Bayesian estimation of predator diet composition from fatty acids and stable isotopes

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Abstract

Quantitative analysis of stable isotopes (SI) and, more recently, fatty acid profiles (FAP) are useful and complementary tools for estimating the relative contribution of different prey items in the diet of a predator. The combination of these two approaches, however, has thus far been limited and qualitative. We propose a mixing model for FAP that follows the Bayesian machinery employed in state-of-the-art mixing models for SI. This framework provides both point estimates and probability distributions for individual and population level diet proportions. Where fat content and conversion coefficients are available, they can be used to improve diet estimates. This model can be explicitly integrated with analogous models for SI to increase resolution and clarify predator-prey relationships. We apply our model to simulated data and an experimental dataset that allows us to illustrate modeling strategies and demonstrate model performance. Our methods are provided as an open source software package for the statistical computing environment R.

Keywords Stable isotope analysis, quantitative fatty acid analysis, QFASA, lipid profile, diet analysis, Bayesian mixing model, fatty acid signature, dietary marker

1 Introduction

Quantitative estimates of an animals diet are a critical component of predator-prey studies, ecosystem models, and ecosystem-based management. Existing methods of estimating diet proportions all have strengths and weaknesses (Bowen & Iverson, 2012). Traditional stomach content and fecal matter analysis represent a brief snapshot of diet at a particularly place and time and can be invasive, time-consuming, and potentially biased by differential rates of digestion of prey or ingestion of identifiable prey parts (Bowen & Iverson, 2012). Chemical markers such as stable isotopes (SI) and fatty acid profiles (FAP) solve some of these problems. For example, both approaches integrate diet composition over an extended time period - typically weeks to months, depending on tissue turnover rates (Tucker *et al.*, 2008). These advantages have led to rapid growth in the use of chemical markers in diet studies (Bowen & Iverson, 2012; Elsdon, 2010; Kelly & Scheibling, 2011; Williams & Buck, 2010). However, chemical dietary markers generally lack the specificity of traditional stomach content analysis. In particular, several prey species often have similar isotopic signatures. More recent studies have sought greater dietary resolution through the use of SI of other elements in addition to carbon and nitrogen(Belicka *et al.*, 2012), compound specific SI

ratios (Budge *et al.*, 2008; Jack & Wing, 2011), or a combination of stomach content analysis and SI or FAP (Pethybridge *et al.*, 2012). The use of SI and FAP in combination also holds great promise; however the few studies to date that have used both chemical markers have been qualitative (Guest *et al.*, 2009) or based on positive correlation of results from both methods (Tucker *et al.*, 2008).

Analysis tools for SI data have become very sophisticated in recent years, starting with the development of general Bayesian analysis tools for estimating diet proportions, and leading to customized (hierarchical) models for individual applications (Hopkins & Ferguson, 2012; Moore & Semmens, 2008; Parnell *et al.*, 2012). The latter models can, for instance, estimate dietary differences of geographically distinct populations (Semmens *et al.*, 2009), accommodate temporal changes in diets or estimate the effect of covariates (e.g., age, size, sex) on diet proportions (Parnell *et al.*, 2012).While these models provide a considerable step towards ecologically relevant models in diet studies, the underlying SI data is limited in the resolution that it can provide. Since typically only 2-3 SI are measured, the contrast that is achievable from such a low number of variables is necessarily limited, especially when the number of potential prey items increases (Phillips & Gregg, 2003; Ward *et al.*, 2011). Optimally

aggregating prey items into prey groups may circumvent this problem (Ward *et al.*, 2011), but may also be less satisfactory in complex food webs. FAP data can, in theory, provide considerably more resolution compared to SI data, due to large number of potential fatty acids that can be measured. Nevertheless, studies employing FAP are either qualitative in their estimates of prey proportions in predator diets, or use Quantitative Fatty Acid Signature Analysis (Iverson *et al.*, 2004) to obtain quantitative estimates of diet proportions. The latter method is the only one available thus far for use with FAP data, and, in contrast to recent (Bayesian) SI mixing models, relies on a distance metric rather than a model based formulation to estimate the most likely diet proportions. This framework provided the first quantitative approach to estimating diet proportions using fatty acids and it has already seen widespread use, particularly in studies of marine mammals (Bowen & Iverson, 2012) and seabirds (Williams & Buck, 2010). Nevertheless, QFASA has a number of limitations. Since it is not based on a probabilistic model, it is difficult to estimate uncertainty associated with estimated diet proportions (Williams & Buck, 2010). The absence of an explicit model also makes it impossible to build ecological mechanisms (e.g., covariates of consumed diets) directly into the model. Furthermore, uncertainty about conversion coefficients

representing enrichment and preferential uptake of fatty acids cannot be considered, yet small changes in these coefficients can lead to differences in inferred diet proportions (Wang *et al.*, 2010). Lastly, the QFASA model assumes constant fat content of consumed items, an assumption that will rarely be met.

Given the discrepancy in methods applied to SI and FAP data, it is perhaps not surprising that their joint application has commonly relied on qualitative comparisons. Because both markers integrate diet composition over often comparable time-scales, however, an explicit integration of these data types could provide substantial benefits. While FAP data could mitigate the resolution problem in SI data, SI data could provide increased resolution and clarify predator-prey relationships, the knowledge of which is usually a pre-requisite for FAP data. For example, for many non-modified fatty acids, FAP alone cannot discriminate between the case of two species which share a common diet and the situation in which one of these species eats the other. In either case, the two species may have similar FAP. The addition of a stable isotope with trophic fractionation (e.g., ^{15}N), however, can readily distinguish predation from dietary overlap.

Here, we present a mixing model for FAP data based on a probabilistic model whose parameters are estimated using Bayesian methods. We

demonstrate the suitability of this model for FAP analysis and highlight the potential benefit of explicit integration with SI data to increase the precision of diet estimates. Using both simulated and published data, we show how this model can be extended to ask ecologically relevant questions.

2 Methods

2.1 A Bayesian mixing model for FAP

Bayesian models for SI data are commonly based on the assumption that SI ratios are normally distributed. This assumption cannot be made for FAP data, since for most methods of analysis, the concentration of individual fatty acids is normalized to the total lipid content of the sample. Thus, the FAP are a collection of proportions (referred to as a composition), which lie between 0 and 1, and are constrained to sum to 1. A common solution to this problem, however, is to consider transformations that make the data approximately normal (Budge *et al.*, 2006). To construct our model, we followed Aitchison & Bacon-Shone (1999) and considered a log ratio transformation, also called alr transformation, such that

$$y_i = alr(\phi_i) = log\left(\frac{\phi_{i,1...p-1}}{\phi_{i,p}}\right) \quad (1)$$

where y_i is the p-variate fatty acid composition of individual i or prey species s, with $i = 1...n$. We then assumed that the distribution of y is multivariate normal, with species specific mean μ_s and covariance matrix Σ_S, or $y \sim N(\mu_s, \Sigma_s)$. A vaguely informative prior on μ_s and Σ_s allows for uncertainty in prey distributions (Ward *et al.*, 2010) to propagate to estimates of diet proportions.

Each predator j consumes a proportion π_j of each prey source, and analogous to stable isotope mixing models, predator FAP are then a linear combination of prey FAPs, normalised to sum to one. Since predators may selectively assimilate or metabolize fatty acids (Budge *et al.*, 2006; Iverson *et al.*, 2004; Rosen & Tollit, 2012), we specify prey-specific conversion coefficients $\kappa_i = \kappa_{i,1}...\kappa_{i,P}$ (Rosen & Tollit, 2012). Furthermore, the n prey species likely have different fat content Φ that will affect the relative amount of fatty acids assimilated by the predator. The FAP of predator j is then a linear combination of the prey FAP, modified by conversion coefficients for each fatty acid p and fat content for each prey i. The

signature of predator j is then:

$$\tau_j = C\left\{\sum_i \left(\pi_i \Phi_i\right)\left(\kappa_i \otimes \phi_{i,j}\right)\right\} \tag{2}$$

Here, C is the closure operation which normalizes the FAP to sum to one, is the outer (element wise) product, are the diet proportions of predator .We again assume that predator signatures are normally distributed after transformation, with mean and covariance matrix . More complex error formulations including measurement error and prey covariances are possible (Hopkins & Ferguson, 2012), but we restrict ourselves here to a simple formulation that does not depend on prey variances. We assume that and are log-normally and gamma distributed around known mean and variance values (estimated or calculated from controlled diet experiments, see below). Since the sum-to-one constraint on the FAP leads to κ being only determined in terms of relative uptake of fatty acids, we add a sum to one constraint to κ, thus restricting the scale (and implicitly making the overall distribution of κ a Dirichlet distribution). While other parameterizations could be formulated here, we found that the above formulation works well in practice, and makes it straightforward to calculate prior parameters from

mean and variance values found in controlled experiments.

The diet proportions predators are the main focus of investigation in diet studies. It is equally possible to estimate individual diet composition by simply If data from individual predators are available, but the focus remains on population level parameters, it is generally advantageous to model individual as draws from a population level distribution of diet proportions . Recent approaches to stable isotope mixing have focused on transformations of to get around the problems associated with the compositional nature of the diet proportions. This approach is analogous to that taken in our model for compositional FAPs. The diet proportions are transformed such that the support of is the real line rather than the interval [0;1]. It is then straightforward to model diet proportions as function of covariates, such as size, sex, or region (i.e., in a regression formulation). While this approach is obviously appealing, it adds considerably to the run-time of Markov Chain Monte Carlo Procedures employed to estimate model parameters. We therefore use a vague Dirichlet prior on the proportions when convenient (e.g., when we estimate only population level parameters), and in our simulations (e.g., Semmens et al. 2009). When estimating individual parameters or for linear model formulations (see Application 1), a clr transformation approach is used.

When estimating population level parameters, the two formulations give near identical results (differences are within the range expected from stochasticity in the MCMC samples).

2.2 Joint diet estimation from FAP and SI

Above, we mentioned three potential benefits of integrating FAP and SI data: i) increased information to discriminate among sources, ii) the potential of SI to resolve predator prey relationships due to trophic enrichment of SI, and iii) the potential reduction in estimation error due to more well-known fractionation coefficients for stable isotopes. It thus appears worthwhile to integrate these types of data in a single model to estimate diet proportions. Our model is conceptually similar to recent models proposed for SI data, and integration of FAP and SI data into a single model is straightforward in the present setting. We again assume that the SI signatures of prey items follow a normal distribution, such that , where the superscript SI denotes that these are stable isotope signatures. Predator SI signatures are again a linear combination of prey SI, this time modified by additive fractionation coefficients and, potentially, by prey C and N concentrations (and/or digestibility, see CITE) . The SI signatures for predator j is then

The exact formulation of this integration depends on the assumptions that one is comfortable with in a given setting: identical dietary proportions may be appropriate if diets (and hence SI and FAP) are thought to be stable, or if both chemical tracers are thought to integrate over similar time-scales. If the time scales of these two elements are thought to be different, individual diet proportions for each tracer may be more appropriate, and may be drawn from an overall population distribution of diet proportions. Our models were implemented in JAGS (Plummer *et al.*, 2003), called from the statistical computing environment R (R Core Team, 2014). Code and data for all models, examples and tutorials are available on the open source repository github.com/philipp-neubauer/fastinR. The models include the above-mentioned formulations for individual diet estimates, population level estimates or both as well as linear model (regression and anova) formulations for diet proportions.

2.3 Simulation studies: accuracy and sensitivities

We explored sensitivities of inferred diet proportions to the source configuration and fatty acid subset selection in a series of simulation experiments. We simulated 100 datasets and varied source separation and the subset of fatty acids retained for the analysis of simulated datasets.

Each dataset was analyzed with subsets retaining 75%-99% of between source variability on the CAP axes, and errors in inferred diet proportions (taken to be the posterior mean of inferred diet proportions) were compared among subsets. We then determined the sensitivity of estimated diet proportions to source separation, collinearity in FAP space and diet evenness (e.g., specialist versus generalist diets). Simulation setup and results are presented in detail in Appendix 1 & 2. To illustrate our method, we also include a simple simulated study, which can repeated in its entirety from Appendix 3 (this file can also be downloaded as a source file from our project repository).

2.4 Application: Estimating predator diets in a controlled experiment

In this application, we use data from an experimental study published by Stowasser *et al.* (2006), which investigated changes in squid FAP and SI as a function on diet treatments. The treatments consisted of exclusive fish and crustacean diets, as well as switched and mixed diets, with the former switching diets from fish (henceforth SF) to crustacean (SC) after 15 days of the 30 day experiment. In our analysis, we analyzed samples from the switched diet treatments, and used both SI and FAP to investigate whether

we can infer diet proportions in either treatments. Since we only had SI for the SC treatment squid, we start by analyzing this treatment in isolation to demonstrate that both SI and FAP can resolve diet proportions, and to demonstrate the benefit of using the two tracers in a joint model. We then analyze the SF and SC treatment squid together in a linear model setup that investigates treatment differences explicitly, and demonstrates how the model based approach to diet estimation can be use to answer ecologically relevant questions about predator diets.

In order to apply our model, we first estimated conversion coefficients of FAP and fractionation in SI, using squid from the 30 day diet treatments feeding exclusively crustacean and fish diets. The model for estimation of SI fractionation followed the model in Hussey *et al.* (2014), and used their results as priors for fractionation parameters for $\delta^{15}N$, and results from Caut *et al.* (2009) to construct priors for $\delta^{13}C$. Estimation of FA conversion coefficients used eq 1 with proportions assumed known from feeding trials. Further details on the estimation of conversion coefficients and fractionation is given in Appendix 4.

3 Results

Simulated test cases and our application to squid diets suggest that our model can estimate diet proportions with high accuracy from both SI and FAP, with accuracy depending mainly on source separation and diet eveness (Appendix 1 & 2). These examples further suggest that our selection procedure for FAP works well, allowed models at a fraction of computational cost with little expected loss in accuracy (Figure S1). At very uneven diet proportions, such as in the feeding trials analyzed in the squid example, we found the choice of point estimate for diet proportions inevitably introduced increased error at the margins of the 0-1 proportion interval. This is due to posterior distribution becoming more skewed toward the limits of this interval, and the mean and median, which are intuitive choices for point estimates in symmetrical posteriors, are often placed in relatively unlikely regions of parameter space.

4 Discussion

We presented here a first and very general framework that combines SI and FAP in an extendable, contemporary Bayesian mixing model. While an increasing number of studies combines these two tracer methods (Bank

et al., 2011; Guest *et al.*, 2008; Guest *et al.*, 2009; Jaschinski *et al.*, 2008; Stowasser *et al.*, 2006; Tucker *et al.*, 2008), we believe that none have done so in an explicitly quantitative way. Indeed, both approaches have their own limits, and, as mentioned above, their combination may help to overcome each tracers shortcomings. We thus suggest that our study and framework provide a substantial step towards building application specific models that explicitly integrate SI and FAP to achieve robust inference of diet proportions and highlight discrepancies in the two methods that need to be addressed through future research.

Recent developments in SI mixing models have led to increasingly realistic models in terms of their error structure (Hopkins & Ferguson, 2012) and incorporation of relevant biology, such as time dependent diet proportions and SI signatures (Parnell *et al.*, 2012). Given that our FAP and combined FAP and SI models are very similar to these models in terms of their underlying structure and assumptions, such developments are readily achievable within this framework. Nevertheless, it should be noted that they present the practitioner with requirements for substantial amounts of data of various kinds (i.e., measurement error estimates, collection of SI and FAP through time, respectively).

When working in high dimensional applications such as FAP, where the

number of measured variables can be large (¿20 FAs is common), one has to balance computation feasibility and model complexity. We opted here for a fully Bayesian analysis that estimates prey and predator distributions, as well as individual proportions. This does not come without a cost: we found that there are limits to the dimensionality that the model (as we formulated it) can deal with. Since the model complexity depends at once on the number of prey items, predators and fatty acids in the analysis, we have found it to be useful to use predator means or relatively few predator signatures first to estimate a single population distribution. Once one has determined that the model can effectively estimate reasonable diet proportions, re-running the model with a larger number of predators is warranted and, although potentially time consuming, may provide additional insights. We also found that using clr (or related) transformations to estimate diet proportions on the real line (Parnell *et al.*, 2012) was a considerable computational burden, and we only used these formulations here when the model structure would not work without such a formulation (i.e., hierarchical models of diet proportions, or a linear model/anova for the diet proportions). Depending on modeling priorities, one may choose to only model predator means, leading to lower computational burden and the possibility to routinely use transformations

for diet proportions. Furthermore, more efficient implementations may be possible with tailored and optimized MCMC approaches.

We presented an approach to variable selection for FAPs in order to further reduce computational burdens of mixing models. Our suggested method, based on CAP, provides a clear advantage over variable selection by discrimination alone (e.g., by classification success in a linear discriminant analysis). Discrimination is highest when the within class variance is lowest relative to the between class variance. A trivial selection would thus be to select a single FA that is slightly different among prey species, but has minimal variance within species. While classification accuracy would approach 100%, n (the number of prey items) would be greater than the number of selected variables, such that there is no unique solution to the diet estimation problem (Phillips & Gregg, 2003). We found that an optimal subset of variables is usually one that explains the bulk of among prey variance (represented by CAP axes), but eliminates FAs that only contribute minimally to separation among sources. In this case estimation errors may be lower than for the full data set.

We refrain from making estimates of relative or absolute error of the proportions themselves, since these depend on the number and configuration of sources in multivariate space in addition to the variance of

conversion coefficients. For many applications, conversion coefficients are not available and would be difficult to obtain, and it is important that practitioners are aware of the increased risk of making erroneous point estimates of diet proportions when setting conversion coefficients to 1. While a similar problem exists with SI fractionation coefficients, more is known about these coefficients, making it easier to construct reasonable priors. Combining SI with FAP may therefore have the additional benefit of reducing errors due to misspecified conversion coefficients.

. What else does this discussion need? A general strategy for modeling FAP and SI data Future research needs: discrepancies and overlap in SI and FAP data. we assumed they integrate over comparable periods and thus represent the same diet proportions CC for FAP desperate need for LOTS of controlled studies. But, when taking into account uncertainty, they are not the deal breakers they appear to be from using just point estimates.

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