Stable isotope mixing models

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Course "Etude des isotopes stables et applications au milieu marin"
What do animals feed on?
What do animals feed on?

There is (nearly) an infinity of ecological questions somehow linked to animal diet

- Which are the resources essential for a consumer’s nutrition?
- Do species A and B feed on the same resources?
- Does species A consumes a different amount of this resource than species B?
- Is the diet of this animal stable in time, or does it shift to match seasonal resource availability?

...
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- Which are the resources essential for a consumer’s nutrition?
- Do species A and B feed on the same resources?
- Does species A consumes a different amount of this resource than species B?
- Is the diet of this animal stable in time, or does it shift to match seasonal resource availability?
- ...

To answer such questions, we need tools that allow us to delineate animal diet, i.e. to quantify the contribution of each potential food item to the diet of a consumer

→ Stable isotope mixing models
Mixing law: stable isotope composition of an animal is a proportional mix of its food sources’ isotopic composition.
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Stable isotopes: you are what you eat

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If you measure the isotopic compositions of an animal and its food item, you can calculate contributions of each food item to this animal’s diet.
A simple mixing model

\[
\begin{align*}
\delta X_{\text{Cons}} &= p_1 \cdot \delta X_{S_1} + p_2 \cdot \delta X_{S_2} \\
p_1 + p_2 &= 1
\end{align*}
\]

\[
\begin{align*}
p_1 &= \frac{\delta X_{\text{Cons}} - \delta X_{S_2}}{\delta X_{S_1} - \delta X_{S_2}} \\
p_2 &= 1 - p_1
\end{align*}
\]
You are what you eat... plus a few ‰!

We need to take into account trophic fractionation (most cases: enrichment in heavy isotope, hence "trophic enrichment factor" or TEF)
You are what you eat... plus a few %!

\[
\begin{align*}
\delta X_{\text{Cons}} - \Delta X &= p_1 \cdot \delta X_{S1} + p_2 \cdot \delta X_{S2} \\
p_1 + p_2 &= 1
\end{align*}
\]

\[
\begin{align*}
p_1 &= \frac{\delta X_{\text{Cons}} - \Delta X - \delta X_{S2}}{\delta X_{S1} - \delta X_{S2}} \\
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\]
What if you have 3 sources?

Just add a second isotopic ratio!

Figure: Newsome et al. 2007 Front Ecol Environ 5: 429-436
What if you have 3 sources?

Just add a second isotopic ratio!

\[
\begin{align*}
\delta X_{\text{Cons}} - \Delta X &= p_1 \cdot \delta X_{S1} + p_2 \cdot \delta X_{S2} + p_3 \cdot \delta X_{S3} \\
\delta Y_{\text{Cons}} - \Delta Y &= p_1 \cdot \delta Y_{S1} + p_2 \cdot \delta Y_{S2} + p_3 \cdot \delta Y_{S3} \\
p_1 + p_2 + p_3 &= 1
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Figure: Newsome et al. 2007 Front Ecol Environ 5: 429-436
What if you have 3 sources?

Just add a second isotopic ratio!

If you have $n$ isotopic ratios, you can deal with $n+1$ sources

Figure: Newsome et al. 2007 Front Ecol Environ 5: 429-436
Real-world food webs are complex: animals feed on many food items...
Most isotopic studies: 2 isotopic ratios (C & N), sometimes 3
Real-world food webs

Many systems are underdetermined: more unknowns then equations
Need of more complex mathematical models
Dealing with underdetermined systems

IsoSource model

Iterative procedure:
- All possible combinations of each source combination (0-100%) are examined in small increments (e.g. 1%).
- Combinations that sum to the consumer’s isotopic composition are considered feasible.
- The program returns the ranges and frequencies of these solutions.

There is no single solution! The model’s "solution" is the full distribution of feasible solutions.
Dealing with underdetermined systems

Source partitioning using stable isotopes: coping with too many sources

Example of IsoSource output: diet of the clam *Ruditapes philippinarum*

Isotopic compositions of consumers and food items are uncertain.
Real-world food webs

Isotopic compositions of consumers and food items are **uncertain**

2 main sources of uncertainty: **natural variability** (holds ecological info - we want to keep it!) and **analytical error** (we aim to minimise it, but we have to deal with it anyway)
Real-world food webs

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2 main sources of uncertainty: **natural variability** (holds ecological info - we want to keep it!) and **analytical error** (we aim to minimise it, but we have to deal with it anyway)

To build more realistic mixing models, we need to take uncertainty into account!
A new family of mixing models

MixSIR (https://conserver.iugo-cafe.org/user/brice.semmens/MixSIR)

Source Partitioning Using Stable Isotopes: Coping with Too Much Variation

SIAR (https://github.com/AndrewLJackson/siar)

Models based on Bayesian inference
Bayesian inference

Method of estimating the probability of an event based on prior knowledge of conditions related to this event.

What’s the probability of Standard de Liège winning the Belgian championship this year?
Bayesian inference

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What’s the probability of Standard de Liège winning the Belgian championship this year, knowing that they won 10 times in 115 championships?
Bayesian inference

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What’s the probability of Standard de Liège winning the Belgian championship this year, knowing that they won 10 times in 115 championships?

What’s the probability of Standard de Liège winning the Belgian championship this year, knowing that they won 10 times in 115 championships and currently rank 8th out of 16?
Bayesian inference

Method of estimating the probability of an event based on prior knowledge of conditions related to this event.

\[
P(A|B) = \frac{P(B|A) \cdot P(A)}{P(B)}
\]

- \( P(A|B) \) : Likelihood of event A occurring given that event B occurred
- \( P(B|A) \) : Likelihood of event B occurring given that event A occurred
- \( P(A) \) : Probability that event A happens independently of B
- \( P(B) \) : Probability that event B happens independently of A
You take a drug test.
This test has 99% sensitivity (i.e. 99% of drug users test positive).
This test has 99% reliability (i.e. 99% of non-drug users test negative).
The tested drug is used by 1% of the population.

Your test comes up positive. What’s the probability that you use the drug?
Bayesian inference

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Intuitive answer: 99%
Bayesian inference

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\[
P(\text{User} | +) = \frac{P(+ | \text{User}).P(\text{User})}{P(+)}
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Bayesian inference

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P(+) = P(+ \mid \text{User}) \cdot P(\text{User}) + P(+ \mid \text{Non-user}) \cdot P(\text{Non-user})
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\]

\[
P(\text{User} \mid +) = 0.5
\]
You have three doors to choose from. Behind one, there is a car. Behind the others, there is a goat.

After you picked one, Monty Hall opens one of the two remaining doors, and shows you that it leads to a goat.

You have two doors remaining: the one you initially picked, and another one. Should you stick to your door or switch?
The Monty Hall problem

winning percentages for each choice

number of simulations

Switch doors

Do not switch doors

The Monty Hall problem

How can you explain this counter-intuitive result?

![Image](https://medium.com/@NickDoesData/applying-bayes-theorem-simulating-the-monty-hall-problem-with-python-5054976d1fb5)

The Monty Hall problem

Assuming you initially picked door A, and Monty opened door B.

To maximise your chances to win, you should always switch doors.
Bayesian mixing models: why?

1) Bayesian methods allow incorporation of *prior* information.

If you have any info about your consumer’s diet (gut contents, functional traits), you can include it as a prior.
Bayesian mixing models: why?

1) Bayesian methods allow incorporation of *prior* information
   If you have any info about you consumer’s diet (gut contents, functional traits), you can include it as a prior.

2) Bayesian methods can integrate *uncertainty* from various sources
   Variability in sources and consumers isotopic ratios, but also in TEFs, can be taken into account in your model.
Bayesian mixing models: why?

1) Bayesian methods allow incorporation of **prior** information
   If you have any info about you consumer’s diet (gut contents, functional traits), you can include it as a prior.

2) Bayesian methods can integrate **uncertainty** from various sources
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3) Bayesian methods explicitly **compare** the strength of **support** for competing models or parameter values
   It is straightforward to compare model solutions (posterior probability distributions), as well as to estimate model performance (using diagnostics)
Bayesian mixing model: an example

Context: These two species of copepods live together in *Posidonia oceanica* litter.

Question: Do they rely on the same resources?

Hypothesis: Differences in their morphology could be linked with different feeding behaviour, and therefore resource partitioning.
Bayesian mixing model: an example

Source contribution to diet of C. aruicornis and E. dentatus from different sources (seagrass epiphytes, seagrass detritus, suspended particulate organic matter).

Mascart et al. 2018 Food webs 16: e00086
Bayesian mixing model: an example

Source contribution to diet

C. arcuicornis

E. dentatus

Source

- Seagrass epiphytes
- Seagrass detritus
- Suspended particulate organic matter

Proportions by group: 1

Proportions by group: 4

MPD, EPI, SPOM

Source
How probable is it that contribution of source is different in the two species?

Epiphytes: 78.16%
Detritus: 99.86%
SPOM: 99.99%
A simple Bayesian mixing model: simmr

Stable Isotope Mixing Models in R with simmr

Andrew Parnell and Richard Inger

https://github.com/andrewcparnell/simmr

Upgrade of SIAR: many common features, plus a few improvements
A complex Bayesian mixing model: MixSIAR

Analyzing mixing systems using a new generation of Bayesian tracer mixing models

Brian C. Stock\textsuperscript{1}, Andrew L. Jackson\textsuperscript{2}, Eric J. Ward\textsuperscript{3}, Andrew C. Parnell\textsuperscript{4}, Donald L. Phillips\textsuperscript{5} and Brice X. Semmens\textsuperscript{1}

Stock et al. (2018), PeerJ, DOI 10.7717/peerj.5096

MixSIAR capabilities:
- Any number of tracers
- Categorical or continuous covariates
- Multiple error structures
- Comparison of model scenarios
- ...

Drawback: computationally intensive

Ontogenetic shift in resource use in \textit{Alligator mississippiensis}
Mixing models can be used to answer many questions in biogeosciences, hydrology, ecology, ...
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What are the sources of organic matter fueling sediment-associated detritus in a seagrass meadow?

Jankowska et al. 2016 J Geophys Res Biogeosci. 121: 2918-2934
Mixing models: beyond diet analysis

Mixing models can be used to answer many questions in biogeosciences, hydrology, ecology, ...

Which commercial mixtures lead to seabass contamination by PCBs?

Schnitzler & Michel, Unpubl.
Building sensible mixing models

"Junk in, junk out" paradigm
"Junk in, junk out" paradigm

- Bad data
- Good model
- Bad results
Building sensible mixing models

"Junk in, junk out" paradigm

Bad data → Good model → Bad results

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Building sensible mixing models

"Junk in, junk out" paradigm

- Bad data → Good model → Bad results
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Building sensible mixing models

- Good characterization of food items (as important as consumers!)
- Sufficient replication (robust error estimates)
- Suitable TEFs (as close as your studied species as possible)
Building sensible mixing models

**Good data**
- Good characterization of food items (as important as consumers!)
- Sufficient replication (robust error estimates)
- Suitable TEFs (as close as your studied species as possible)

**Good model**
- Set the models parameters sensibly, and assess model performance
- Include all relevant food items (and only them)
- Make sure your model assumptions are met: plot your data
Mixing polygons

Source 1
Source 2
Source 3
Source 4
Your mixing model can only work with consumers that are within the "mixing polygon" defined by the sources’ isotopic values.
Mixing polygons

\[ \delta X \text{ (‰)} \]

\[ \delta Y \text{ (‰)} \]

Source 1

Source 2

Source 3

Source 4

Consumer 1

Consumer 2

Consumer 3
Mixing polygons

Consumers’ isotopic ratios taking into account trophic fractionation
Mixing polygons

\[ \delta X (\% \text{‰}) \]

\[ \delta Y (\% \text{‰}) \]

Source 1
Source 2
Source 3
Source 4
Consumer 1
Consumer 2
Consumer 3
If, after correcting for fractionation, your consumers do not fit in the mixing polygon, it makes no sense to run a mixing model.
Isotopic similarity of food items
Isotopic similarity of food items

Common problem: some potential food items have the same isotopic composition...
If isotopic compositions of food items are similar, it makes no sense to use them separately as input to a mixing model. The model will not be able to tell them apart from one another.
Isotopic similarity of food items

Solution 1: Aggregate the similar sources
Isotopic similarity of food items

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Realistic from a modeling point of view, but can lead to loss of ecological information
Isotopic similarity of food items

Solution 1: Aggregate the similar sources

Solution 2: Combine SI with other tracers that can discriminate the food items
Alternative tracers: fatty acids

"Building blocks" of lipids

Long carbon chain with a final acid group

Saturated

Unsaturated

Source: www.nature.com
During digestion, lipids are degraded but fatty acids are incorporated in the consumer’s tissues in a conservative way

➡️ A consumer’s fatty acid composition is similar to the one of its food sources

➡️ Fatty acids can be used as trophic markers and combined to stable isotopes to build mixing models
During digestion, lipids are degraded but fatty acids are incorporated in the consumer's tissues in a conservative way. A consumer's fatty acid composition is similar to the one of its food sources. Fatty acids can be used as trophic markers and combined to stable isotopes to build mixing models.

"Building blocks" of lipids

Long carbon chain with a final acid group

Saturated

Unsaturated

+: Limits loss of ecological info

-: More assumptions (what about fatty acid biosynthesis?)

A consumer’s fatty acid composition is similar to the one of its food sources.

Fatty acids can be used as trophic markers and combined to stable isotopes to build mixing models.
1. Use prior knowledge to identify relevant questions
2. Consider what’s known about the animal’s diet
3. Plan your sampling design well
4. Use appropriate trophic fractionation factors
5. Plot your data before running your model
6. Include all relevant food items, in an informed way
7. Group your sources when isotopically and/or ecologically relevant
8. Don’t forget about concentration dependence and isotopic routing
9. Consider and incorporate uncertainties
10. Report distribution of results
11. Your model will always be an oversimplification of a complex ecological reality. Assess its performance. Remember its limitations!
"Remember that all models are wrong; the practical question is how wrong do they have to be to not be useful."

George E.P. Box
1919-2013
Building sensible mixing models

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Mixing models are not "magic wands", nor perfect tools

However, when used sensibly, they offer an efficient way to assess animal diet and explore ecological patterns
They say, "You are what you eat!" That's funny. I don't remember eating a SEXY BEAST this morning.

You are what you eat?
Well, I'd rather be a cupcake than a freakin' carrot...

Thanks for your attention