeneous residual variance. In certain situations, however, using a more complex covariance structure might be needed. For example, if within individual residuals are correlated, then an autoregressive correlation structure might be appropriate to obtain an adequate fit of the model (Pineiro and Bates 2000). If the primary interest is to estimate intercept and slope effects, the LMM with an unstructured variance should provide acceptable estimates of variance even if a more complex variance structure actually holds (Lange and Laird 1989). However, we urge researchers to properly evaluate the covariance structure and fit the model as adequately as possible to increase the accuracy of their estimates.

Adding other covariates as fixed effects in the LMM has the potential to increase the accuracy of individual estimates. In our simulations, we considered an age effect that varied among individuals but not within. Our results clearly showed that including fixed effects which varied only among individuals did not increase the accuracy of individual estimates. In a mixed model, or hierarchical model with individual as a random effect, including fixed effects that vary only among individuals does not affect the residual variance but does affect the individual variance (Pineiro and Bates 2000). To increase accuracy of individual estimates, fixed effects included

Fig. 4. Distribution of the difference in mean square error (Δ MSE) between linear mixed model (LMM) and linear regression (LM) estimates as a function of the number of observations per simulated individual. Black vertical bars represent equivalence between LMM and LM estimates (Δ MSE = 0). Note that negative values represent a better fit of LMM than LM. Δ MSE were estimated for 500 models corresponding to 2000 simulated individuals with a large intercept and slope variance for each sampling size.



in the model used for the standardization should decrease residual variance. Thus, only fixed effects that vary within individuals would increase accuracy of individual estimates. However, if variance components are estimated with a large uncertainty—because of small sample size or limited number of individuals—including fixed effects varying only among individuals might increase estimates accuracy of estimates by decreasing variance associated with the individual random effects. Our simulations were based on an average of 80 observations for 20 individuals, which is a reasonable sample size allowing a good estimation of components of variance of the model. With smaller sample sizes or if individuals have few repeated observations (2–3), using a Bayesian approach to fit a LMM might also be useful because additional information could be provided to the model through the use of informative priors.

Although standardization is often inevitable, it also has limitations. It is generally encouraged to avoid standardizing a response variable and to prefer a multivariate analysis without need to standardize variables (Hadfield et al. 2010). Indeed, using standardized traits as dependent variables in subsequent analysis is associated with the so-called “stats on stats” problem, i.e., cumulating statistical uncertainty through successive models, because trait standardization includes a quite large uncertainty around the estimate of each individual (e.g., Hadfield et al. 2010). Furthermore, standardization methods make different statistical assumptions, such as independence of data between individuals, that might be violated (e.g., related individuals) and could be problematic in subsequent analysis (e.g., quantitative genetics). However, when a multivariate analysis cannot be done, mixed model standardization should be preferred over the classical linear regression.

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In this article, we emphasized the importance of using linear mixed models to standardize traits at a given point in time. This approach can be easily extended to any plastic trait with a predictable relationship to another variable that a researcher seeks to standardize (e.g., standardization of size for age (McRoberts et al. 1998), parturition date for climate (Nussey et al. 2005), laying date for temperature (Brommer et al. 2005), and exploration for number of tests (Martin and Réale 2008)). Despite some potential problems with BLUPs estimation within mixed models (Hadfield et al. 2010), the mixed model approach should be preferred over other methods when standardizing a trait with repeated measurements. We therefore encourage ecologists to use this approach instead of the more classical least regression methods.

Acknowledgements

We thank Marco Festa-Bianchet for his help and support and for providing access to the Ram Mountain data set. We also thank Denis Réale and Dany Garant for providing comments on an earlier draft. Financial support was provided by the Natural Sciences and Engineering Research Council of Canada through a Discovery Grant to F.P. and a scholarship to J.M. F.P. holds the Canada Research Chair in Evolutionary Demography and Conservation.

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