

Assessing the robustness of quantitative fatty acid signature analysis to assumption violations

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Summary

1. Knowledge of animal diets can provide important insights into life history and ecology, relationships among species in a community and potential response to ecosystem change or perturbation. Quantitative fatty acid signature analysis (QFASA) is a method of estimating diets from data on the composition, or signature, of fatty acids stored in adipose tissue. Given data on signatures of potential prey, a predator diet is estimated by minimizing the distance between its signature and a mixture of prey signatures. Calibration coefficients, constants derived from feeding trials, are used to account for differential metabolism of individual fatty acids. QFASA has been widely applied since its introduction and several variants of the original estimator have appeared in the literature. However, work to compare the statistical properties of QFASA estimators has been limited.

2. One important characteristic of an estimator is its robustness to violations of model assumptions. The primary assumptions of QFASA are that prey signature data contain representatives of all prey types consumed and the calibration coefficients are known without error. We investigated the robustness of two QFASA estimators to a range of violations of these assumptions using computer simulation and recorded the resulting bias in diet estimates.

3. We found that the Aitchison distance measure was most robust to errors in the calibration coefficients. Conversely, the Kullback–Leibler distance measure was most robust to the consumption of prey without representation in the prey signature data.

4. In most QFASA applications, investigators will generally have some knowledge of the prey available to predators and be able to assess the completeness of prey signature data and sample additional prey as necessary. Conversely, because calibration coefficients are derived from feeding trials with captive animals and their values may be sensitive to consumer physiology and nutritional status, their applicability to free-ranging animals is difficult to establish. We therefore recommend that investigators first make any improvements to the prey signature data that seem warranted and then base estimation on the Aitchison distance measure, as it appears to minimize risk from violations of the assumption that is most difficult to verify.

Key-words: Aitchison, bias, diet composition, diet estimation, Kullback–Leibler, QFASA, simulation

Introduction

Quantitative fatty acid signature analysis (QFASA) has become a common method of estimating animal diet composition (Iverson *et al.* 2004). Ecologists studying marine species have implemented the method most widely (Bowen & Iverson 2013), likely due to the structural diversity of fatty acids in marine ecosystems (Thiemann *et al.* 2007) and the importance of lipids for energy storage in marine food webs. QFASA requires data on the fatty acid composition, proportions that sum to 1.0 collectively termed a signature, of all prey types potentially consumed by a predator. A predator signature is then modelled as a linear mixture of the prey signatures. The mixture proportions that minimize a measure of distance

between observed and modelled predator signatures are an estimate of diet composition. Constants termed calibration coefficients, which are derived from feeding trials, are used to account for the differential metabolism of individual fatty acids by a predator.

The QFASA model is based on two primary assumptions (Iverson *et al.* 2004). The first is that the prey signature data contain representatives of all prey types consumed by a predator. The degree to which this assumption is satisfied will depend on such factors as prior knowledge of prey types likely to be consumed, the diversity of prey available, the degree to which a predator is a specialist or generalist, and within-species heterogeneity (temporal, spatial, between age classes, etc.) in prey signatures. Therefore, this assumption ultimately can be satisfied by adequately sampling prey and grouping them into suitable categories to minimize heterogeneity within prey types

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and maximize heterogeneity between prey types. The second assumption is that the calibration coefficients are known without error. The validity of this assumption is conceptually impossible to establish in most investigations. Feeding trials have not been conducted for many predator species, in which case investigators must use calibration coefficients developed for a surrogate species (e.g. Thiemann, Iverson & Stirling 2008; Meynier *et al.* 2010; Haynes *et al.* 2015). Even if a feeding trial has been conducted with the subject species, characteristics of its experimental design, including the experimental diet and the length of time a diet was fed, could limit the applicability of the resulting calibration coefficients for investigations of free-ranging predators (Budge, Penney & Lall 2012; Rosen & Tollit 2012). Furthermore, the metabolism of free-ranging predators may differ from that of captive individuals.

Variants of the QFASA estimator may have differential robustness to assumption violations. For example, in an investigation comparing the statistical properties of six QFASA estimators, Bromaghin *et al.* (2015) observed substantial differences between estimates of mean diet for Chukchi Sea polar bears (*Ursus maritimus*) and hypothesized that the underlying cause was differential robustness to assumption violations. However, the degree to which assumption violations may bias diet estimates has not been studied in a comprehensive manner. Iverson *et al.* (2004) performed limited simulations to investigate how diet estimates were affected by the consumption of prey not included in the prey signature data, which we term 'ghost' prey. However, we are unaware of any systematic investigation of estimator robustness to errors in calibration coefficients, although several investigators have reported that diet estimates can be sensitive to the selection of calibration coefficients (e.g. Meynier *et al.* 2010; Wang, Hollmén & Iverson 2010; Budge, Penney & Lall 2012; Rosen & Tollit 2012; Haynes *et al.* 2015).

We explored the robustness of QFASA estimators to violations of model assumptions using computer simulation. The relative contribution of a ghost prey type to predator diets and the magnitude of error in calibration coefficients were jointly varied and the resultant bias of estimators based on the Kullback–Leibler and Aitchison distance measures, the two measures most commonly used (e.g. Iverson *et al.* 2004; Stewart & Field 2011; Bromaghin *et al.* 2015), was observed. Simulations were conducted with two prey signature data sets having differing complexity to enhance the general applicability of our results.

Materials and methods

DATA INPUTS

We used two prey signature data sets of differing complexity to construct predator signatures, given specified diet mixtures of the prey, and subsequently estimated diets. The marine mammal data set, previously used to estimate the diets of polar bears in the Chukchi Sea (Rode *et al.* 2014), contained signatures from 357 prey representing 7 species. The second prey data set, previously used to estimate the diet of grey seals (*Halichoerus grypus*; Beck *et al.* 2007), was considerably more complex,

containing signatures from 957 prey representing 29 species. Bromaghin *et al.* (2015) used both of these data sets in their simulations to compare QFASA estimators (see their tables S1 and S2 for details regarding species and sample sizes).

We wanted to evaluate estimator robustness to assumption violations using diets that were biologically plausible for each prey data set. For that reason, we used the same diet mixtures for adult female and adult male polar bears and spring-sampled female and male grey seals that were used by Bromaghin *et al.* (2015). Those diets were approximated from published estimates, Rode *et al.* (2014) for polar bears and Beck *et al.* (2007) for grey seals, by eliminating some prey with minor contributions to diets and rounding diet proportions to three decimals (tables S1 and S2 of Bromaghin *et al.* (2015)).

Fatty acid signatures were based on the 41 fatty acids in the extended dietary set (Appendix A of Iverson *et al.* (2004)), which are commonly used in QFASA investigations (e.g. Wang, Hollmén & Iverson 2010; Haynes *et al.* 2015). Prey signatures for this set of fatty acids were scaled so that their proportions summed to 1. Because the Kullback–Leibler and Aitchison distance measures are not defined for proportions of 0, values of 0 were replaced with $1 \cdot 0 \cdot e^{-5}$ and the signature proportions were rescaled to sum to 1 using the multiplicative method (Martín-Fernández, Palarea-Albaladejo & Olea 2011).

GHOST PREY SIGNATURES

A ghost prey signature was established to be somewhat distinct from the other prey types, but not so different that it shared none of the characteristics of the other prey signatures. For each prey data set, we computed the mean signature for each prey type (species) and established box constraints for each fatty acid proportion as 75% of the minimum proportion and 125% of the maximum proportion observed among prey types. The signature that maximized the Kullback–Leibler distance between itself and the mean prey signatures, subject to being within the box constraints, was obtained using the `solnp` function of the R package `Rsolnp` (Ghalanos & Theussl 2014). The signature that maximized the Aitchison distance between itself and the prey signatures was similarly obtained. The ghost prey signature was established as the average of these two signatures. This method produced a ghost prey signature as different as possible from the prey signature data overall, as jointly measured by the Kullback–Leibler and Aitchison distances, but within the limits formed by the box constraints.

CALIBRATION COEFFICIENT ERROR

We used the 'all mink' calibration coefficients of Thiemann, Iverson & Stirling (2008) as the 'true' values used to construct predator signatures (details below), multiplicatively scaled to sum to the number of fatty acids (41), while 'hypothesized' calibration coefficients incorporating various levels of error were used to estimate diets. Given the true values of the calibration coefficients for each fatty acid (CC), lower and upper bounds for the hypothesized calibration coefficients were computed as $(1-k) \cdot CC$ and $(1+k) \cdot CC$, respectively. Error in the hypothesized calibration coefficients was incorporated by randomly selecting a value uniformly distributed between the lower and upper bounds for each calibration coefficient, after which the values were multiplicatively scaled to sum to the number of fatty acids. Because only the relative magnitudes of calibration coefficients are important in diet estimation, rescaling the values to a common reference point was necessary for our measure of calibration coefficient error (below) to be meaningful. The degree of error was loosely controlled by letting the constant k range from 0 (no error) to 0.75 (maximum 75% error) in increments of 0.05.

The error in any one set of hypothesized calibration coefficients was measured as the absolute value of the difference between the true and hypothesized calibration coefficients divided by the true values, averaged over all fatty acids.

SIMULATION DESIGN

The simulation design was based on factorial-like combinations of calibration coefficient error and levels of ghost prey in the diet. For each of the 16 values of the constant controlling calibration coefficient error, k (from 0.00 to 0.75 in increments of 0.05), 50 sets of hypothesized

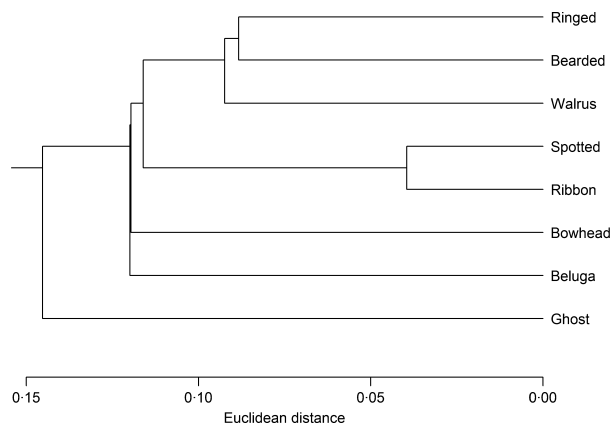


Fig. 1. A Euclidean-distance dendrogram of the mean fatty acid signatures in the marine mammal prey data set. Predator diets contained various amounts of the ghost prey, but the ghost prey signature was not used in diet estimation, allowing the evaluation of estimator robustness to that assumption violation.

calibration coefficients were randomly generated using the method described above. For each set of hypothesized calibration coefficients, the proportion of ghost prey in the diet (p) was varied from 0 to 0.5 in increments of 0.025 (21 unique values). A predator diet was established by first setting the proportion for the ghost prey as p and then multiplying the realistic polar bear or grey seal diet proportions by $1-p$ so that all diet proportions summed to 1.

Given a predator diet, predator signatures were randomly constructed from the prey signature data, augmented with the ghost prey signature, using a method similar to that of Bromaghin *et al.* (2015). A bootstrap sample was randomly selected with replacement from the signatures of individual non-ghost prey animals, with prey-type sample sizes equal to the observed sample size in the prey signature data, and the mean signature was computed for each prey type from the bootstrap sample. Because we were primarily interested in diet estimation bias, using relatively large prey sample sizes effectively minimized a source of variation that was not of interest. A predator signature in the prey space was then computed as a linear mixture of the mean bootstrapped prey signatures, with the predator diet providing the mixture proportions, after which the true calibration coefficients were used to transform the resulting signature to the predator space (Bromaghin *et al.* 2015). For each combination of a set of hypothesized calibration coefficients and a predator diet, 30 predator signatures were randomly established in this manner. Therefore, a total of $16 \times 50 \times 21 \times 30 = 504\,000$ predator signatures were constructed for each of the four realistic polar bear and grey seal diets.

The simulation was implemented in R 3.1.2 (R Core Team 2014). Diet estimates were obtained using a dynamic-link library compiled from Fortran code and called from an R script file to increase computational speed (Bromaghin *et al.* 2015). Estimation was performed in the prey space (Bromaghin *et al.* 2015) using hypothesized calibration coefficients, mean non-ghost prey signatures, and both the Kullback–Leibler and Aitchison distance measures. The total estimation error

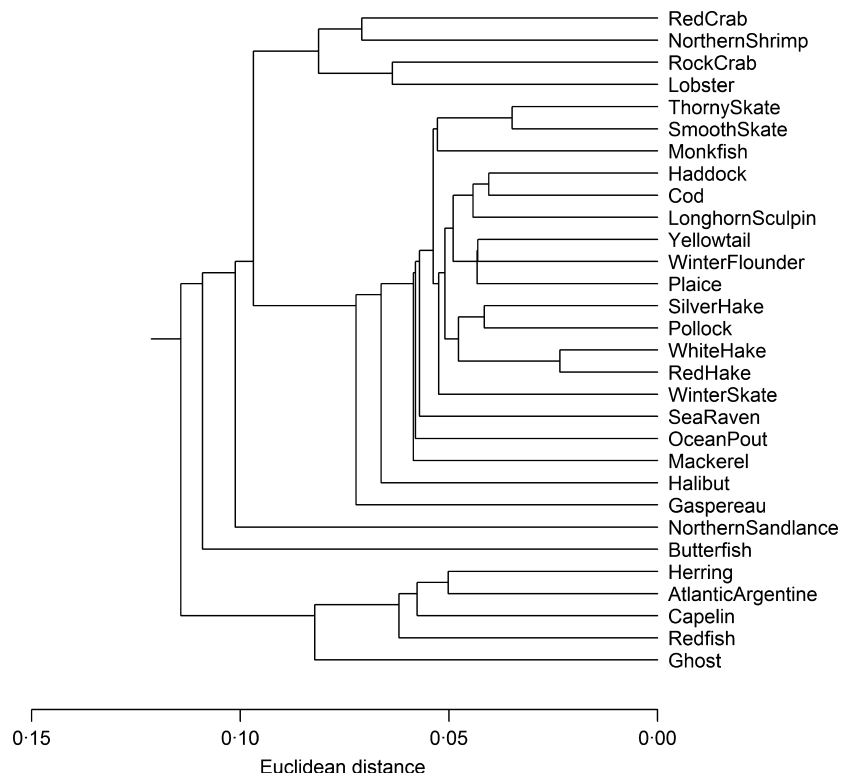


Fig. 2. A Euclidean-distance dendrogram of the mean fatty acid signatures in the fish and shellfish prey data set. Predator diets contained various amounts of the ghost prey, but the ghost prey signature was not used in diet estimation, allowing the evaluation of estimator robustness to that assumption violation.

(bias) in a diet estimate was measured as the sum of the absolute values of the differences between the true and estimated diet proportions for each prey type, including the ghost prey which were not included in the prey data and therefore always had an estimate of 0. For that reason, the minimum and maximum values of the bias statistic were $2p$ and 2, respectively.

Results

The procedure for establishing a ghost prey signature performed approximately as we intended. Because of the small number of prey types in the marine mammal prey data set, and their relative distinctiveness, the ghost prey was reasonably distinct from other prey types (Fig. 1). The ghost prey for the fish prey data set was less distinct from all other prey types (Fig. 2), which was expected because

the greater complexity of that data set made it more difficult to construct a ghost prey signature different from all other prey types.

The simulation results revealed patterns that were reasonably consistent across both prey data sets and all four realistic diets. To illustrate these patterns, we constructed scatterplots of bias versus calibration coefficient error for each realistic diet and four levels of ghost prey contribution to diet (0.0%, 7.5%, 15.0% and 22.5%), using loess smoothers to summarize overall trend (Figs 3–6). These four levels of ghost prey were subjectively selected because the greatest differences between the estimators were observed at low to moderate levels of ghost prey consumption, and both estimators performed poorly when contributions of ghost prey to diet were larger. When model assumptions were perfectly satisfied, that is there were no

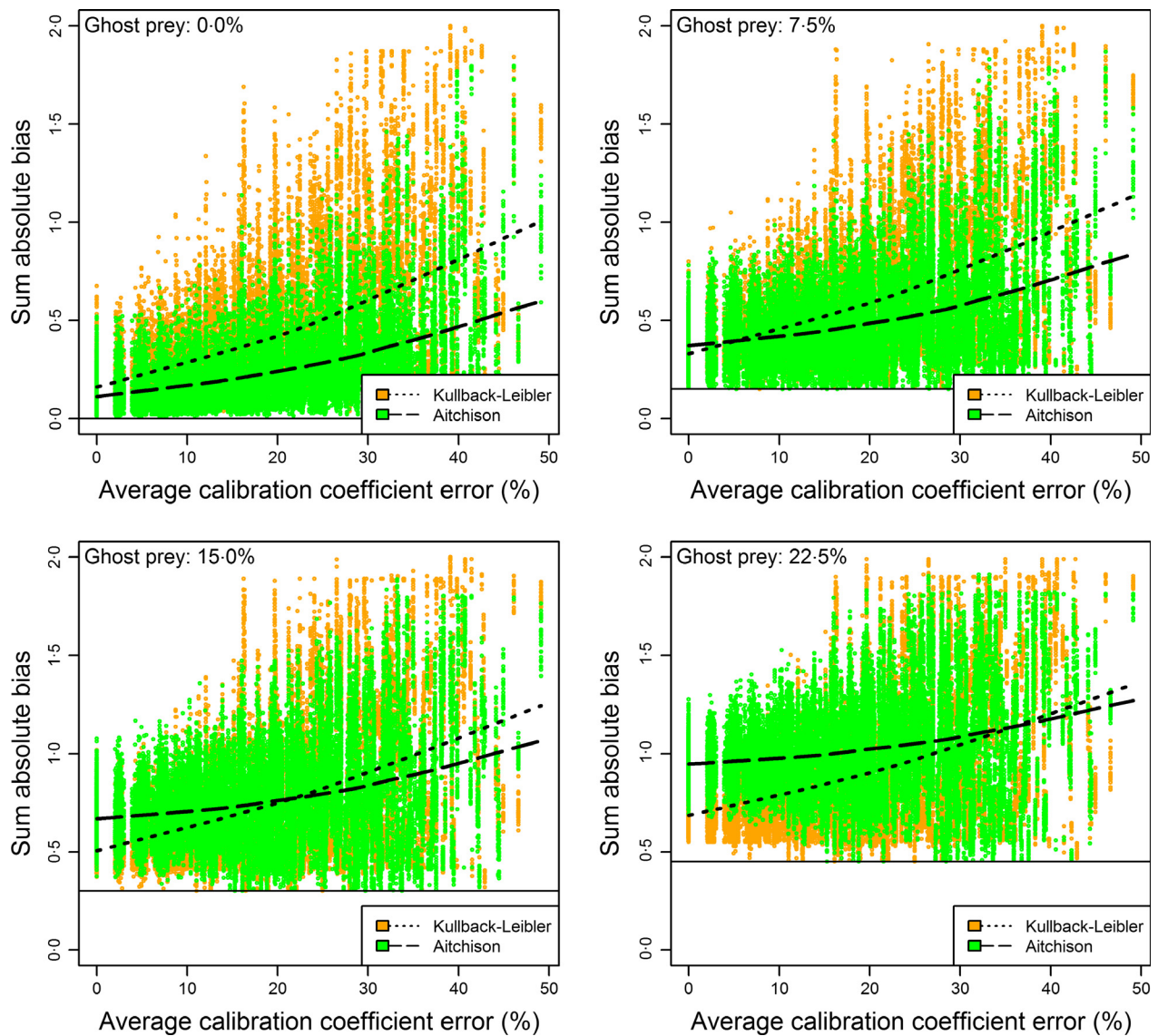


Fig. 3. Scatterplots of observed bias of the Kullback–Leibler and Aitchison diet estimators versus calibration coefficient error for selected contributions of ghost prey (0.0%, 7.5%, 15.0%, and 22.5%) to the diets of adult female polar bears (*Ursus maritimus*). The broken lines were independently generated for each estimator using loess smoothers to capture overall patterns in bias as a function of error.

ghost prey in the diet and calibration coefficients contained no error (leftmost portion of upper left panels of Figs 3–6), both estimators had the lowest bias observed in the simulations. Under these conditions, the Aitchison estimator tended to have less bias than the Kullback–Leibler estimator, consistent with prior findings (Bromaghin *et al.* 2015), and the bias is caused by the degree of similarity among prey-type signatures.

When predator diets contained no ghost prey, the Kullback–Leibler estimator appeared more sensitive to errors in the calibration coefficients than the Aitchison estimator (upper left panel of Figs 3–6). As the degree of calibration coefficient error increased, the bias of both estimators also increased. However, the bias of the Kullback–Leibler estimator increased more rapidly than that of the Aitchison estimator, with all four realistic diets.

Conversely, the Aitchison estimator appeared most sensitive to the presence of ghost prey in predator diets. When there was no error in the calibration coefficients (leftmost portion of all panels, Figs 3–6), the bias of both estimators increased as the contribution of ghost prey to predator diets increased, and the increase in bias was greater than the increase in the minimum possible bias (2*p*). However, bias of the Aitchison estimator increased more rapidly than that of the Kullback–Leibler estimator with three of the four diets, the exception being the female grey seal diet (Fig. 5).

When both model assumptions were simultaneously violated, the bias of both estimators increased and tended to converge as the severity of the violations increased (all but the upper left panel, Figs 3–6). The female grey seal diet was again a partial exception, in that the degree of convergence was reduced (Fig. 5).

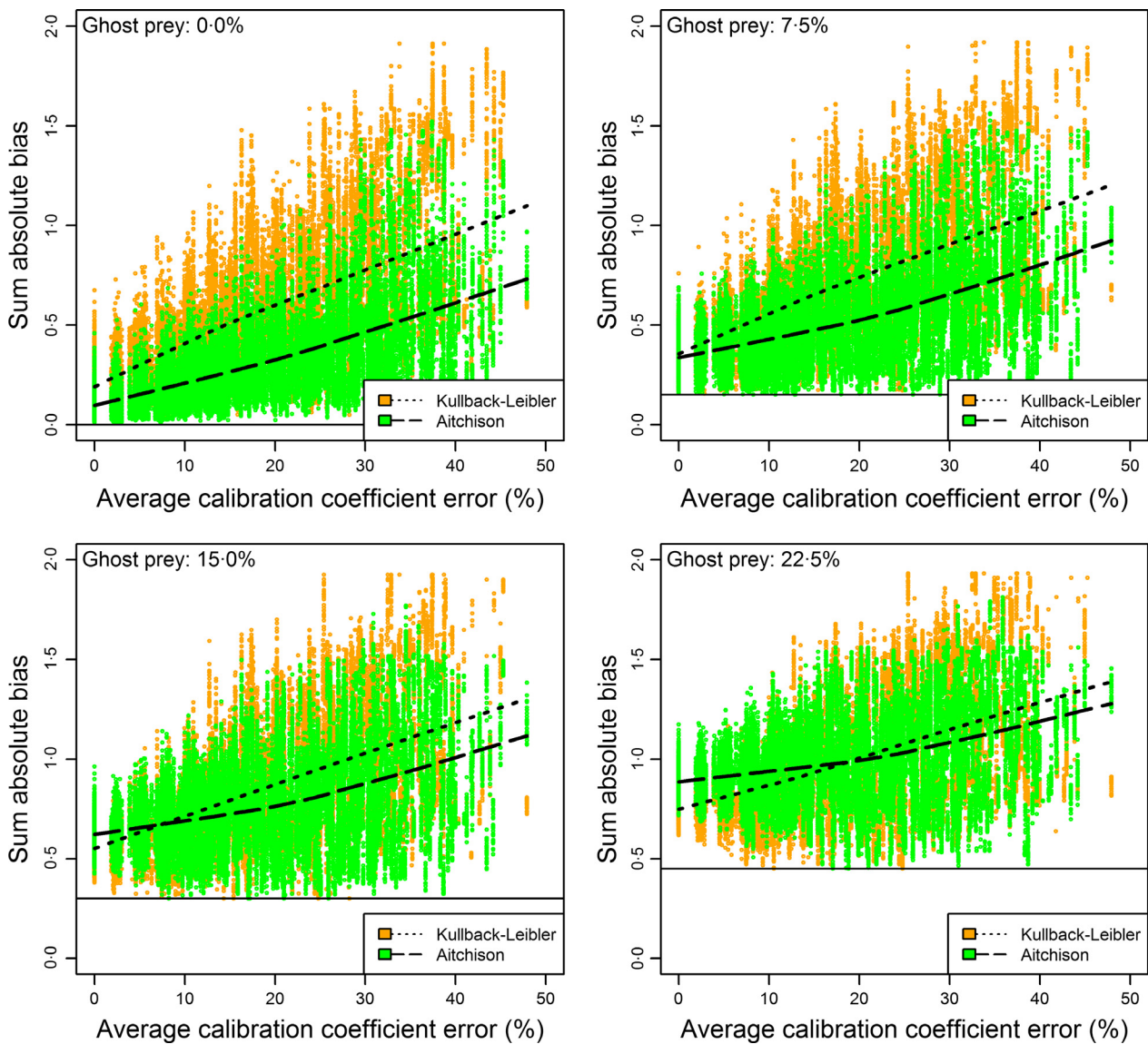


Fig. 4. Scatterplots of observed bias of the Kullback–Leibler and Aitchison diet estimators versus calibration coefficient error for selected contributions of ghost prey (0.0%, 7.5%, 15.0%, and 22.5%) to the diets of adult male polar bears (*Ursus maritimus*). The broken lines were independently generated for each estimator using loess smoothers to capture overall patterns in bias as a function of error.

Discussion

This work is the first to investigate the robustness of QFASA diet estimators to violations of model assumptions under a reasonably comprehensive suite of conditions. Our findings that the Kullback–Leibler and Aitchison estimators respond differently to violations of the two primary assumptions confirm prior hypotheses that these two distance measures are sensitive to different characteristics of fatty acid signatures (Bromaghin *et al.* 2015). Collectively, these results provide new information to help guide selection of an estimator and may provide insights that will ultimately lead to future improvements in diet estimation.

The causes underlying the differential robustness to assumption violations we observed are not known with certainty; however, there is basis for reasonable speculation. The way in

which calibration coefficients account for differential metabolism of fatty acids directly alters the magnitudes of signature proportions, with the proportions of fatty acids that are present at greater than trace amounts and have particularly small or large calibration coefficients (relative to 1) changing the most. Errors in the calibration coefficients may therefore produce large errors in some components of the transformed signatures. Consequently, prior findings that the Kullback–Leibler distance measure is most sensitive to large absolute differences between observed and modelled signature proportions (Bromaghin *et al.* 2015) likely explains its greater sensitivity to violations of this assumption. Conversely, the consumption of ghost prey would be expected to more subtly influence all components of a predator signature, except in the seemingly unlikely case where the ghost prey was a major contributor to diet and its signature differed substantially from the

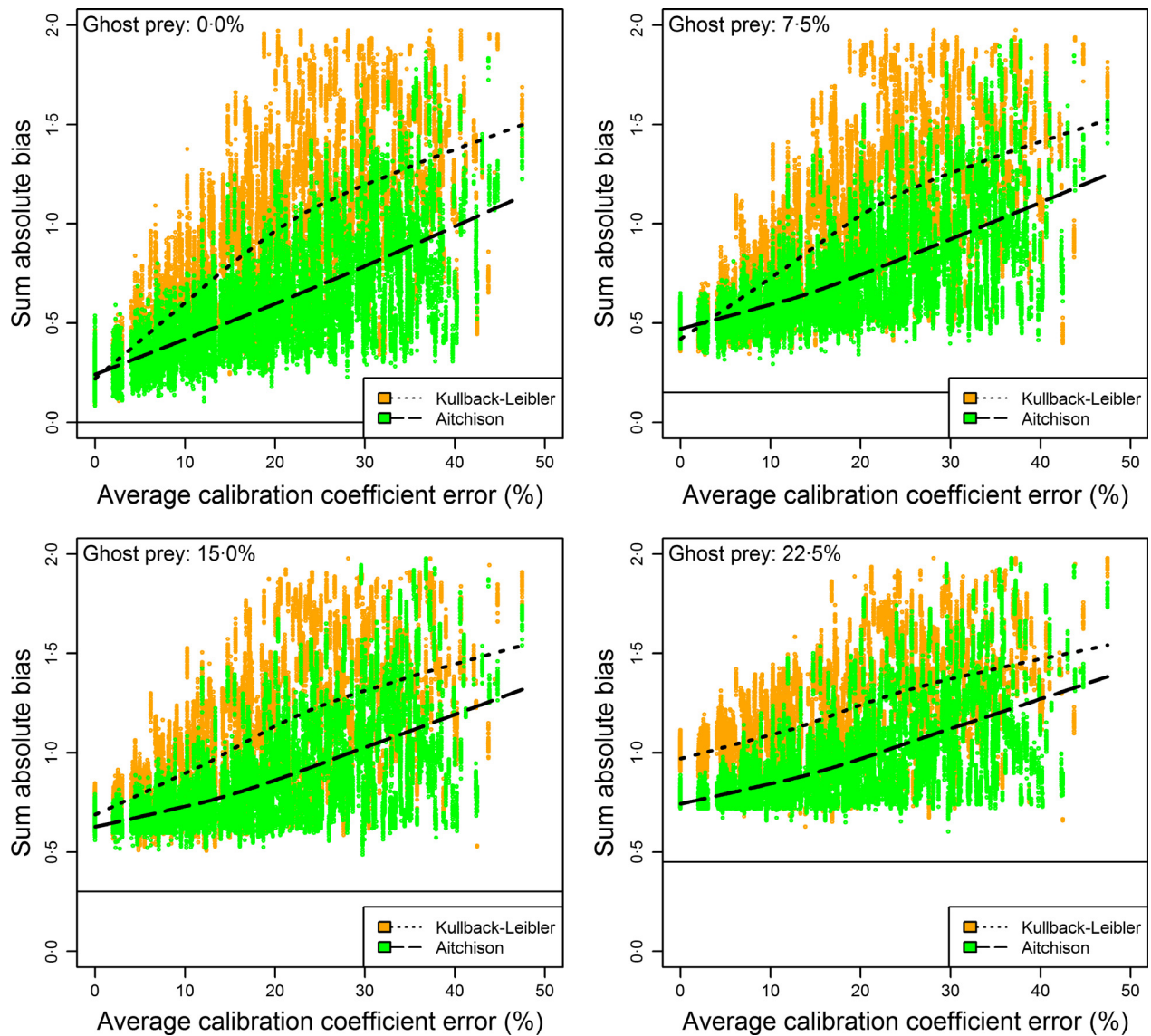


Fig. 5. Scatterplots of observed bias of the Kullback–Leibler and Aitchison diet estimators versus calibration coefficient error for selected contributions of ghost prey (0.0%, 7.5%, 15.0% and 22.5%) to the diets of spring-sampled female grey seals (*Halichoerus grypus*). The broken lines were independently generated for each estimator using loess smoothers to capture overall patterns in bias as a function of error.

signature of any other prey type. Such a subtle influence has the greatest potential to alter the relative magnitude of signature components for fatty acids having the smallest proportions. The reported sensitivity of the Aitchison distance measure to large relative differences between observed and modelled predator fatty acid signature proportions (Broaghin *et al.* 2015) may therefore translate into the greater sensitivity to the consumption of ghost prey that we observed.

Our finding that neither estimator performed well when both assumptions were badly violated was not surprising, but nevertheless serves as a useful reminder of an obvious fact. If an investigator has cause to suspect that key assumptions cannot be satisfied, at least approximately, use of the method would be difficult to justify and the availability of alternative methods should be explored. However, the cases in which

violations were less severe, perhaps with only one assumption violated, may better represent conditions faced by practitioners, and our findings are informative for such instances. In particular, the fact that the Aitchison estimator was most robust to errors in the calibration coefficients, while the Kullback–Leibler estimator was most robust to the incompleteness of the prey signature data, suggests a strategy to minimize the potential for diet estimates to be biased by potential assumption violations.

Use of the Aitchison distance measure appears to offer a degree of protection from potential errors in the calibration coefficients. The accuracy of calibration coefficients, derived from feeding trials with captive animals, for application to free-ranging predators is difficult to explicitly validate. Even if a feeding trial has been conducted with the study species,

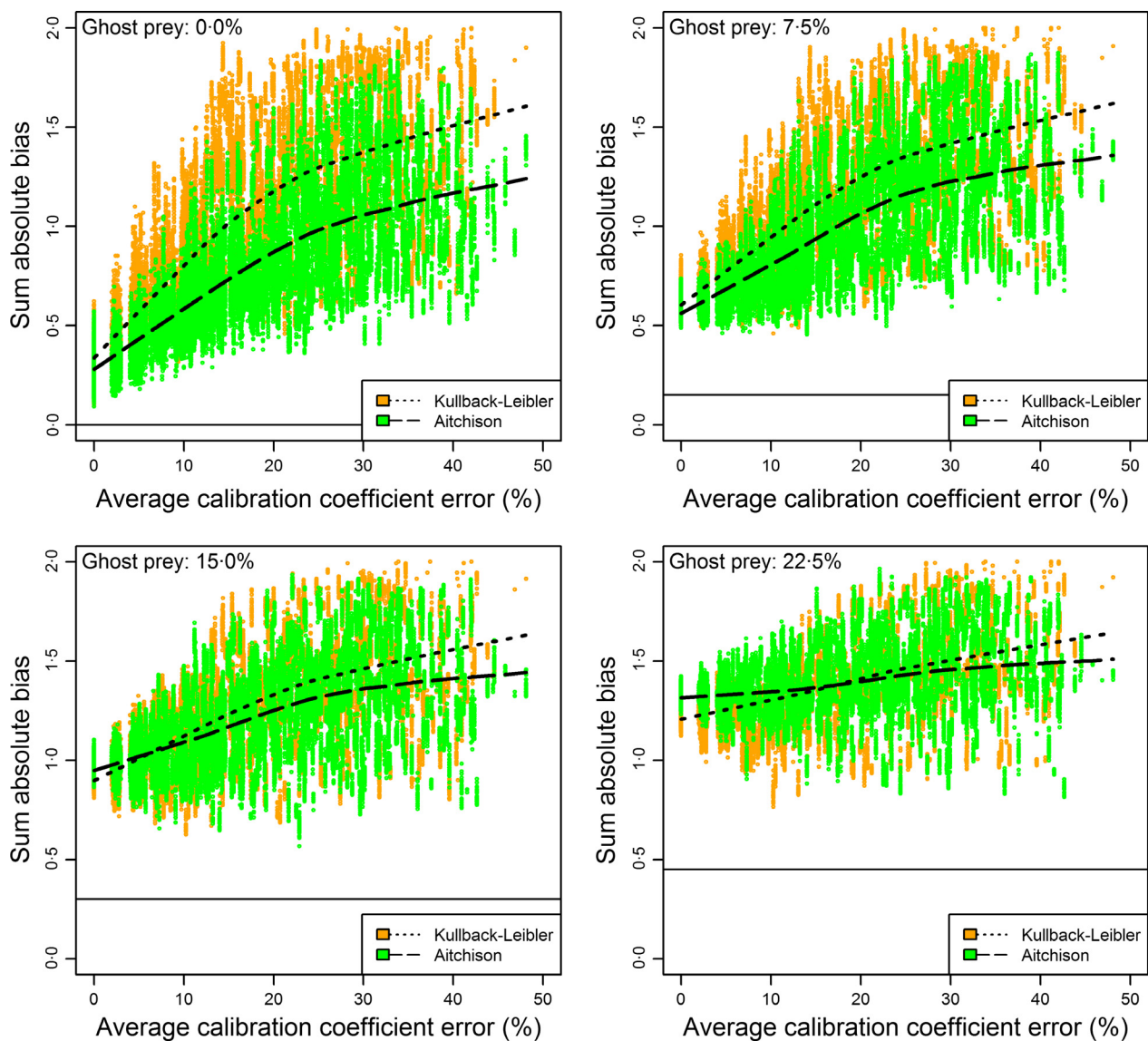


Fig. 6. Scatterplots of observed bias of the Kullback–Leibler and Aitchison diet estimators versus calibration coefficient error for selected contributions of ghost prey (0.0%, 7.5%, 15.0%, and 22.5%) to the diets of spring-sampled male grey seals (*Halichoerus grypus*). The broken lines were independently generated for each estimator using loess smoothers to capture overall patterns in bias as a function of error.

differences in the diets or metabolism of captive and wild animals may cause errors in the calibration coefficients that could bias diet estimates. For example, Budge, Penney & Lall (2012) reported that calibration coefficients resulting from feeding trials of Atlantic salmon (*Salmo salar*) were dependent on the fatty acid composition of foods used in the trial. Several aspects of the design of a feeding trial, such as inadequate time for signatures to stabilize, properties of available food sources, the representativeness of available predators, or limitations imposed by ethical considerations associated with use of captive animals, may reduce the applicability of calibration coefficients to free-ranging predators. It is perhaps most common for investigators to use calibration coefficients derived from a feeding trial with a surrogate species (e.g. Thiemann, Iverson & Stirling 2008; Meynier *et al.* 2010; Rode *et al.* 2014; Haynes *et al.* 2015), which may further reduce the applicability of the calibration coefficients for the study species.

If multiple sets of calibration coefficients are available and there is uncertainty regarding which set might be most suitable, a reasonable approach might be to estimate diets using each set. If the resulting estimates were similar, or if prominent features of the estimates such as the dominant prey types were similar, one would have some assurance that the selection of calibration coefficients had not substantially influenced the findings. For example, Thiemann, Iverson & Stirling (2008) used two sets of mink calibration coefficients and derived polar bear diet estimates by averaging the estimates obtained with each set of calibration coefficients. However, it is perhaps more common to find that different calibration coefficients lead to differences in diet estimates that are meaningful in a particular application (e.g. Meynier *et al.* 2010; Haynes *et al.* 2015). In this case, one can only describe the level of uncertainty that exists in the diet estimates.

Conclusions

The greater sensitivity of the Aitchison distance measure to inadequate breadth of the prey signature data and relative robustness to errors in the calibration coefficients, compared to the Kullback–Leibler measure, is an interesting and important finding. In most QFASA applications, investigators will be better able to assess the adequacy of the available prey signature data and obtain any additional prey samples deemed necessary, than to conduct a feeding trial with the study species or otherwise verify the applicability of published calibration coefficients for the study species.

Our recommendation for minimizing risk to potential violations of QFASA assumptions is to first assess the completeness of the prey signature data. The acquisition of additional prey samples may be needed to increase the breadth of coverage with respect to potential prey types that are completely missing from the data or whose sample size is smaller than desired. Investigators should also assess whether established prey types should be further divided to account for heterogeneity in signatures within a prey type potentially caused by such factors as sex, size or age, and season or location of sample acquisition, with the goal of minimizing heterogeneity within prey types

and maximizing heterogeneity between prey types. Completing this first step will obviously be advantageous regardless of the estimator to be used, potentially reducing both bias and estimation variance. Once the prey signature data have been deemed adequate, our results suggest that use of the Aitchison distance measure can be expected to limit bias arising from potential errors in the calibration coefficients, which are generally more difficult to rectify than weaknesses in the prey signature data.

Our findings, together with those of Bromaghin *et al.* (2015), suggest that making strong and universal recommendations regarding estimation methods is difficult due to the multivariate nature of the problem. Perhaps not surprisingly, estimator performance seems to depend on a complex interaction of the suite of fatty acids used, the calibration coefficients, prey and predator signature data, predator diets and the selection of estimation methods. Despite this complexity, our work provides new insights into the performance of QFASA models that we hope will prove useful to QFASA practitioners and also stimulate additional research into this class of models that will ultimately advance the capabilities of quantitative methods in the study of animal ecology.

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Data accessibility

The prey signature data for fish species and a portion of the marine mammal data are available from Dalhousie University (<http://dx.doi.org/10.15273/10222/57254>). The remainder of the marine mammal signature data is available from the U.S. Geological Survey, Alaska Science Center (<https://dx.doi.org/10.5066/F7PR7T2W>). The R and FORTRAN code is available from the U.S. Geological Survey, Alaska Science Center (<https://dx.doi.org/10.5066/F7N877TK>). Links to both data files and the code are also available at http://alaska.usgs.gov/science/biology/quantitative_ecology/products.php.

References

- Beck, C.A., Iverson, S.J., Bowen, W.D. & Blanchard, W. (2007) Sex differences in grey seal diet reflect seasonal variation in foraging behaviour and reproductive expenditure: evidence from quantitative fatty acid signature analysis. *Journal of Animal Ecology*, **76**, 490–502.
- Bowen, W.D. & Iverson, S.J. (2013) Methods of estimating marine mammal diets: a review of validation experiments and sources of bias and uncertainty. *Marine Mammal Science*, **29**, 719–754.
- Bromaghin, J.F., Rode, K.D., Budge, S.M. & Thiemann, G.W. (2015) Distance measures and optimization spaces in quantitative fatty acid signature analysis. *Ecology and Evolution*, **5**, 1249–1262.
- Budge, S.M., Penney, S.N. & Lall, S.P. (2012) Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses: validation with controlled feeding studies. *Canadian Journal of Fisheries and Aquatic Sciences*, **69**, 1033–1046.

- Ghalanos, A. & Theussl, S. (2014) Rsolnp: General non-linear optimization using augmented Lagrange multiplier method. R package version 1.15.
- Haynes, T.B., Schmutz, J.A., Bromaghin, J.F., Iverson, S.J., Padula, V.M. & Rosenberger, A.E. (2015) Diet of breeding yellow-billed loons on the Arctic Coastal Plain, Alaska. *Polar Biology*, **38**, 1239–1247.
- Iverson, S.J., Field, C., Bowen, W.D. & Blanchard, W. (2004) Quantitative fatty acid signature analysis: a new method of estimating predator diets. *Ecological Monographs*, **74**, 211–235.
- Martín-Fernández, J.A., Palarea-Albaladejo, J. & Olea, R.A. (2011) Dealing with zeros. *Compositional Data Analysis: Theory and Application* (eds V. Pawlowsky-Glahn & A. Buccianto), pp. 43–58. John Wiley, Chichester.
- Meynier, L., Morel, P.C.H., Chilvers, B.L., Mackenzie, D.D.S. & Duignan, P. (2010) Quantitative fatty acid signature analysis on New Zealand sea lions: model sensitivity and diet estimates. *Journal of Mammalogy*, **91**, 1484–1495.
- R Core Team. (2014) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing, Vienna, Austria. <http://www.R-project.org/>.
- Rode, K.D., Regehr, E.V., Douglas, D.C., Durner, G., Derocher, A.E., Thiemann, G.W. & Budge, S.M. (2014) Variation in the response of an arctic top predator experiencing habitat loss: feeding and reproductive ecology of two polar bear populations. *Global Change Biology*, **20**, 76–88.
- Rosen, D.A.S. & Tollit, D.J. (2012) Effects of phylogeny and prey type on fatty acid calibration coefficients in three pinniped species: implications for the QFASA dietary quantification technique. *Marine Ecology Progress Series*, **467**, 263–276.
- Stewart, C. & Field, C. (2011) Managing the essential zeros in quantitative fatty acid signature analysis. *Journal of Agricultural, Biological, and Environmental Statistics*, **16**, 45–69.
- Thiemann, G.W., Iverson, S.J. & Stirling, I. (2008) Polar bear diets and arctic marine food webs: insights from fatty acid analysis. *Ecological Monographs*, **78**, 591–613.
- Thiemann, G.W., Budge, S.M., Iverson, S.J. & Stirling, I. (2007) Unusual fatty acid biomarkers reveal age- and sex-specific foraging in polar bears (*Ursus maritimus*). *Canadian Journal of Zoology*, **85**, 505–517.
- Wang, S.W., Hollmén, T.E. & Iverson, S.J. (2010) Validating quantitative fatty acid signature analysis to estimate diets of spectacled and Steller's eiders (*Somateria fischeri* and *Polysticta stelleri*). *Journal of Comparative Physiology B*, **180**, 125–139.

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