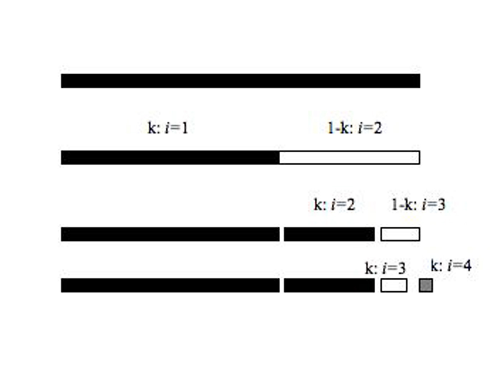
*1. Question from Merav Ben-David (only part 5 is new).*

1. Using the rKIN package (<https://github.com/salbeke/rKIN>) please create an isospace for prey in one of your river study sites. You may select any location with 3 or more prey species.
2. After modifying the attached MatLab code to R, please model the feeding behavior of one of your predator species. These are called broken stick models and were first proposed by McArthur (1957, 1961) to describe the packing of species by niche partitioning (see illustration below). Nonetheless, they have been used to model dietary contributions (Flaherty and Ben-David 2010). The model that best fits your assignment is model 4 (one habitat and multiple prey). In the attached MatLab code the “kestrels” are randomly foraging on each prey type drawing from a normal distribution of prey isotopic signatures. You should be using the results from rKIN such that the predator consumes prey according to their real distribution. For example, if your predator consumes 20% of prey A, it should be consuming 10% of that prey within the 50% contour of the KUD.



1. Please generate 25 “virtual” predators. You may repeat this exercise with several discrimination factors as you see fit (at least until you complete your captive experiments). Then compare the simulated data to the OBSERVED isotopic signatures for that species in that location.
2. Please use programs MixSIAR and SISUS (mixing models) to estimate the relative contribution of the different prey to those of your OBSEREVED predators.
3. How does your interpretation of dietary contributions of prey to the OBSERVED predator from the simulation data differ from those of mixing models? In your written response, please explain the problems with mixing models, explain the methods you used, describe your results and offer an opinion on whether predictive isoscape analyses are superior (or not) to traditional mixing models.

**Question 1, Part 1-4:** Response can be found Supplement No. 1.

**Question 1, Part 5:** Mixing models that use stable isotope data are a powerful tool for examining animal diets and inferring trophic structure in ecological communities (Martínez del Rio et al. 2009, Phillips et al. 2014). They are also an increasingly common tool used by ecologists, with use rising rapidly since 2008 (Fig. 1). I will begin with a brief background on the use of mixing models in the context of trophic ecology, highlighting key assumptions and drawbacks in their application, before wading into an alternative approach for examining foraging ecology in animals (i.e., Predictive Isoscape Analysis).

Predation and other top-down trophic processes can directly and indirectly shape communities (Pace et al. 1999, Tunney et al. 2012, Giam and Olden 2016). So a critical first step toward describing community structure is quantifying the proportional contribution of different prey types to consumer diets. If we know something about the isotopic signatures of a predator and its prey, we can estimate the proportional contributions of *n* + 1 prey in that predator’s diet by using *n* different isotope systems (e.g., δ13C, δ15N, δ18O) with linear mixing models based on mass balance equations (Phillips and Gregg 2003). This is because isotopic signatures of prey vary across taxa and trophic levels, and are maintained once prey are consumed and incorporated into consumer tissues (after accounting for trophic discrimination; Gannes et al. 1997, Phillips et al. 2014). For example, consider a mixture (i.e., a predator) and two sources (i.e., its prey). A simple one-dimensional linear mixing model can estimate the proportional contribution of source one and two to the mixture, given the mixture falls within the mixing space of sources one and two (Koch and Phillips 2002, Phillips and Gregg 2003, Flaherty and Ben-David 2010, Phillips et al. 2014). But what if a consumer falls outside of the prey mixing space, or sources have a large degree of variability (uncertainly) about them?

Prior to 2008, these sources of uncertainty were not accounted for in mixing models, and thus hindered their development and application. But since 2008, Bayesian statistical methods have been incorporated into stable isotope mixing models because they can integrate prior information (Moore and Semmens 2008), hierarchical data structures (Semmens et al. 2009), source uncertainly (Ward et al. 2010), and continuous effects (Francis et al. 2011). Such models have been incorporated into numerous packages developed for implementation in Program R (Jackson et al. 2009, Semmens et al. 2009, Parnell et al. 2010, Hopkins and Ferguson 2012, Fernandes et al. 2014, Stock and Semmens 2016a), which produce as output a posterior distribution of possible solutions to a mixing model. Posterior distributions can then be used to estimate 95% credible intervals for the proportional contribution of prey types to consumer diets – which are often very large and thus disregarded in interpretations of dietary contributions, with many researchers relying simply on point estimates such as mean, median, and mode (Ramírez et al. 2014, Resano-Mayor et al. 2014). But uncertainly around point estimates of dietary contributions are key to ecologically meaningful interpretations, not to mention conservation and management efforts (Bond and Diamond 2011), and is thus a primary benefit of using Bayesian estimation methods; that is, one can incorporate prior information to inform posterior distributions (but more on this below).

To use stable isotope mixing models, the following data are required: (1) isotopic signatures of consumers, (2) isotopic signatures of sources, (3) values for trophic discrimination factors (hereafter TDFs), and (4) optional external or prior information (e.g., gut contents or observations). TDFs refer to the differences between stable isotope values found in consumers and sources. TDF values should be experimentally determined (Martínez del Rio et al. 2009), but almost always come from literature values (e.g. Post 2002). This is arguably one of the most important weaknesses in stable isotope mixing models because TDFs vary among species, regions, and diet quality, among other factors (Pinnegar and Polunin 1999, Sweeting et al. 2007, Colborne et al. 2017). Accordingly, the use of even slightly different TDFs can significantly alter mixing model results and lead to fallacious interpretations of diet. But once TDFs are chosen, they are used to correct consumer isotopic signatures, which are then plotted in dual isotopic niche space to determine whether consumers are within the source mixing space. As mentioned above, consumers must fall within this mixing space, else no solution can be achieved (e.g., Fig. 2). Assuming one has reasonable TDF values and confirmed that the consumer falls within the mixing space of prey, a Bayesian mixing model can be implemented given these additional assumptions are met: (1) all consumers have the same diet, (2) all sources are equally available (in terms of biomass), (3) no prey are missing from the analysis, and (4) the source parameters are exactly known (i.e., one has a large sample size, generally > 10; Gannes et al. 1997, Boecklen et al. 2011, Nielsen et al. 2017). In reality, most of these assumptions are not met, but mixing models are nonetheless used to make inference into the trophic ecology of animals.

As alluded to above, perhaps the most commonly cited advantage of Bayesian mixing models is the ability to include informative priors, which improve both precision and accuracy of mixing model results. For example, Moore and Semmens (2008) show how incorporating an informative prior of gut content analysis improved the precision of mixing model results estimating diet to marten on an Alaskan island (Fig. 3). But in many ecosystems, factors such as a large number of potential prey and variation or correlation among isotopic signatures of prey can lead to erroneous interpretations of model results. To illustrate with a recent example, take the case in which researchers tested the efficacy of using Bayesian mixing models to estimate the diet of Arctic Peregrine Falcon (*Falco peregrinus tundrius*) nestlings in Nunavut, Canada. Robinson et al. (2018) found that when uninformed, mixing models produced wide credible intervals and suggested lemmings (*Lemmus trimucronatus* and *Dicrostonyx groenlandicus*) were the main contributors to falcon diets (Fig. 4). Conversely, diets estimated using observational methods had high precision and suggested insectivorous birds were the dominant prey of falcons. Finally, when informative priors were incorporated from observational analyses into the mixing models, diet estimates, while precise, were simply a reflection of the priors. Thus, stable isotope mixing models failed to estimate the diet of these avian predators, and when given prior information, simply reflected those distributions, ostensibly because the weight of the prior outweighed the likelihood of the observed data.

It is therefore evident that, despite growing in popularity and use (e.g., Fig. 1), mixing model analyses still suffer from assumptions and methodological choices that can, and do, affect the interpretations researchers make into the trophic ecology of animals. This is a non-trivial problem that requires a novel solution, and in particular one which moves away from an *a posteriori* approach and instead focuses on an *a priori*, predictive approach. One such alternative approach is Predictive Isoscape Analysis (hereafter PIA). Rather than simply inferring trophic ecology from observed data, PIA allows researchers to predict the isotopic niche and dietary contributions of prey to a population of predators given observed prey isotopic signatures. These predictions can then be compared to observed predators, from which inferences about deviation from the observed can be made. Details of a first implementation of this approach are in the accompany R Markdown HTML document (Supplement No. 1). In the following paragraphs, I highlight key methods and results from this first implementation, compare results to those obtained through traditional mixing models, and offer an opinion on the relative advantages of this novel approach.

Stable isotope data were compiled for one study site, LR05, located on the Laramie River in Laramie, Wyoming. Fish and invertebrate samples were collected during summer 2016. For the purposes of this exam question, I chose to focus on Brown Trout (*Salmo trutta*) as the predator species of interest, and five putative prey items previously identified to be important to Laramie River Brown Trout diets (Dugan 2015). Prey categories included: (1) crayfish (Crustacea: *Orconectes* spp.), (2) “minnows” (i.e., a prey fish category composed of Brassy Minnow [*Hybognathus hankinsoni*] and Fathead Minnow [*Pimephales promelas*]), (3) flathead mayflies (Ephemeroptera: Baetidae), (4) net-spinning caddisflies (Tricoptera: Hydropsychidae), and (5) isopods (Crustacea: Isopoda). Brassy Minnows and Fathead Minnows were grouped together given their similar trophic ecology and overlapping isotopic signatures.

The R package *rKIN* (Eckrich et al. *in revision*, Albeke 2017) was then used to create an isospace for prey at this study site (Fig. 5). *rKIN* uses the Kernel Utilization Density (KUD) method to estimate isotopic niche metrics. In contrast to other niche-estimation methods such as convex hulls (Layman et al. 2007) or standard ellipses (Jackson et al. 2011), the KUD precisely describes the distribution of bivariate isotopic data using a nonparametric estimator, yielding an accurate delineation of isotopic niche space (Eckrich et al. *in revision*). Once the prey utilization density kernels were estimated, I randomly sampled 100 times each prey’s kernel to generate true distributions of isotopic values for each prey category, which were then used to simulate the expected isotopic signatures of individual Brown Trout (as opposed to drawing isotopic values from a standard normal distribution).

To simulate Brown Trout dietary contributions and isotopic niches, I adapted Matlab code written to model the foraging behavior of a consumer across an isotopically heterogeneous landscape and the dietary contributions of different prey items to simulated predators (MacArthur 1957, Flaherty and Ben-David 2010). Specifically, Model 4 (a predator foraging in one habitat on multiple prey) was coded in Program R, where “Brown Trout” are randomly foraging on each of five prey types such that the predator consumes prey according to their real isotopic distributions (*sensu* MacArthur 1957). This model (1) simulates the relative dietary proportions of the five prey items to 25 simulated Brown Trout individuals, and (2) estimates the expected isotopic signature of each Brown Trout given isotopic distributions estimated though *rKIN* KUDs. The simulated predator population’s isotopic niche was then visually compared to the observed predator population’s isotopic niche (Fig. 6). This Predictive Isoscape Analysis indicated that crayfish and “minnows” were the greatest contributor to Brown Trout diets, but mayflies and caddisflies also contributed small portions (Table 1).

The Bayesian mixing model analysis was implemented in Program R with the MixSIAR package (Stock and Semmens 2016b). Wholly uninformative priors were used, as well as TDFs of 1.5 ‰ and 3.4 ‰ (for δ13C and δ15N, respectively). The TDFs chosen are commonly cited TDF values for freshwater fish from the literature (DeNiro and Epstein 1981, Minagawa and Wada 1984, Post 2002) but note here that they are likely erroneous. MCMC sampling parameters included 3 chains of length 10,000 with a burn-in of 5,000. Model diagnostics suggested chains were fully converged. Posterior distributions were diffuse with most 95% credible intervals as large as 0.4 (Table 1, Fig. 7). Bayesian mixing models indicated that crayfish and “minnows” dominated Brown Trout diets, with negligible contributions from other prey types (Table 1).

Point estimates of dietary proportions from the Predictive Isoscape Analysis (PIA) were similar to those produced using the uninformed Bayesian mixing model. Both indicated that crayfish and “minnows” dominated Brown Trout diets, however the PIA produced higher proportional estimates of the remaining prey categories than did MixSIAR, which effectively indicated a negligible contribution of benthic macroinvertebrates to diet. Not surprisingly, both methods indicated a high degree of variability around point estimates, perhaps due to small sample sizes in some prey groups. In reality, Brown Trout likely rely heavily on crayfish, but also to a lesser extent on small fish, benthic macroinvertebrates, and anurans (Dugan 2015). Thus, solely using a traditional mixing model would lead to the interpretation that benthic macroinvertebrates are not important to Brown Trout diets, whereas the PIA suggested a more realistic dietary breakdown among the different prey types.

Importantly, each method employed very different approaches to the problem of investigating consumer trophic ecology; one an *a priori* framework in which predictions are made about the isotopic/ecological niche of consumer species and then compared to observed data, while the other operates in an *a posteriori* framework, in which the observed data are simply used to estimate the diet of consumers, from which interpretations about foraging ecology are made. The PIA allows visual comparison of a simulated consumer population to an observed population (e.g., Fig. 6), from which in this example we might infer that most Brown Trout individuals feed predominately on crayfish, with some also relying on small minnows, mayflies, and caddisflies. In contrast, results from the Bayesian mixing model leave little room for interpretation, and simply suggest crayfish and small minnow constitute Brown Trout diets.

Thus, the PIA approach offers a more robust framework in which to predict and interpret stable isotope data and examine foraging behavior than traditional mixing models. Particularly given the numerous limitations and assumptions required to perform mixing model analyses, and the increasing rate at which studies suggest mixing models provide fallacious interpretations of diet contributions and foraging behavior (e.g., Robinson et al. 2018).