Scientific Research Publishing

Open Journal of Ecology, 2022, 12, 333-359
https://www.scirp.org/journal/oje
ISSN Online: 2162-1993
ISSN Print: 2162-1985

Foodweb Trophic Level and Diet Inference Using an Extended Bayesian Stable Isotope Mixing Model

Erik Barry Erhardt[1], Rachel Marie Wilson[2]

[1]Department of Mathematics and Statistics, University of New Mexico, Albuquerque, NM, USA
[2]Department of Earth Ocean and Atmospheric Sciences, Florida State University, Tallahassee, FL, USA
Email: erike@stat.unm.edu, rmwilson@fsu.edu

How to cite this paper: Erhardt, E.B. and Wilson, R.M. (2022) Foodweb Trophic Level and Diet Inference Using an Extended Bayesian Stable Isotope Mixing Model. *Open Journal of Ecology*, **12**, 333-359.
https://doi.org/10.4236/oje.2022.126020

Received: March 30, 2022
Accepted: June 17, 2022
Published: June 20, 2022

Abstract

You are *what you eat* (diet) and *where you eat* (trophic level) in the food web. The relative abundance of pairs of stable isotopes of the organic elements carbon (e.g., the isotope ratio of ^{13}C vs ^{12}C), nitrogen, and sulfur, among others, in the tissues of a consumer reflects a weighted-average of the isotope ratios in the sources it consumes, after some corrections for the processes of digestion and assimilation. We extended a Bayesian mixing model to infer trophic positions of consumer organisms in a food web in addition to the degree to which distinct resource pools (diet sources) support consumers. The novel features in this work include: 1) trophic level estimation (vertical position in foodweb) and 2) the Bayesian exposition of a biologically realistic model [1] including stable isotope ratios and concentrations of carbon, nitrogen, and sulfur, isotopic fractionations, elemental assimilation efficiencies, as well as extensive use of prior information. We discuss issues of parameter identifiability in the complex and most realistic model. We apply our model to simulated data and to bottlenose dolphins (*Tursiops truncatus*) feeding on several numerically abundant fish species, which in turn feed on other fish and primary producing plants and algae present in St. George Sound, FL, USA. Finally, we discuss extensions from other work that apply to this model and three important general ecological applications. Online supplementary materials include data, OpenBUGS scripts, and simulation details.

Keywords

Stable Isotope, Animal Ecology, Trophic Level, Animal Diet, Informative Priors

1. Introduction

1.1. Scientific Background

Stable isotope sourcing models are widely used to understand, among other things, animal diets and food webs [1] [2] [3] [4]. Because chemical elements have multiple isotopic forms (same number of protons, different numbers of neutrons, **Figure 1(a)**), and their relative abundance is a function of chemical and biological processes, the natural abundance of stable isotopes ratios varies geographically and among species (**Figure 1(b)**) (data from [5]). Unlike radioactive elemental isotopes, stable isotopes do not naturally decay, so the persistence of stable isotopes of hydrogen (H), carbon (C), nitrogen (N), oxygen (O), and sulfur (S) has found widespread use as biological tracers in studies of animal diets. The isotope ratio, $\delta = 1000\left(R_{\text{sample}} / R_{\text{standard}} - 1\right)‰$, is a normalized ratio of an estimate of the number of rarer to common isotope atoms in a sample (R_{sample}, such as $^{13}C/^{12}C$) relative to an international standard (R_{standard}) given in parts per thousand (‰) [6]. A mass spectrometer is typically used to measure isotope ratios from tissue or blood samples after combustion, vaporization, and ionization (**Figure 1(c)**). The concentration of an element is the proportion of that element in a sample, which can be measured jointly with the isotope ratio given that the mass of the sample is measured prior to analysis. The relative concentration of carbon to nitrogen among plant leaves in terrestrial environments can vary by two orders of magnitude and animal tissues have much greater concentrations of N than plant material [7].

The **assimilation efficiency** is the proportion of each element consumed that is incorporated into consumer's tissues (**Figure 1(d)**). For example, a fish (or a population of fish) ingests plant matter (ingestion) and a proportion of this material is processed by the digestive system and used to make new cells or tissues (assimilation) and the remaining undigested material exits the fish's body (excretion). The efficiency varies greatly, from 15% to 50% for plant material, and 60% to 100% for animal material. Furthermore, the process of assimilation preferentially incorporates heavier isotopes into the animal's tissues (trophic fractionation) (**Figure 1(e)**).

A food chain represents a succession of organisms that eat another organism and may be, in turn, eaten themselves [8]. A food web consists of all the food chains in a single ecosystem (**Figure 1(f)**) [9]. The number of steps an organism is from the start of the chain is a measure of its trophic level [10] and can be inferred from the diet and trophic fractionation.

Stable isotope analysis of a consumer animal's tissues (the mixture) and their potential prey and diet (the sources) is a leading strategy to gain insight about the trophic structure of food webs, because stable isotopes contain both time- and space-dependent information among organisms. The model presented here acknowledges that "you are what you assimilate". In particular, the isotope ratios of the elements of carbon, nitrogen, and sulfur, in the tissues (e.g., blood, muscle, bone, or hair) of a consumer reflect the isotope ratios in the sources it

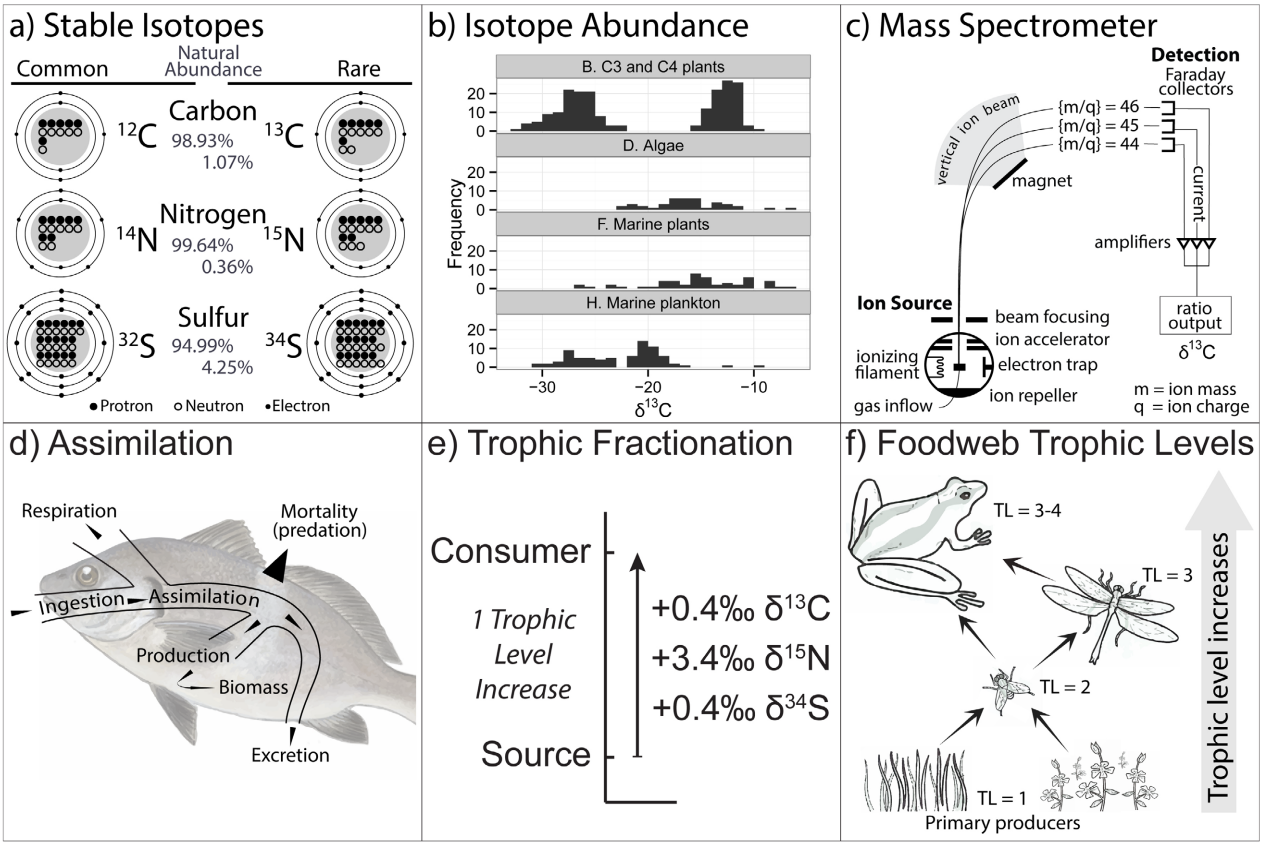


Figure 1. Stable isotope sourcing and trophic level background. (a) The carbon atom is defined by having six protons. The common isotope with six neutrons is called "carbon-12" (^{12}C) with a natural abundance of 98.93% and the rare isotope has seven neutrons (^{13}C) with a natural abundance of 1.07%. (b) Different photosynthetic pathways lead to distinct isotope ratios. This partial reproduction of Figure 9-3 from Deines (1980) illustrates B. known C3 and C4 terrestrial plants (C3 photosynthesis captures less ^{13}C than C4 photosythesis), D. algae, F. marine plants exclusive of plankton, and H. marine plankton. (c) Using a magnetic force on moving charged particles, a mass spectometer separates molecules by mass measuring the relative concentrations of isotopes in a sample. (d) Within a species, we are interested in the process of ingesting sources, assimilating a proportion of each source into the consumer's tissues while excreting the remaining proportion of the source, and ultimately possibly being consumed by a higher species. (e) Trophic fractionation is the result of digestion and assimilation where lighter isotopes are preferentially excreted with the result that the consumer's tissues are isotopically heavier than the sources consumed. (f) The food web is a directed graph defining the flow of nutrients from sources to consumers. Trophic level increases by one unit for each step up a food chain.

consumes weighted by the proportion of each in the diet, after corrections for the processes of digestion and assimilation. While the use of stomach and fecal contents still provides critical insight into feeding relationships, there are some very well-understood limitations for evaluating dietary breadth [11] making stable isotope analysis preferred in many contexts [11] [12] [13]. The model developed here provides direct answers to the two core ecological questions necessary for food web reconstruction: 1) What is the trophic position of a consumer species in a food web? 2) What are the proportional contributions of sources to consumer diets?

1.2. Literature Background

Analysis of food web structure has increasingly relied upon stable isotope data

and models because temporal and spatial trophic relationships can be inferred among species. While many of the early (1970s) innovating stable isotope applications were qualitative, more recent (2000s) quantitative methods have become numerous and specific and have greatly increased our understanding of food webs. Three recent reviews discuss the primary analytical tools for, Bayesian models for, and best practices for applying stable isotope models for understanding food web structure.

A variety of analytical methods for estimating species trophic level, estimating species diet, refining foodweb structure, and understanding intrapopulation trophic variability have been proposed [14]. When estimating trophic level, metaanalysis is dependable when averaging over many food chains [15], but literature values are currently unreliable for estimating trophic level for individuals or single species [16].

Inference on diet has focused on using a mass-balance mixing model as the primary defining relationship between the consumer and sources isotope ratios (basic mixing model, BMM) [2]. Extensions soon followed to incorporate elemental concentrations (concentration mixing model, CMM) [17] and assimilation efficiencies (extended mixing model, EMM)) [1]. The mixing model defines the mean isotope ratio of the consumer as a convex combination of the mean isotope ratios of the sources, where the weights in the convex combination are the proportional contributions of the sources to the consumer's diet. For discussion, in the context of a single consumer, let I be the number of isotopes measured and S be the number of sources considered. The diet probability vector starts with $S-1$ degrees-of-freedom (given the simplex constrain that the sum of proportions equals 1), and each isotope provides an equation in a linear system reducing the degrees-of-freedom by one. Thus, given I and S, three situations for the diet probability vector are possible: 1) when $S = I+1$ the diet is perfectly constrained with a unique solution; 2) when $S < I+1$ the diet is overconstrained and there are generally no solutions (provided no equations are collinear); and 3) when $S > I+1$ the diet is underconstrained and there are infinitely many solutions. Different methods apply to one or more of these situations.

Frequentist methods apply to cases (1) and (2). In the perfectly constrained case (1), [17] applied the delta method to situations with $S = 2$ or 3 for the BMM and [18] extended this for arbitrary S for the EMM using the implicit function theorem. In the overconstrained case (2), [19] provide a detailed frequentist approach deriving the asymptotic distributions using weighted least squares (WLS) of both non-temporal and temporal models, show that including "uninformative" isotopes in the model does not reduce the efficiency in the WLS estimator (thus isotopes that do not distinguish sources well may still improve estimate precision), and include a lack-of-fit strategy.

Solution-polytope methods apply to case (3). In the underconstrained case (3), which is the most common case of having many sources but few measured isotopes, the first strategy by [20] ignored variation and returned approximate de-

terministic solutions for the mixing model by testing every possible combination of source proportions in small increments implemented in software. This strategy was improved upon by [21] in software (available since 2007), which returns exact probabilistic solutions by quickly sampling uniformly over the solution polytope; this method is an approximate Bayesian large sample procedure.

Bayesian methods apply to all three cases (1), (2), and (3). [3] discusses a Bayesian stable isotope mixing model (SIMM) with shared random effects and the previous decade of development by their coauthors in this area. [22] formulates a Bayesian model similar to [3] but without shared random effects, and discuss how the shared random effects may be problematic. [23] provides a Bayesian R package for trophic estimation for a version of the BMM.

There are suggestions regarding best practices for using SIMMs, including using prior knowledge for foodweb structure and model parameters, including isotope ratios, fractionation, concentrations, assimilation efficiency, diet proportions, and trophic level, data collection strategies, plotting data, grouping sources when reasonable, reporting uncertainty in estimates, and being explicit about limitations of the analysis [24].

The innovations in our model include the: 1) simultaneously inference of diet and trophic level in a 2) multi-level foodweb using a 3) Bayesian EMM. This is a Bayesian formalization and extension of the idea by [25] who used a heuristical two-step approach first to estimate diet using δ^{13}C and δ^{34}S, then to estimate trophic level using δ^{15}N within a single-level foodweb. In our model, trophic level is estimated by averaging over all the food chains represented in the hypothesized foodweb. We extend the model by [22] for simultaneous trophic-level modeling with a detailed explanation of the incorporation of prior information. Additional features include: 1) prediction (imputation) of missing values at each step in the MCMC so incomplete information can be used; 2) treatment of the mean assimilation efficiency as random and potentially correlated between isotopes; 3) inclusion of correlation between any data measured together and transformation to a sensible scale, such as isotope and (logit) concentration values; 4) ease to include discrimination estimated from diet experiments, regression, or other methods, with the appropriate uncertainty; and 5) consolidation of source estimates into a "combined" source, if desired. We apply the model to a previously published bottlenose dolphin and fish foodweb [25] [26] [27] [28].

2. Extended Bayesian Stable Isotope Mixing Model

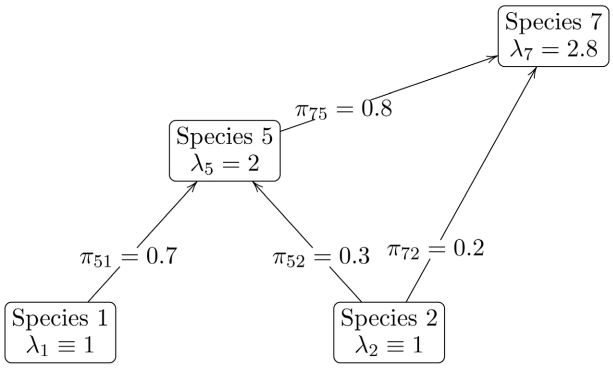
Our model is composed of four hierarchical submodels to estimate parameters for source isotope ratio and concentration (L_S), fractionation (L_F), assimilation efficiency (L_S), and consumer isotope ratio (L_C), parameters of which are connected through a defining relationship with trophic level and diet. The full posterior distribution is the product of the submodels,
$g(\text{parameters} \mid \text{data}) \propto L_S L_F L_E L_C$. Each submodel has a sampling model for the data given parameters and a prior distribution for the parameters. While there

are many parameters, the relationships of individual components are simple. Our basic sampling models assume random samples $\mathcal{D}$ from multivariate $N(\mu, \Sigma)$ distributions, but alternate distributions may be substituted. We use $f(\cdot)$ and $g(\cdot)$ to identify a generic function and a probability distribution, respectively.

In the following sections we specify the defining relationship (the functional relationship of model parameters), the four hierarchical submodels, considerations for Bayesian inference, and results of simulation studies. Simulation Scenario 1 presented in Figure 2 is used for model exposition illustration and model validation, defining a simple foodweb containing all the modeling complexities in the dolphin example; **Appendix B** includes two additional simulation scenarios for further model validation.

2.1. Defining Relationship

Following [22], we formulate a general extended mixing model (EMM) for trophic level inference in terms of population means. In this section, to illustrate diet and trophic level parameters, we refer to the simulation scenario in Figure 2 with two primary sources (s1 and s2), one consumer (s5) of primary sources, and a consumer (s7) of a primary source and the other consumer. Given a directed adjacency matrix for the hypothesized food web graph, let m indicate a particular consumer (mixture) species with source species listed in the vector ℓ_m of length S_m indicating the non-zero column numbers for row m. For example, the two consumers are $m=5$ with $\ell_5=\{1,2\}$ and $m=7$ with $\ell_7=\{2,5\}$. Let π_m be the probability vector of proportional contributions of the S_m sources to the mean diet for consumer m [e.g., $\pi_7=(\pi_{72}=0.2, \pi_{75}=0.8)^{\mathrm{T}}$]. Let λ_m be the mean trophic level of consumer m or λ_s be the mean or defined trophic level of source s (e.g., $\lambda_1 \equiv 1$, $\lambda_2 \equiv 1$, $\lambda_5=2$, and $\lambda_7=2.8$). Let δ_{is} be the mean isotope ratio for isotope i in source s. Let Δ_i be the mean per trophic level enrichment for isotope i, previously illustrated in Figure 1(d). Let



m \ s	1	2	5
5	1	1	0
7	0	1	1

Figure 2. Simulation Scenario 1. (left) Species 1 and 2 are producers (primary autotroph sources) and are both trophic level 1 by definition. Species 5 consumes a diet consisting of 70% Species 1 and 30% Species 2, and is trophic level 2. Species 7 consumes a diet consisting of 80% Species 5 and 20% Species 2, thus its trophic level is $0.8(2)+0.2(1)+1=2.8$. (right) The associated directed adjacency matrix.

κ_{is} be the typical concentration of element i in source s, and η_{mis} be the typical assimilation efficiency for consumer m of element i for source s.

The population mean vector isotope ratio (δ_m) within consumer population (species) m is assumed to be a convex combination of the mean isotope ratios (δ_m) in the source populations, $s \in \ell_m$, accounting for trophic fractionation (Δ), elemental concentration (κ), and assimilation efficiency (η). Assuming there are I isotopes (three in our applications: C, N, and S), the defining relationship for the EMM [1], is

$$\text{EMM}: \delta_{mi} = \frac{\sum_{s\in\ell_m}\left\{\delta_{is} + \left(\lambda_m - \lambda_s\right)\Delta_i\right\}\kappa_{is}\eta_{mis}\pi_{ms}}{\sum_{s\in\ell_m}\kappa_{is}\eta_{mis}\pi_{ms}}, \quad i = 1,\cdots,I. \tag{1}$$

Two special cases include the (CMM), where $\eta_{mis} \equiv 1$ for all i and s, and the (BMM) in (2), where both $\kappa_{is} \equiv 1$ and $\eta_{mis} \equiv 1$ for all i and s,

$$\text{BMM}: \delta_{mi} = \sum_{s\in\ell_m}\left\{\delta_{is} + \left(\lambda_m - \lambda_s\right)\Delta_i\right\}\pi_{ms}, \quad i = 1,\cdots,I. \tag{2}$$

Note that while the EMM interpretation of π_m is "how much did the consumer eat", the BMM interpretation is "how much did the consumer assimilate", especially when the concentration and assimilation assumptions for the BMM are not sensible.

The next several subsections make explicit standard stable isotope mixing model assumptions; we discuss the trophic level innovation last.

2.2. Source Isotope Ratio and Concentration Model, L_S

The source mean isotope ratio and concentration vectors, $\mu_{\delta\kappa s} = \left(\delta_s^{\mathrm{T}}, f\left(\kappa_s\right)^{\mathrm{T}}\right)^{\mathrm{T}}$, $s \in \ell_m$, are estimated based on independent samples from each of the source populations. Let $\mathcal{D}_s = \left\{\left(\mathbf{d}_{sk}^{\mathrm{T}}, \mathbf{c}_{sk}^{\mathrm{T}}\right)^{\mathrm{T}}\right\}_{k=1}^{K_s}$ be a random sample of size K_s from source population s, with

$$\begin{bmatrix} \mathbf{d}_{sk} \\ f\left(\mathbf{c}_{sk}\right) \end{bmatrix} \Bigg| \mu_{\delta\kappa s}, \Sigma_{\delta\kappa s} \overset{\text{ind}}{\sim} \text{Normal}\left(\mu_{\delta\kappa s}, \Sigma_{\delta\kappa s}\right), \tag{3}$$

$k = 1,\cdots,K_s$, where $\Sigma_{\delta\kappa s}$ is the covariance matrix. Each $\mathbf{d}_{sk}$ and $\mathbf{c}_{sk}$ have I elements, one for each isotope. Assuming independence across samples and priors, the source isotope ratio and concentration model for sources contributing to consumer m is $L_{\mathrm{S}} = \prod_{s\in\ell_m} g\left(\mathcal{D}_s \mid \mu_{\delta\kappa s}, \Sigma_{\delta\kappa s}\right) g\left(\mu_{\delta\kappa s}, \Sigma_{\delta\kappa s}\right)$, where $g\left(\mathcal{D}_s \mid \mu_{\delta\kappa s}, \Sigma_{\delta\kappa s}\right)$ is a product of K_s multivariate normal densities and the prior $g\left(\mu_{\delta\kappa s}, \Sigma_{\delta\kappa s}\right)$ is to be specified in Section 2.6.

2.3. Per Trophic Level Fractionation Model, L_F

Ideally, fractionation for each consumer-source pair is determined by conducting a diet experiment and estimating the difference between the mean consumer and mean source isotope ratio vectors. In this case, the isotope partition of model (3) can be used independently for each of the consumer and diet tissues, where the difference between the mean vectors is the trophic fractionation, Δ.

The more common situation is to use trophic fractionation estimates from prior studies. In this case, a prior for Δ can be specified, and is often assumed to be the same for multiple consumer-source pairs, $L_{\mathrm{F}} = g\left(\Delta, \Sigma_{\Delta}\right)$, see Section 2.6.

2.4. Assimilation Efficiency Model, L_{E}

Recall, the assimilation efficiency for consumer m is the proportion of each element $i = 1, \cdots, I$ in the diet portion from source s that is digested and becomes incorporated in the measured tissue of consumer m. Assimilation efficiencies can vary by consumer-source pairs, by element, and by consumer tissue type (blood, muscle, fat, hair, etc.).

The assimilation efficiency vectors, $\mu_{\eta e}$, $e = 1, \cdots, E$, are estimated based on samples from E diet experiments or other estimation technique. Let $\mathcal{E}_e = \left\{\mathbf{e}_{ek}\right\}_{k=1}^{K_e}$ be a random sample of size K_e from the eth experiment, with

$$f\left(\mathbf{e}_{ek}\right) \mid \mu_{\eta e}, \Sigma_{\eta e} \overset{\text{ind}}{\sim} \text{Normal}\left(\mu_{\eta e}, \Sigma_{\eta e}\right), \tag{4}$$

$k = 1, \cdots, K_e$ and $e = 1, \cdots, E$. Each $\mathbf{e}_{ek}$ has I elements, one for each isotope. Assuming independence across samples and priors, the assimilation efficiency model is $L_{\mathrm{E}} = \prod_{e=1}^{E} g\left(\mathcal{E}_e \mid \mu_{\eta e}, \Sigma_{\eta e}\right) g\left(\mu_{\eta e}, \Sigma_{\eta e}\right)$. Finally, assimilation efficiencies for each source are $\eta_{ms} = f^{-1}\left(\mu_{\eta e}\right)$ for an appropriate pairing of consumer m and source s with experiment e.

Note that both concentration and assimilation efficiency are proportions. Thus, if either are not normally distributed, for example because the proportions are near the 0 or 1 boundary, the specification of $f(\cdot)$ might be $\text{logit}(\cdot)$ or another appropriate transformation so that the data on the transformed scale are normal.

2.5. Consumer Model, L_{C}

We obtain a random sample $\mathcal{B}_m = \left\{\mathbf{b}_{mj}\right\}_{j=1}^{J_m}$ of isotope (and concentration values, if consumer m is a source to another consumer) responses from each consumer population, $m = 1, \cdots, M$, with $\mathbf{b}_{mj} \mid \pi_m, \Sigma_{bm}, \lambda_m, \Theta_m \sim \text{Normal}\left(\delta_m, \Sigma_{bm}\right)$, where Θ_m includes the δ, κ, η, Δ, and λ means related to consumer m. The population mean response δ_m depends on π_m and Θ_m through the in (1), so the normal sampling model $g\left(\mathcal{B}_m \mid \pi_m, \Sigma_{bm}, \Theta_m\right)$ is conditional on $\left(\pi_m, \Sigma_{bm}, \Theta_m\right)$ but independent of Σ_{Θ_m}, the covariance matrices associated with Θ_m. The consumer model is $L_{\mathrm{C}} = \prod_{m=1}^{M} g\left(\mathcal{B}_m \mid \pi_m, \Sigma_{bm}, \Theta_m\right) g\left(\pi_m\right) g\left(\Sigma_{bm}\right) g\left(\lambda_m\right)$, where the priors on π_m and Σ_{bm} are independent, and independent of Θ_m. We also assume $g\left(\Sigma_{bm}\right)$ is defined as in Section 2.6.

2.6. Prior Distributions

The consumer sample $\mathcal{B}$ and each source and assimilation efficiency sample $\mathcal{D}_s$ and $\mathcal{E}_e$ assumes a $\text{Normal}\left(\mu, \Sigma\right)$ model. It remains to specify prior distributions for the μ and Σ parameters to complete the L_{S}, L_{F}, and L_{E} model components, and the covariance component of L_{C}. For convenience, we follow

common practice and use the conjugate prior $\mu \mid \Sigma \sim \text{Normal}\left(\mu_0, \Sigma/\nu_0\right)$ and $\Sigma \sim \text{Inv-Wishart}\left(\Sigma_0, \nu_0\right)$, the product of which is called the distribution. A limiting form of the conjugate prior is Jeffrey's prior, $g\left(\mu, \Sigma\right) \propto \left|\Sigma\right|^{-(I+1)/2}$.

2.7. Diet Model

For the diet model there are several options for modeling a probability vector on the simplex. One option is to assume $\pi_m \sim \text{Dirichlet}\left(J_{0m}\pi_{0m}\right)$, where π_{0ms} is the prior knowledge for the *s*th diet proportion and J_0 is the effective sample size for the prior. Choosing $J_0\pi_{0ms} = 1$, $s = 1, \cdots, S$, defines a uniform distribution over the simplex. While the Dirichlet imposes a restrictive correlation structure, it requires only S_m parameters, the number of diet sources for consumer *s*.

For a more general covariance structure, options include the generalized Dirichlet distribution [29] or logistic-normal distribution [30]. Modeling the covariance structure between elements of the diet probability vector will be most useful in the case when the model is identifiable (the (1) perfectly constrained and (2) overconstrained cases) or when strong prior information exists (e.g., that pairs of sources are commonly consumed together or substituted for each other).

2.8. Trophic Level Model

The consumer trophic level model, $g\left(\lambda_m \mid \lambda_{0m}\right)$, can be flexible to accommodate any distribution summarizing the best knowledge. For example, if trophic values are equally likely within bounds, a uniform distribution may be specified; for example, $\lambda_m \mid \lambda_{0m1}, \lambda_{0m2} \sim \text{Uniform}\left(\lambda_{0m1}, \lambda_{0m2}\right)$, where $\lambda_{0m1} = 1$ and $\lambda_{0m1}, \lambda_{0m2}$ represents four trophic levels of uncertainty. Other sensible choices include a normal distribution or a location-scale beta distribution.

Source trophic levels, λ_s, might be considered fixed and known, particularly primary producer sources ($\lambda_s = 1$) or sources feeding exclusively on primary producers ($\lambda_s = 2$). But, they may also be given distributions to represent uncertainty in their values.

2.9. Modeling Consumers of Consumers

We consider two possible strategies for modeling trophic level and diet in a multilevel foodweb. The first strategy (A) models each consumer as a function of the primary sources (those sources on which all consumers depend) with potential intermediate consumers implicitly modeled. The diet graph for this strategy has primary sources connect directly to each consumer bipassing intermediate consumers. The second strategy (B) models a higher consumer in the hypothesized foodweb by treating the specified intermediate consumers explicity as sources to that higher consumer. This is the strategy described in the model exposition. **Figure 4** illustrates both models using the example described in Section 3. In general, the foodweb can be defined as a directed acyclic graph, with cycles being modeled implicitly (such as a canibalistic population), thus a higher consumer

can consume primary sources or explicitly-specified intermediate consumers.

Given the model specifications above it remains to specify informative priors for model parameters and fit the joint posterior distribution given the data and priors. After model considerations in Section 2.10, and model checking of simulated scenarios in Section 2.12, we make trophic level inference for an aquatic foodweb in Section 3.

Modeling Proportions on the Logit Scale

The concentration and assimilation efficiency proportion parameters can be modeled on the logit scale using the multivariate logistic normal distribution [30] [31] and, for concentrations, incorporated in the multivariate normal model. This strategy meets the desire to compute on MVN distributions with covariation within concentrations and with isotopes while imposing a sensible distribution on the proportion scale.

2.10. Bayesian Inference: General Considerations

Many of the parameters of the full posterior distribution $g(\text{parameters} \mid \text{data}) \propto L_{\text{S}} L_{\text{F}} L_{\text{E}} L_{\text{C}}$ are of minor interest. A more focused analysis considers the parameters $\left(\left\{\lambda_{\beta m}, \pi_m, \Sigma_{bm}\right\}_{m=1}^{M}, \Theta_m\right)$ that index the sampling distribution of each consumer isotope ratio distribution. The source samples and diet experiments can then be viewed as primarily needed to generate prior information for Θ_m, which is required to estimate λ_m and π_m, the features of primary interest. In particular, each source and diet sample $\mathcal{D}$ contributes $g(\mathcal{D} \mid \mu, \Sigma) g(\mu, \Sigma) = g(\mu, \Sigma \mid \mathcal{D}) g(\mathcal{D})$ to the joint distribution for some (μ, Σ). As Σ does not appear in the consumer model, it can be integrated out of $g(\mu, \Sigma \mid \mathcal{D})$, giving $g(\mu \mid \mathcal{D})$ which can be used as an "updated prior" along with the consumer model; see [22] for an exact importance sampling algorithm. The full posterior can be simulated in available software, such as WinBUGS [32], OpenBUGS, or JAGS, provided all priors are proper. Finally, inference for the parameters of interest can be summarized from their posterior distributions.

The Bayesian method will use all observations, including those with missing values, by predicting (imputing) the missing data on each MCMC step then using the full "augmented" dataset for parameter estimation.

2.11. Parameter Identifiability and MCMC Convergence

Convergence is a problem in implementations of the full EMM. The defining relationship in (1) specifies a product of the three proportion parameters for diet (π), concentration (κ), and assimilation efficiency (η). The MCMC chains for these parameters endlessly drift aimlessly over the entire range of their support, possibly due to identifiability issues. In simulations, this is true even when the priors for η are extremely informative (e.g., nearly a point mass worth 1000 s of observations). The BMM does not have this challenge. It converges quickly to the correct posterior. Thus, successful application of the EMM requires addi-

tional constraints, such as setting κ and/or η to constants or more stringently bounding their support. Such more stringent bounds would not be unreasonable, for example the observed ranges of concentrations of carbon or nitrogen in a particular source population is relatively narrow (maybe 20%) over the [0, 1] interval, similarly for assimilation efficiencies. More work to address this issue is required.

2.12. Model Checking for Simulation Scenario 1

To check both that model inferences make sense and that the model is consistent with the data, posterior predictive distributions and posterior densities are assessed. Results from Simulation 1 shown in **Figure 3**, detailed in the Supplementary Materials Section A, indicate concordance between the posterior and true values, and the bias that exists is a function of the simulated data having a mean slightly different from the true mean. See Appendix Section B for results of more extensive simulation Scenarios 2 and 3.

3. Bottlenose Dolphin BMM Example

3.1. Description of Data and Goals

Data collection methods and elemental analysis are described elsewhere [25] [26], and we limit ourselves to summarizing the data and goals for this example. Potential organic matter sources were collected from St. George Sound, just offshore of the Florida State University Coastal and Marine Laboratory, St. Teresa, Florida from April 2007 through July 2009. Potential sources collected included seagrass epiphytes, macroalgae, benthic microalgae, plankton, and seagrass. Consumer samples were collected by otter trawl during April through November of the sampling years (2007-2009). Consumers sampled included numerically abundant species: Atlantic croaker (*Micropogonias undulatus*), pigfish (*Orthopristis chrysoptera*), pinfish (*Lagodon rhomboides*), mojarra (*Eucinostomus gula*), and silver perch (*Bairdiella chrysoura*), among others. Fish were gutted and muscle tissue was removed from bones and skin, dried, and ground to a fine

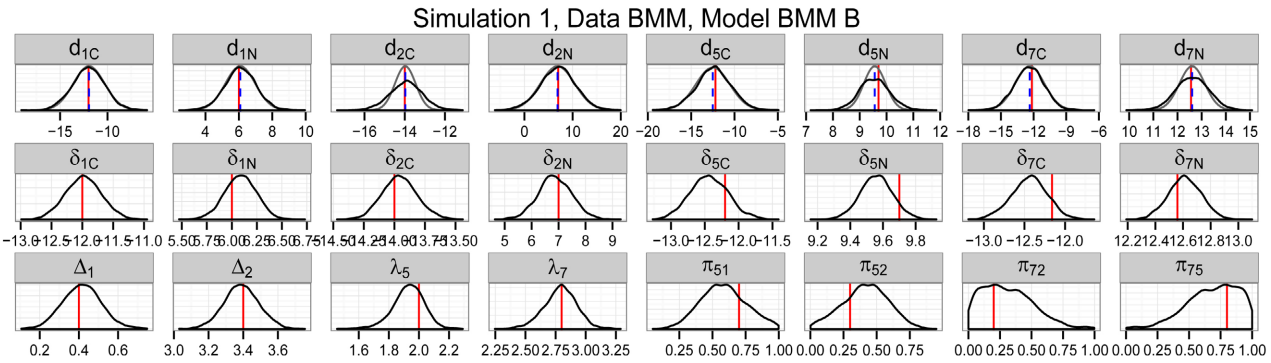


Figure 3. Simulation 1 Strategy B posterior predictive distributions (top row) and posterior densities. Black curves are the posterior distributions, gray curves are the data distributions (top row), red solid lines indicate the true parameter value input to the simulation, and blue dashed lines indicate the observed mean data value.

powder for isotopic analysis. Bottlenose dolphin (*Tursiops truncatus*) skin tissues were obtained via remote dart biopsy, dried, and ground to a homogeneous powder for isotopic analysis. Stable isotope and elemental concentration analyses for carbon (δ^{13}C, [C]), nitrogen (δ^{15}N, [N]), and sulfur (δ^{34}S, [S]) were conducted.

We ask one set of questions using two complimentary models of the foodweb discussed in Section 2.9 and shown in Figure 4. "For each consumer (mixture), what proportion of the consumer's mean population diet (π_m) comes from each source and what is its mean trophic level (λ_m)?" Model A describes the proportion of a consumer's diet (Atlantic croaker, pigfish, pinfish, mojarra, silver perch, and bottlenose dolphin) originating from the primary sources (seagrass epiphytes, macroalgae, benthic microalgae, plankton, and seagrass) implicitly via intermediate consumers, and the trophic level of each consumer. Model B is the same except dolphin are explicitly modeled using the five consumer fish as sources.

The data plotted in Figure 5 are the source and consumer samples in isotopic space. The (δ^{13}C, δ^{15}N) plot indicates that nitrogen will provide the primary information regarding trophic level because of the large fractionation from sources to consumers and the narrow source convex hull. Some sources and consumers have strong correlations between measurements, others do not. There isn't strong evidence of violation of the multivariate normal distributional assumptions for isotope ratios. Missing data (benthic microalgae has 4 missing sulfur values, plankton has 2 missing sulfur values) are imputed at each MCMC step, so no data are discarded due to partial missingness.

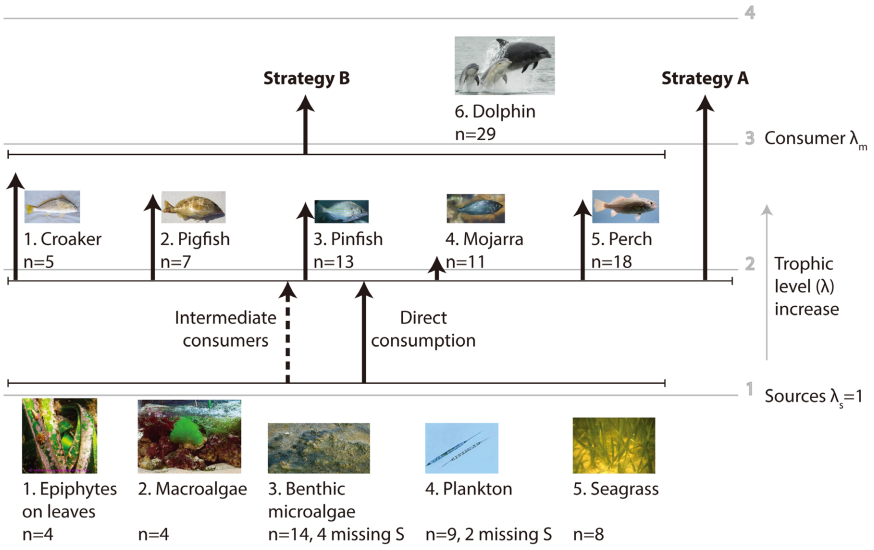


Figure 4. Primary producers (sources) are at trophic level 1. Strategy A describes the proportion of a consumer's diet originating from the primary sources, implicitly via intermediate consumers. Strategy B explicitly models the dolphin diet as proportions of five consumer fish. For each organism, the sample size and information about missing values are provided.

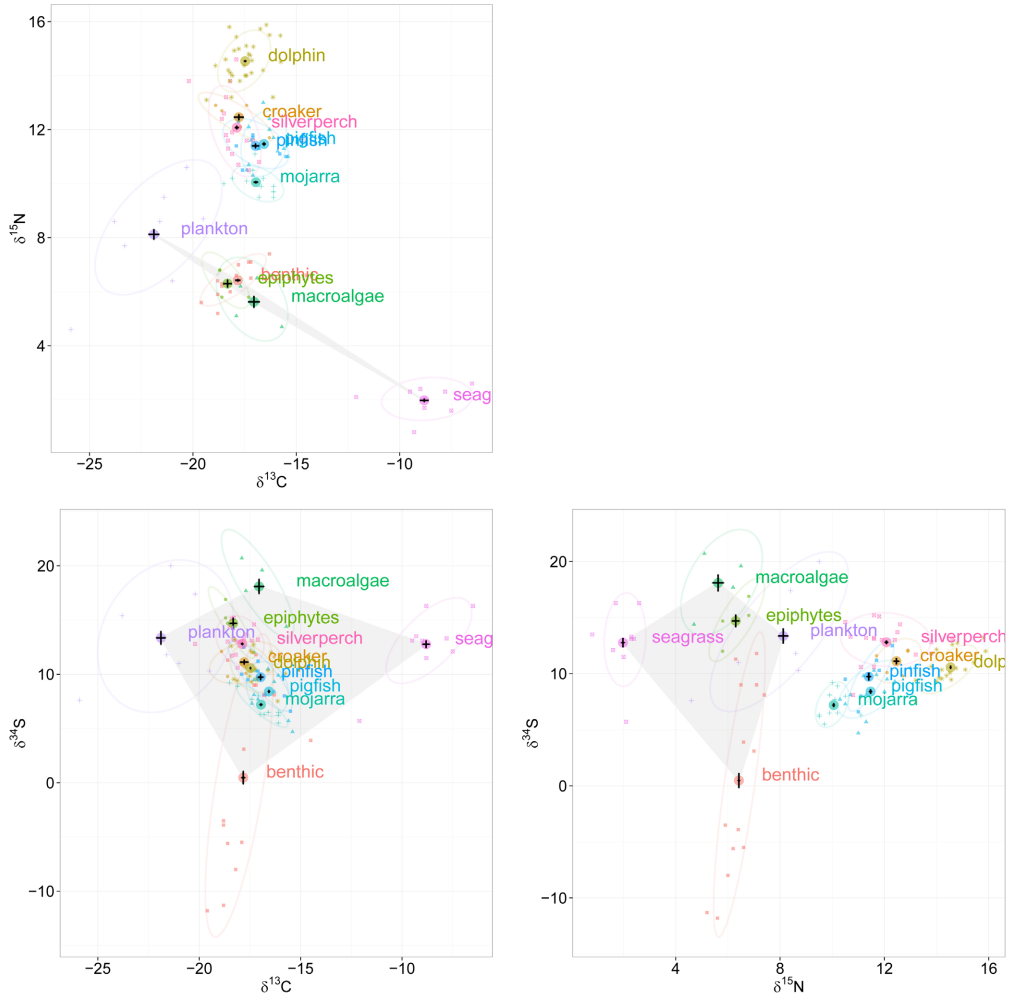


Figure 5. Dolphin dataset isotope ratio observations for the five sources, five fish consumers, and the dolphin consumer. Small points indicate observations, large points indicate the sample mean with 95% confidence intervals for the mean and 75% confidence ellipse for the data distribution, and the shaded region is the convex hull of the source means where, after adjusting for trophic fractionation, the consumer means must lie under the basic mixing model.

To explore a factorial design of model choices, we present the results from the BMM using the following options for comparisons (many others were also run): isotope covariance as independent or selected covariance relationships; diet prior either using a vague or informative prior; trophic level prior either using a vague or informative prior; and Model strategies A and B are fit as separate models (16 combinations). The selected covariance relationships we choose to model depends on the sample size for each source and on the observed covariance relationships among the three-dimensional isotope observations. We have support for modeling all covariance terms for benthic and plankton sources, all for mojarra and dophin consumers, but not enough data or covariance to support modeling other terms. It is not necessary to model all covariances or none. Given enough data we would model all covariances and let the data decide, but when data is sparce fewer parameters will improve model estimation.

Number of Parameters vs Observations

In our example, three isotope ratio and three concentration measurements are jointly taken on each source observation. For a three-dimensional multivariate normal model there are 9 parameters to estimate: 3 means, 3 variances, and 3 covariances. While we typically would like at least as many observations as parameters estimated, the Bayesian model allows for fewer, though estimation may be strongly influenced by the prior (even with a weakly informative prior). Simplifing the multivariate normal model can reduce the number of parameters substantially (since most parameters are variance/covariance terms) by treating certain values as being independent. For example, if isotope ratios are assumed independent, the number of parameters reduces to 6 (3 × (1 mean and 1 variance)). Note that in some cases, even an independence model has more parameters than we have observations. Most marine studies will ordinarily not have many source samples, as they are much more time consuming to both collect and process than fish samples. J.W. Tukey suggests that the calculation of the k^{th} moment ought to be based on at least 5^k observations, as found on p. 1.72 of [33], thus at least 25 observations should be used to estimate variances and covariances. Therefore, for best estimation of sources, consider sample sizes of at least a few more than the number of parameters in the model, which would be 15 - 25 or more for the full multivariate normal model with three isotope ratios and three concentrations. Parameter estimates will be more precise if correlation is modeled when it exists.

3.2. Informative Prior, Distributions

While the general model provides the flexibility for any covariance structure among parameters informed by multivariate data, many of the prior distributions have a simplier structure (Section 3.1.1) because covariation information is either absent from the summaries in the literature and/or because there is insufficient evidence in the data to model dependencies. Since consumer (mixture) data is plentiful and because of observed relationships we model the full covariance matrix for consumer isotopes. Since source data is scarce for some sources, and because insufficient evidence exists to suggest nonzero correlation between some pairs of isotopes and concentrations, we reduce the number of parameters by modeling only specific relationships.

Prior information from seven literature sources to inform all the model parameters is provided in **Table 1** for sources and **Table 2** for consumers.

3.3. Results

We focus on the two primary quantities of interest: trophic level and diet proportions. While meaningful informative trophic level priors were provided for all consumers, informative diet proportions priors were provided for only pinfish and dolphin.

3.3.1. Trophic Level Results

Selected trophic level posterior distributions are presented in **Figure 6**. We

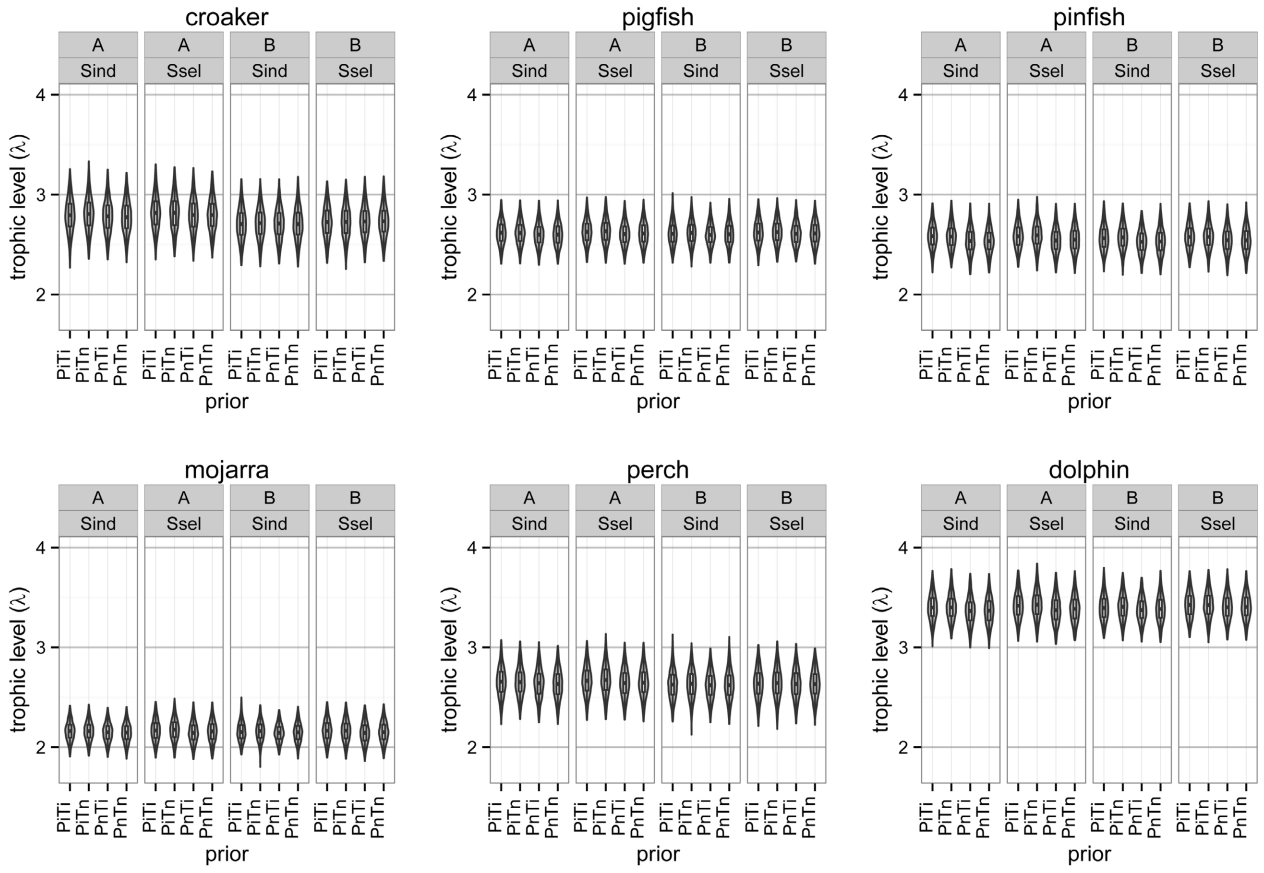


Figure 6. Posterior distributions for trophic level (λ_m) for all consumers and model types: model , independent (Sind) or selected (Ssel) covariance relationships, vague (Pn) or informative (Pi) diet prior, vague (Tn) or informative (Ti) trophic level prior, and model strategies A or B.

Table 1. Prior information used to specify source-related model hyperparameters.

Source isotope ratios for L_S					Citations
s	name	$\mu_{0\delta s}^{\mathrm{T}}$	$\Sigma_{0\delta s}$	$\upsilon_{0\delta s}$	Isotopes
1	epiphytes	$(-17.55, 5.85, 18.20)$	$\mathrm{diag}\left(1.97^2, 1.05^2, 2.05^2\right)$	1 + 2	$\mathrm{CNS}^{\mathrm{MS01}}$
2	macroalgae	$(-16.75, 7.00, 18.18)$	$\mathrm{diag}\left(0.50^2, 2.82^2, 1.60^2\right)$	1 + 2	$\mathrm{CNS}^{\mathrm{MS01}}$
3	benthic	$(-15.80, 7.25, 3.90)$	$\mathrm{diag}\left(1.56^2, 0.78^2, 3.68^2\right)$	1 + 2	$\mathrm{CN}^{\mathrm{MS01}}\ \mathrm{S}^{\mathrm{CNP95}}$
4	plankton	$(-21.77, 9.92, 15.38)$	$\mathrm{diag}\left(0.77^2, 0.96^2, 2.55^2\right)$	1 + 2	$\mathrm{CNS}^{\mathrm{MS01}}$
5	seagrass	$(-12.20, 6.05, 11.48)$	$\mathrm{diag}\left(1.32^2, 1.22^2, 4.10^2\right)$	1 + 2	$\mathrm{CNS}^{\mathrm{MS01}}$
Per-trophic level fractionation factors for L_F					
i	name	$\mu_{0\Delta}$	$\sigma_{0\Delta}^2$	$\upsilon_{0\Delta}$	
1	carbon	0.4	1.3^2	107	$\mathrm{C}^{\mathrm{P02}}$
2	nitrogen	3.4	1^2	56	$\mathrm{N}^{\mathrm{P02}}$
3	sulphur	0.4	$1.9^2 = \left(0.52\sqrt{13}\right)^2$	13	$\mathrm{S}^{\mathrm{ML03}}$

$^{\mathrm{CNP95}}$[34], $^{\mathrm{ML03}}$[35], $^{\mathrm{MS01}}$[36], $^{\mathrm{P02}}$[37].

Table 2. Prior information used to specify consumer-related model hyperparameters.

	Consumer isotope ratio variances for L_C				
m	name		Σ_{0bm}	ν_{0bm}	
1	croaker		$\text{diag}\left(2.75^2, 0.50^2, 1.49^2\right)$	3	CNS[SM93]
2	pigfish		$\text{diag}\left(0.85^2, 0.87^2, 1.71^2\right)$	6	CNS[SM93]
3	pinfish		$\text{diag}\left(0.35^2, 0.57^2, 0.81^2\right)$	2 + 1	CNS[SM93]
4	mojarra		$\text{diag}\left(0.35^2, 0.57^2, 0.81^2\right)$	3	as pinfish[g]
5	perch		$\text{diag}\left(2.75^2, 0.50^2, 1.49^2\right)$	3	as croaker[g]
6	dolphin		$\text{diag}\left(1.00^2, 1.00^2, 1.73^2\right)$	3	inflating sample var[g]
	Consumer diet proportions for L_C				
m	name	π_{0m}^{T}		J_0	
1	croaker	$(0.20, 0.20, 0.20, 0.20, 0.20)$		$5 = S$	diet[g]
2	pigfish	$(0.20, 0.20, 0.20, 0.20, 0.20)$		5	diet[g]
3	pinfish	$(0.20, 0.20, 0.20, 0.20, 0.20)$		5	diet[g]
4	mojarra	$(0.20, 0.20, 0.20, 0.20, 0.20)$		5	diet[g]
5	perch	$(0.20, 0.20, 0.20, 0.20, 0.20)$		5	diet[g]
6	dolphin	$(0.20, 0.20, 0.20, 0.20, 0.20)$	(of fish consumers 1 - 5)	5	diet[g]
3	pinfish	$(0.47, 0.30, 0.05, 0.06, 0.12)$		10	diet[WAG07]
6	dolphin	$(0.23, 0.23, 0.23, 0.08, 0.23)$	(of fish consumers 1 - 5)	10	diet[g]
	Consumer trophic levels for L_C		**Source trophic levels**		
m	name	$\text{Uniform}\left(\lambda_{0m1}, \lambda_{0m2}\right)$	s name	λ_{0s}	
1	croaker	(1, 5)	1 epiphytes	1	Consumer[g]
2	pigfish	(1, 5)	2 macroalgae	1	Source[g]
3	pinfish	(1, 5)	3 benthic	1	
4	mojarra	(1, 5)	4 plankton	1	
5	perch	(1, 5)	5 seagrass	1	
6	dolphin	(2, 6)			
m	name	$\text{Normal}\left(\lambda_{0m1}, \lambda_{0m2}\right)$			Consumer[AW06,AW08]
1	croaker	$(2.87, 0.19^2)$ from $n = 175$			
2	pigfish	$(2.16, 0.41^2)$ as pinfish			
3	pinfish	$(2.16, 0.41^2)$ from $n = 316$			

Continued

4	mojarra	$(2.16, 0.41^2)$ as pinfish
5	perch	$(2.87, 0.19^2)$ as croaker
6	dolphin	$\left(2.16+1, \left(0.41+0.2\right)^2\right)$ as pinfish

[AW06, AW08][38] [39], using summer mean estimates of trophic level from [38] (**Table 2**, no uncertainty), so we use SEs from [39] which include both summer and winter so will overestimate uncertainty, and assume that pigfish is similar to pinfish. [SM93][40], [WAG07][41] we allocated their algae, benthic, plankton, and seagrass, then the remainder (C3 and C4) was assigned to epiphytes [g]guess by the authors.

observe generally that pinfish are estimated to be between trophic level 2.4 and 2.8, indicating reliance on both primary sources and intermediate consumers; mojarra are between 1.8 and 2.4, indicating reliance principally on primary sources; and dolphin are between 2.9 and 3.7. Trophic levels are estimated slightly lower for vague diet priors (Pn vs Pi). Model strategies A and B give roughly equal inference regarding location and spread for trophic level.

3.3.2. Diet Proportion Results

Selected diet proportion posterior distributions are presented in **Figure 7**. For pinfish, the diet posteriors are more concentrated with the informative diet prior (Pi), and not influenced by the trophic prior. For pinfish, shile benthic is the primary source with a vague diet prior, an informative prior indicates epiphytes are the primary source. For mojarra, benthic is the primary source. Model strategies A and B give the same inference for all consumers. Note that dolphin model strategies A and B are not comparable since the sources considered are different, dolphin Model strategy B can be thought of as weighted averages of the fish, which in turn are weighted averages of the primary sources.

The mojarra and pigfish diet proportion results reveal a reliance on benthic sources regardless of the model chosen. These results are consistent with stomach content analyses that found mojarra in the size ranges we sampled (7.5 - 9 cm) are primary carnivores feeding largely on polychaetes [42] [43] and that pigfish in the size range we sampled (10.5 - 16.5 cm) largely consume benthic invertebrates [43]. The primary source for pinfish varied between models depending on the informativeness of the priors. The informative priors for pinfish were established from published results of the implementation of a previous isotope model [41]. The observed change in the relative rankings of sources in our analyses suggests that pinfish in St. George Sound may have a different diet than those studied by [41]. Pinfish diets are notoriously inclusive or opportunistic [43]. Some studies based on stomach content analyses suggest that seagrass (and associated epiphytes) make up a large proportion of pinfish diets (e.g., [44] [45] [46] [47]), while others suggest that pinfish ingest seagrass material incidentally with other food items [48]. Our vague prior results suggest that benthic sources, rather than seagrass or associated epiphytes, dominate pinfish diets, a result consistent with reports from Crystal River, FL by [43] that pinfish of the size

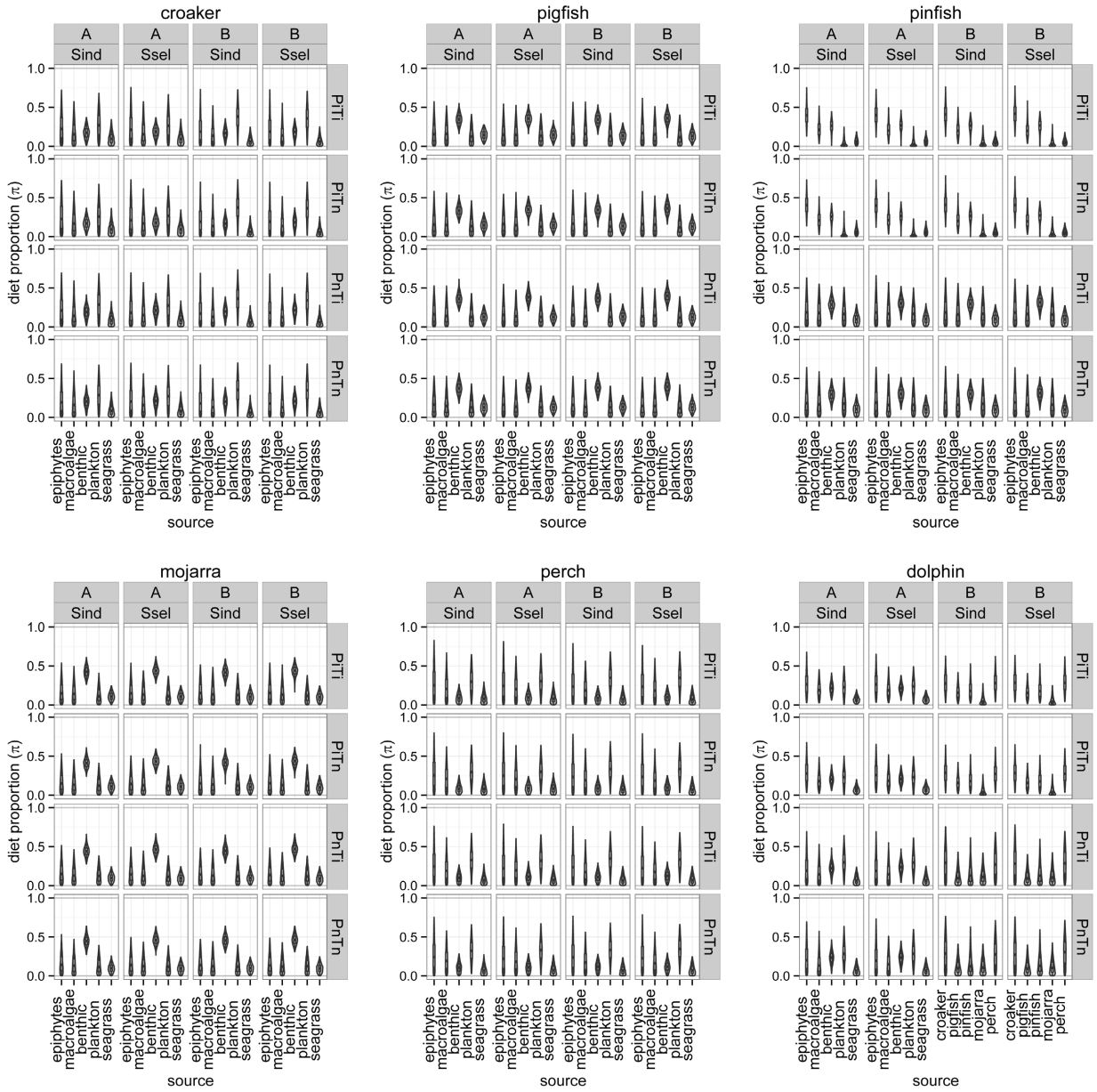


Figure 7. Posterior distributions for diet proportions (π_s) for selected consumers and model types. For illustration we present the results for three consumers, independent (Sind) or selected (Ssel) covariance relationships, vague (Pn) or informative (Pi) diet prior; vague (Tn) or informative (Ti) trophic level prior; and Model strategies A or B.

range collected in our study (>8 cm) primarily consume shrimp and other fishes. Further complicating dietary estimates, pinfish are also known to undergo a progression of ontogenetic dietary changes [43] [44] where the earliest stages are planktonic, followed by an herbivorous stage, and finally by carnivory. Thus size differences (as well as geographic differences) among studies may render the prior information invalid or misleading.

3.4. Discussion

Our results differ from previous analyses, which is not surprising given the dif-

ferent model structure and inclusion of prior information. For example, we estimate croaker to be roughly between trophic levels 2.4 and 3, while previous estimates were a third of a trophic level higher between 2.7 and 3.4 [25] [26]. One explanation is that these previous models estimated that croaker's primary source was benthic, while our model indicates that plankton is the primary source. This difference in trophic level, therefore, could be the result of ^{15}N differences between basal plankton and basal benthic organic matter.

4. Discussions

This model is easily extended to include covariates such as consumer sex and age, time of year, and sources that change over time [22]. Subject-specific trophic level and diet can also be estimated via hierarchical Dirichlet distributions [18].

Results from one study (e.g., FL dolphin) may be used to inform priors for other studies (e.g., TX dolphins) when the systems are very similar; otherwise vague priors may be more appropriate. The adequacy of selected priors cannot be tested by Bayesian or any other methods if we have only a small amount of data. But with enough data this becomes possible; for then the posterior density of the parameter from a vague prior, such as Jeffreys, becomes sharply peaked, with the data alone pointing to a well-defined value of the parameter. An informative prior sharply peaked at a very different value thus stands in conflict with the evidence of the data; intuitively, we would be led to doubt the validity of our prior information or model.

The most important prior information comes from the ecological expert about which sources to include in the diet model and the foodweb graph structure. The choice of sources can come from direct observation, the analysis of stomach or fecal contents, or contextual speculation. A missing source may be inferred if the consumer's isotope ratio is not a convex combination of the included discrimination-corrected sources. Rejecting a source is more difficult since it depends on the relative positions of the source isotope ratio means and the consumer mean, and there is often some overlap between sources or, in the underconstrained situation, multiple convex combinations that explain the consumer isotope ratios. In our example, seagrass is a potential source, but is estimated as a low diet contributer. Having a principled strategy for testing and excluding unimportant sources would be valuable since the dimension of the model space is determined by the number of sources.

The mixing model relationships mentioned in Section 2.1 can simplify modeling when appropriate. When assimilation efficiencies for sources are all equal (such as carnivores, when all approximately 100%) then using the CMM instead of the EMM will reduce the number of parameters and the need for prior information to inform the efficiencies. Furthermore, if elemental concentrations are similar among sources, then using the BMM instead of the CMM will simplify modeling similarly. However, the more complicated EMM model is justified in situations where efficiencies are low or vary among potential sources. For exam-

ple, when determining the dietary proportions of omnivores that feed on both plant and animal prey sources, or when elemental concentrations among potential sources vary, as when considering potential contributions of plankton and plant material.

The BMM and EMM will yield different results, and the decision on which to use will depend on the context and the nature of the research question. For example, the EMM results are useful for estimating primary production rates necessary to support secondary production in a system or for energy transfer calculations or to calculate nutrient uptake. The BMM, which specifically estimates assimilated proportions, may prove more useful for studies related to bioaccumulation of organic contaminants or heavy metal uptake.

As discussed in Section 2.11, the full EMM has convergence issues possibly due to identifiability issues. Use of the CMM or EMM is currently restricted to strategies that impose additional constraints, such as setting concentration and assimilation efficiencies to constants or by stringently bounding their support.

From an ecological perspective, there are at least three important applications for this model: assigning trophic niche, estimating energy flow, and assessing contaminant entry routes and bioaccumulation. 1) A trophic niche is the place or function of an organism in terms of organic matter utilization and trophic level. By grouping organisms by specialization (planktivorous-grass eaters, etc.) select species from a given niche can be monitored that encompassed the range of trophic habit in the area to provide information about the health of the ecosystem. For example, choosing a planktivore can inform about a system's pelagic health. 2) Because energy flows from the primary producers up the food chain, a sample of seagrass with size and abundance data can help estimate the primary production necessary to support the estimated number of fish living in a given area. 3) Contaminants accumulate up the food chain, such as mercury in fish. In an effort to assess the relative health of ecosystems and potential ill-effects of contaminants, given an assessment of whether organic matter utilization contributes to organic loading (for example, are benthic feeders more contaminated?), and by correlating concentration with trophic level, we can derive a bioaccumulation factor to compare between systems.

Acknowledgements

Sincere thanks to JA Nelson and JP Chanton for assistance with sample collection and isotope analysis.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] Martínez del Rio, C. and Wolf, B.O. (2005) Mass-Balance Models for Animal Iso-

topic Ecology. In: Starck, J.M., Wang, T. and Wang, T., Eds., *Physiological and Ecological Adaptations to Feeding in Vertebrates*, Science Publishers, Enfield, Chapter 6, 141-174.

[2] Phillips, D.L. (2001) Mixing Models in Analyses of Diet Using Multiple Stable Isotopes: A Critique. *Oecologia*, **127**, 166-170. https://doi.org/10.1007/s004420000571

[3] Parnell, A.C., Phillips, D.L., Bearhop, S., Semmens, B.X., Ward, E.J., Moore, J.W., Jackson, A.L., Grey, J., Kelly, D.J. and Inger, R. (2013) Bayesian Stable Isotope Mixing Models. *Environmetrics*, **24**, 387-399. https://doi.org/10.1002/env.2221

[4] McCormack, S.A., Trebilco, R., Melbourne-Thomas, J., Blanchard, J.L., Fulton, E.A. and Constable, A. (2019) Using Stable Isotope Data to Advance Marine Food Web Modelling. *Reviews in Fish Biology and Fisheries*, **29**, 277-296. https://doi.org/10.1007/s11160-019-09552-4

[5] Deines, P. (1980) The Terrestrial Environment. In: Fritz, P. and Fontes, J.C., Eds., *Handbook of Environmental Isotope Geochemistry*, Volume 1, *The Isotopic Composition of Reduced Organic Carbon*, Elsevier Science, New York, 329-406. https://doi.org/10.1016/B978-0-444-41780-0.50015-8

[6] Kendall, C. and McDonnell, J.J. (Eds.) (1998) Isotope Tracers in Catchment Hydrology. Elsevier, Amsterdam, New York.

[7] Sterner, R.W. and Elser, J.J. (2002) Ecological Stoichiometry: The Biology of Elements from Molecules to the Biosphere. Princeton University Press, Princeton. https://doi.org/10.1515/9781400885695

[8] Briand, F and Cohen, J.E. (1987) Environmental Correlates of Food Chain Length. *Science*, **238**, 956-960. https://doi.org/10.1126/science.3672136

[9] Allesina, S., Alonso, D. and Pascual, M. (2008) A General Model for Food Web Structure. *Science*, **320**, 658-661. https://doi.org/10.1126/science.1156269

[10] Pauly, D. and Palomares, M.-L. (2005) Fishing Down Marine Food Web: It Is Far More Pervasive than We Thought. *Bulletin of Marine Science*, **76**, 197-212.

[11] Votier, S.C., Bearhop, S., MacCormick, A., Ratcliffe, N. and Furness, R.W. (2003) Assessing The Diet of Great Skuas, Catharacta Skua, Using Five Different Techniques. *Polar Biology*, **26**, 20-26. https://doi.org/10.1007/s00300-002-0446-z

[12] Hobson, K.A. and Wassenaar, L.I. (Eds.) (2008) Tracking Animal Migration with Stable Isotopes. Academic Press, Amsterdam.

[13] Hobson, K.A. and Wassenaar, L.I. (1999) Stable Isotope Ecology: An Introduction. *Oecologia*, **120**, 312-313. https://doi.org/10.1007/s004420050864

[14] Layman, C.A., Araujo, M.S., Boucek, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., Matich, P., Rosenblatt, A.E., Vaudo, J.J., Yeager, L.A., Post, D.M. and Bearhop, S. (2012) Applying Stable Isotopes to Examine Foodweb Structure: An Overview of Analytical Tools. *Biological Reviews*, **87**, 545-562. https://doi.org/10.1111/j.1469-185X.2011.00208.x

[15] Post, D.M. (2002) The Long and Short of Food-Chain Length. *Trends in Ecology & Evolution*, **17**, 269-277. https://doi.org/10.1016/S0169-5347(02)02455-2

[16] Martínez Del Rio, C., Wolf, N., Carleton, S.A. and Gannes, L.Z. (2009) Isotopic Ecology Ten Years after a Call for More Laboratory Experiments. *Biological Reviews*, **84**, 91-111. https://doi.org/10.1111/j.1469-185X.2008.00064.x

[17] Phillips, D.L. and Koch, P.L. (2002) Incorporating Concentration Dependence in Stable Isotope Mixing Models. *Oecologia*, **130**, 114-125. https://doi.org/10.1007/s004420100786

[18] Erhardt, E.B. (2009) Stable Isotope Sourcing Using Sampling. PhD Dissertation,

University of New Mexico, Albuquerque.

[19] Erhardt, E.B. and Bedrick, E.J. (2014) Inference for Stable Isotope Mixing Models: A Study of the Diet of Dunlin. *Journal of the Royal Statistical Society*: *Series C*, **63**, 579-593. https://doi.org/10.1111/rssc.12047

[20] Phillips, D.L. and Gregg, J.W. (2003) Source Partitioning Using Stable Isotopes: Coping with Too Many Sources. *Oecologia*, **136**, 261-269. https://doi.org/10.1007/s00442-003-1218-3

[21] Erhardt, E.B., Wolf, B.O., Ben-David, M. and Bedrick, E.J. (2014) Stable Isotope Sourcing Using Sampling. *Open Journal of Ecology*, **4**, 289-298. https://doi.org/10.4236/oje.2014.46027

[22] Erhardt, E.B. and Bedrick, E.J. (2013) A Bayesian Framework for Stable Isotope Mixing Models. *Environmental and Ecological Statistics*, **20**, 377-397. https://doi.org/10.1007/s10651-012-0224-1

[23] Quezada-Romegialli, C., Jackson, A.L., Hayden, B., Kahilainen, K.K., Lopes, C. and Harrod, C. (2018) Trophicposition, An R Package for the Bayesian Estimation of Trophic Position from Consumer Stable Isotope Ratios. *Methods in Ecology and Evolution*, **9**, 1592-1599. https://doi.org/10.1111/2041-210X.13009

[24] Phillips, D.L., Inger, R., Bearhop, S., Jackson, A.L., Moore, J.W., Parnell, A.C., Semmens, B.X. and Ward, E.J. (2014) Best Practices for Use of Stable Isotope Mixing Models in Food-Web Studies. *Canadian Journal of Zoology*, **92**, 823-835. https://doi.org/10.1139/cjz-2014-0127

[25] Wilson, R.M., Chanton, J., Lewis, F.G. and Nowacek, D. (2009) Combining Organic Matter Source and Relative Trophic Position Determinations to Explore Trophic Structure. *Estuaries and Coasts*, **32**, 999-1010. https://doi.org/10.1007/s12237-009-9183-7

[26] Wilson, R.M., Chanton, J., Lewis, F.G. and Nowacek, D. (2010) Concentration Dependent Stable Isotope Analysis of Consumers in the Upper Reaches of a Freshwater-Dominated Estuary: Apalachicola Bay, FL, USA. *Estuaries and Coasts*, **33**, 1406-1419. https://doi.org/10.1007/s12237-010-9304-3

[27] Wilson, R.M., Kucklick, J.R., Balmer, B.C., Wells, R.S., Chanton, J.P. and Nowacek, D.P. (2012) Spatial Distribution of Bottlenose Dolphins (*Tursiops truncatus*) Inferred from Stable Isotopes and Priority Organic Pollutants. *Science of the Total Environment*, **425**, 223-230. https://doi.org/10.1016/j.scitotenv.2012.02.030

[28] Wilson, R.M., Nelson, J.A., Balmer, B.C., Nowacek, D.P. and Chanton, J.P. (2013) Stable Isotope Variation in the Northern Gulf of Mexico Constrains Bottlenose Dolphin (*Tursiops Truncatus*) Foraging Ranges. *Marine Biology*, **160**, 297-2980. https://doi.org/10.1007/s00227-013-2287-4

[29] Wong, T.-T. (1998)Generalized Dirichlet Distribution in Bayesian Analysis. *Applied Mathematics and Computation*, **97**, 165-181. https://doi.org/10.1016/S0096-3003(97)10140-0

[30] Aitchison, J. (2003) The Statistical Analysis of Compositional Data. Blackburn Press, West Caldwell.

[31] Aitchison, J. (1982) The Statistical Analysis of Compositional Data. *Journal of the Royal Statistical Society. Series B* (*Methodological*), **44**, 139-160. https://doi.org/10.1111/j.2517-6161.1982.tb01195.x

[32] Lunn, D.J., Thomas, A., Best, N. and Spiegelhalter, D. (2000) WinBUGS—A Bayesian Modelling Framework: Concepts, Structure, and Extensibility. *Statistics and Computing*, **10**, 325-337. https://doi.org/10.1023/A:1008929526011

[33] Sachs, L. (1992) Applied Statistics. Application of Statistical Methods [Angewandte Statistik. Anwendung Statistischer Methoden]. Springer, Berlin, New York.

[34] Currin, C.A., Newell, S.Y. and Paerl, H.W. (1995) The Role of Standing Dead *Spartina alterniflora* and Benthic Microalgae in Salt Marsh Food Webs: Considerations Based on Multiple Stable Isotope Analysis. *Marine Ecology Progress Series*, **21**, 99-116. https://doi.org/10.3354/meps121099

[35] McCutchan Jr., J.H., Lewis Jr., W.M., Kendall, C. and McGrath, C.C. (2003) Variation in Trophic Shift for Stable Isotope Ratios of Carbon, Nitrogen, and Sulfur. *Oikos*, **102**, 378-390. https://doi.org/10.1034/j.1600-0706.2003.12098.x

[36] Moncreiff, C.A. and Sullivan, M.J. (2001) Trophic Importance of Epiphytic Algae in Subtropical Seagrass Beds: Evidence from Multiple Stable Isotope Analyses. *Marine Ecology Progress Series*, **215**, 93-106. https://doi.org/10.3354/meps215093

[37] Post, D.M. (2002) Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumption. *Ecology*, **83**, 703-718. https://doi.org/10.1890/0012-9658(2002)083[0703:USITET]2.0.CO;2

[38] Akin, S. and Winemiller, K.O. (2006) Seasonal Variation in Food Web Composition and Structure in a Temperate Tidal Estuary. *Estuaries and Coasts*, **29**, 552-567. https://doi.org/10.1007/BF02784282

[39] Akin, S. and Winemiller, K.O. (2008) Body Size and Trophic Position in a Temperate Estuarine Food Web. *Acta Oecologica*, **33**, 144-153. https://doi.org/10.1016/j.actao.2007.08.002

[40] Sullivan, M.J. and Moncreiff, C.A. (1993) Trophic Importance of Epiphytic Algae in Mississippi Seagrass Beds. Technical Report, Mississippi-Alabama Sea Grant Publication No MASGP-92-018, Mississippi-Alabama Sea Grant Consortium, Ocean Springs.

[41] Winemiller, K.O., Akin, S. and Zeug, S.C. (2007) Production Sources and Food Web Structure of a Temperate Tidal Estuary: Integration of Dietary and Stable Isotope Data. *Marine Ecology Progress Series*, **343**, 63-76. https://doi.org/10.3354/meps06884

[42] Livingston, R.J., Niu, X., Lewis III, F.G. and Woodsum, G.C. (1997) Freshwater Input to a Gulf Estuary: Long-Term Control of Trophic Organization. *Ecological Applications*, **7**, 277-299. https://doi.org/10.1890/1051-0761(1997)007[0277:FITAGE]2.0.CO;2

[43] Carr, W.E.S. and Adams, C.A. (1973) Food Habits of Juvenile Marine Fishes Occupying Seagrass Beds in The Estuarine Zone Near Crystal River, Florida. *Transactions of the American Fisheries Society*, **102**, 511-540. https://doi.org/10.1577/1548-8659(1973)102<511:FHOJMF>2.0.CO;2

[44] Stoner, A.W. (1980) Feeding Ecology of Lagodon-Rhomboides (Pisces, Sparidae) Variation and Functional Responses. *Fishery Bulletin - National Oceanic and Atmospheric Administration*, **78**, 337-352.

[45] Springer, V.G. and Woodburn, K.D. (1960) An Ecological Study of The Fishes of The Tampa Bay Area. Florida State Board of Conservation Professional Papers Series. Florida Department of Natural Resources Marine Research Laboratory.

[46] Darnell, R.M. (1961) Trophic Spectrum of an Estuarine Community, Based on Studies of Lake Pontchartrain, Louisiana. *Ecology*, **42**, 553-568. https://doi.org/10.2307/1932242

[47] Darnell, R.M. (1958) Food Habits of Fishes and Larger Invertebrat of Lake Pontchartrain, Louisiana, an Estuarine Community. *Institute of Marine Science*, **5**,

353-416.

[48] Caldwell, D.K. (1957) The Biology and Systematics of The Pinfish, Lagodon Rhomboides (Linnaeus). *Bulletin of the Florida State Museum Biological Sciences*, **6**, 77-173.

Appendix A. Supplementary Materials

Please see the zip file for all examples in this paper, including well-documented OpenBUGS model, data, script, and init files.

Supplemental Simulations: Simulation Scenarios 1, 2, and 3 use sample sizes of 30 for each component with varying amounts of correlation and priors are vague. Report and R code are available at https://statacumen.com/?p=3884.

Dolphin Example: Application data and OpenBUGS code for each model scenario is available at https://statacumen.com/?p=3884.

Appendix B. Simulation Scenarios 2 and 3

Details for the simulation scenarios are provided in the Supplementary Materials Section A.

The Simulation 2 and 3 foodweb graphs are given in Figure A1. The results for Strategy B in Figure A2 indicate concordance between the posterior and true values, and the bias that exists is a function of the simulated data having a mean slightly different from the true mean. The results for Strategy A in Figure A3 are similar.

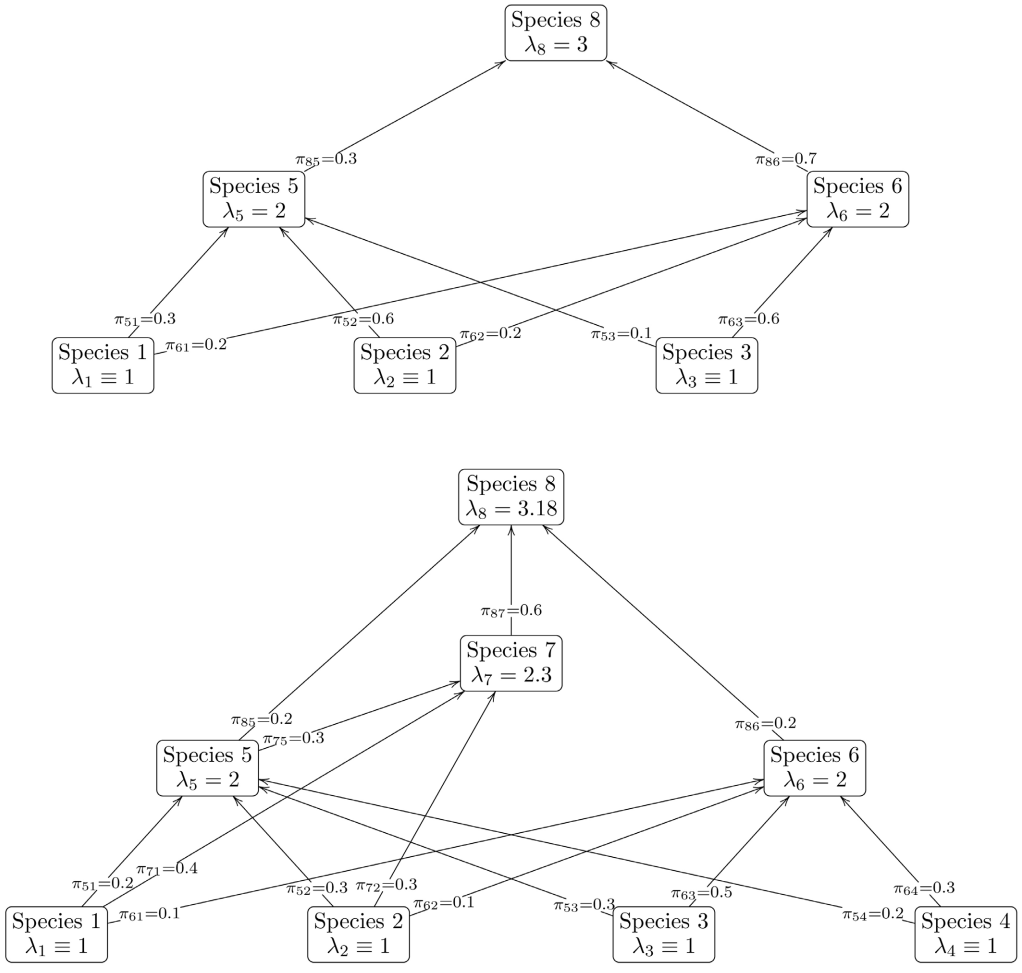


Figure A1. Simulation Scenarios 2 and 3.

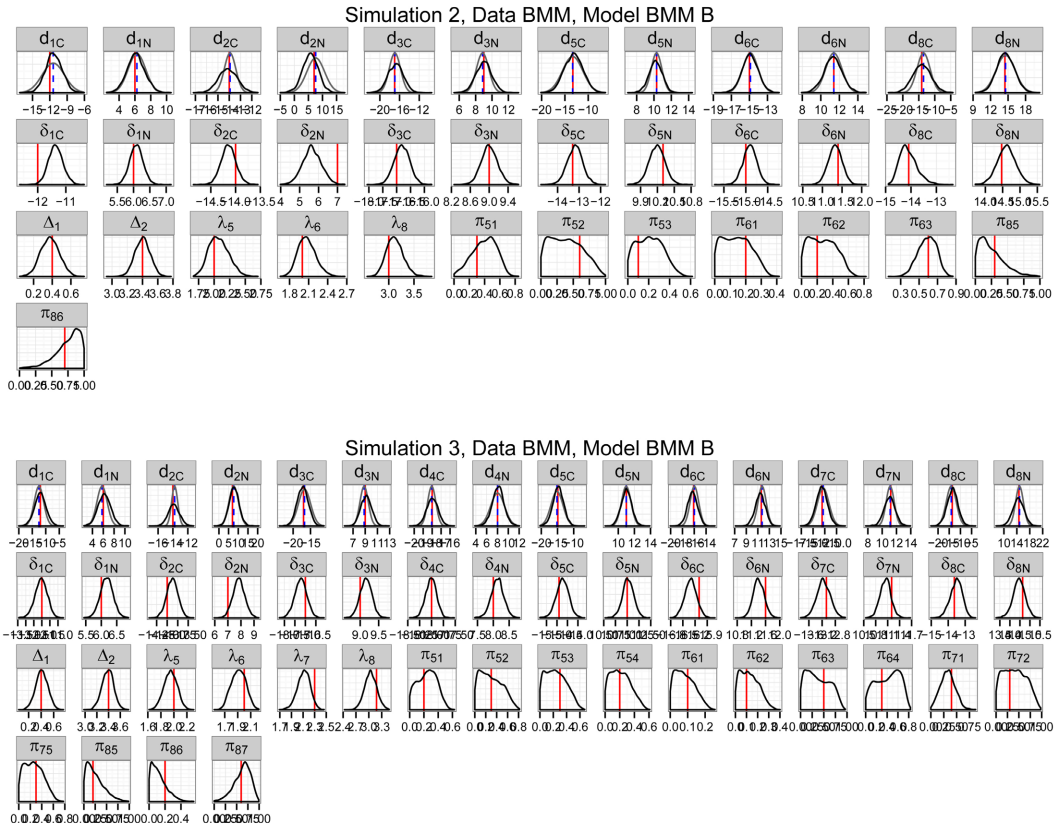


Figure A2. Simulations 2 (top) and 3 (bottom) Strategy B, posterior predictive distributions (top row of each) and posterior densities. Black curves are the posterior distributions, gray curves are the data distributions (top row of each), red solid lines indicate the true parameter value input to the simulation, and blue dashed lines indicate the observed mean data value.

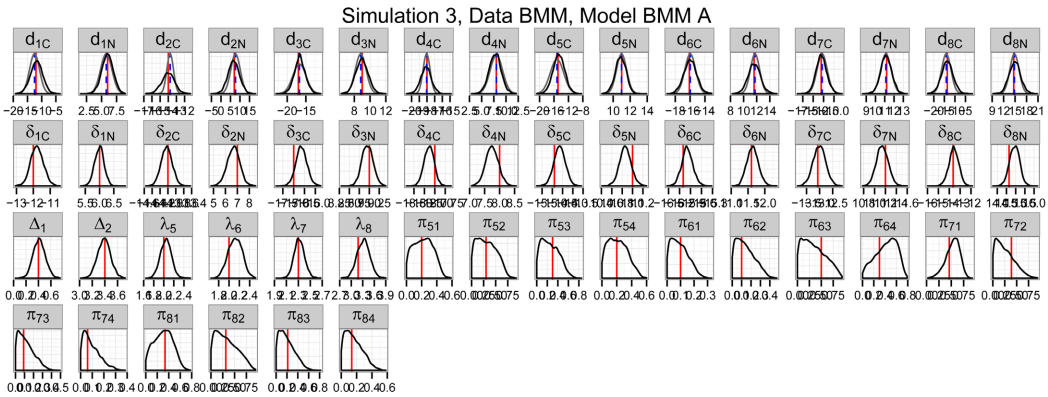


Figure A3. Simulations 1, 2 and 3 Strategy A, posterior predictive distributions (top row of each) and posterior densities. Black curves are the posterior distributions, gray curves are the data distributions (top row of each), red solid lines indicate the true parameter value input to the simulation, and blue dashed lines indicate the observed mean data value.

Appendix C. Isotope Ratio Correlations

Table A1 gives the observed pairwise correlations between the isotope ratio observations.

Table A1. Source and consumer correlations between isotope ratio observations.

Sources					Consumers				
		d_C	d_N	d_S			d_C	d_N	d_S
epiphytes	d_C	1.00	−0.63	−0.91	croaker	d_C	1.00	−0.76	0.21
	d_N	−0.63	1.00	0.77		d_N	−0.76	1.00	0.22
	d_S	−0.91	0.77	1.00		d_S	0.21	0.22	1.00
macroalgae	d_C	1.00	−0.40	−0.81	mojarra	d_C	1.00	−0.33	−0.86
	d_N	−0.40	1.00	0.44		d_N	−0.33	1.00	0.19
	d_S	−0.81	0.44	1.00		d_S	−0.86	0.19	1.00
benthic	d_C	1.00	0.63	0.57	pigfish	d_C	1.00	0.16	−0.38
	d_N	0.63	1.00	0.78		d_N	0.16	1.00	0.57
	d_S	0.57	0.78	1.00		d_S	−0.38	0.57	1.00
plankton	d_C	1.00	0.66	0.33	pinfish	d_C	1.00	−0.35	−0.82
	d_N	0.66	1.00	0.74		d_N	−0.35	1.00	0.73
	d_S	0.33	0.74	1.00		d_S	−0.82	0.73	1.00
seagrass	d_C	1.00	0.17	0.78	silverperch	d_C	1.00	−0.44	−0.39
	d_N	0.17	1.00	0.01		d_N	−0.44	1.00	0.06
	d_S	0.78	0.01	1.00		d_S	−0.39	0.06	1.00
					dolphin	d_C	1.00	0.35	−0.35
						d_N	0.35	1.00	0.66
						d_S	−0.35	0.66	1.00