

Introduction

Stable isotope ratios are being increasingly used to explore feeding relationships in natural ecosystems. However, their utility has been critically hindered because it is difficult to construct meaningful food-webs from the two-dimensional ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) data which result from this technique.

In 2003 we argued that per-trophic level enrichment, which is usually assumed to be constant (3.4‰ for $\delta^{15}\text{N}$, 1‰ for $\delta^{13}\text{C}$) actually results from a dynamic equilibrium between fractionation vectors associated with assimilation and excretion (Olive *et al.* 2003). We described a new linear model whereby the equilibrium isotope signature of the consumer could be predicted given the feeding rate (Ω) and factors describing isotopic discrimination during assimilation (q) and the discrimination during excretion (Z).

In the present study we apply the approach of Olive *et al.* (2003), and explore how this technique, together with isotope 'mixing models' might be used to investigate a Mediterranean littoral food-web. We discuss: (a) what sensible values of Ω , Z and q might look like, and (b) we demonstrate how Ω , Z and q might be derived for marine and freshwater fishes.



Sarpa salpa, *Mullus surmuletus*, *Spicara maena* and *Oblada melanura*.
All photographs by Robert A. Patzner, (Courtesy of Fishbase)

Box 1: The Model

The abundance of any pair of isotopes in delta (δ) notation units expresses the ratio of heavy to light isotopes with reference to an international standard. Thus for nitrogen:

$$\delta^{15}\text{N} = \left(\frac{^{15}\text{N}/(^{14}\text{N}+^{15}\text{N})_{\text{sample}}}{^{15}\text{N}/(^{14}\text{N}+^{15}\text{N})_{\text{standard}}} - 1 \right) \times 1000 \quad \text{[Equation 1]}$$

where the international standard is naturally occurring N_2 in the atmosphere.

The model of Olive *et al.* considers a whole heterotrophic organism to be a single 'black box' system. There is net mass transfer inwards due to feeding and outwards due to excretion

The fractionation events for the system at equilibrium, can be described as:

$$\frac{d\delta_a}{dt} = Y + Z = 0 \quad \text{[Equation 2]}$$

where $\frac{d\delta_a}{dt}$ is the rate of change in isotopic signature for any pair of stable isotopes e.g. $\delta^{15}\text{N}$ of the homogenized animal tissue, Y represents the rate of change in isotope signature consequent upon feeding and Z represents the rate of change in isotopic signature consequent upon excretion. Y and Z have opposite sign and Z will be positive.

Equation 2 will be disturbed by any change in isotopic signature of the food (δ_{food}) and by changes in feeding rate (see Olive *et al.* 2003). Assuming a constant rate of enrichment due to excretion (i.e. Z), Y in equation 2 can be expanded to represent the instantaneous rate of change in the isotopic signature of a whole animal (δ_a) at some time t following a change in the diet:

$$\frac{d\delta_a}{dt} = \Omega_a (q\delta_{\text{food}} - \delta_a) + Z \quad \text{[Equation 3]}$$

The term Ω_a has a minimum value of zero for an animal that does not feed and is the dimensionless ratio of the mass of an element (e.g. nitrogen) in the ingested food in relation to the mass of the element in the animal as a whole. The term q is defined such that $q\delta_{\text{food}} = \delta_{\text{food-product}}$. When assimilation for a particular element is 100%, the fraction of unused substrate $f = 0$ and $q = 1$, but when assimilation is $<100\%$, then $0 < f < 1$, $\delta_{\text{food-product}} < \delta_{\text{food}}$ and $q < 1$. The integral of equation 3 can be found by calculus and solved for time t as:

$$\delta_a = -\frac{Z + \Omega_a q \delta_{\text{food}}}{\Omega_a} (e^{-\Omega_a t} - 1) + \delta_a e^{\Omega_a t} \quad \text{[Equation 4]}$$

Given sensible values of Ω_a , q , Z and δ_a (the isotopic signature of the animal) it is possible to predict the isotope signature of the animals' diet (δ_{food}), through a further rearrangement of equation 4:

$$\delta_{\text{food}} = \left(\frac{(-\delta_a - \delta_a e^{\Omega_a t}) / (\Omega_a - Z)}{\Omega_a} \right) e^{-\Omega_a t} \quad \text{[Equation 5]}$$

Deriving sensible values for Ω , q and Z

Ω can be derived from estimates of daily ration (R_d), i.e. food consumption expressed as % body-weight per day, as well as the nitrogen or carbon content of the food (as a proportion) and the nitrogen or carbon content of the consumer:

q can be derived from Nitrogen Absorption Efficiency (NAE) or Carbon Absorption Efficiency (CAE) estimates.

Z can be derived either through: (a) direct measurements of nitrogen or carbon excretion rates and the isotope signature of the excreted products, or (b) diet-switch experiments and rearrangement of equation 4 using sensible values for Ω and q .

Here we used data on Mediterranean fishes collected by Pinnegar *et al.* (2000) together published estimates of R_d ($n = 227$), NAE ($n = 151$) and CAE ($n = 84$) from the literature to derive sensible values for Ω , q and Z . We have attempted to use these values in equation 5 to predict the aggregate isotope signature of the animal's diet and also the per-trophic step fractionation factor (usually assumed to be +3.4‰ for $\delta^{15}\text{N}$ and +1‰ for $\delta^{13}\text{C}$).

Using these predictions for the isotope signature of the animal's diet it is possible to use isotope 'mixing models' to estimate the diet composition of fishes (i.e. the % represented by different prey types).

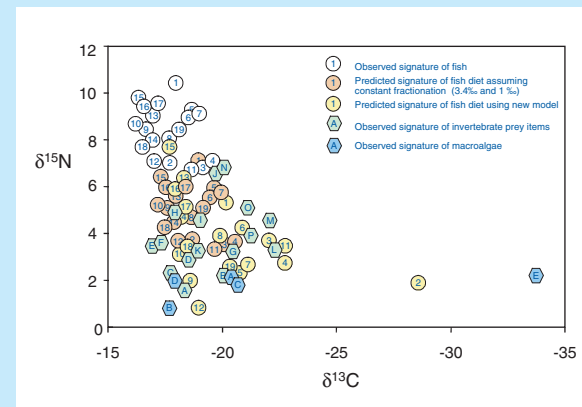


Figure 1: Here we compare the observed isotope signature of the predator (○), with the predicted isotope signature of predator's diet assuming a per-trophic-level fractionation factor of +3.4‰ for $\delta^{15}\text{N}$ and +1‰ for $\delta^{13}\text{C}$ (○), and the predicted isotope signature of the predator's diet using the model of Olive *et al.* (○), numbers coincide for the same predator species. We also compare the predicted isotope signature of the predator's diet with the observed signature of different prey types (○).

Results & Discussion

- The predicted diet signature for a particular predator can be very different if you simply assume a constant fractionation, or use sensible values for feeding and assimilation within the model of Olive *et al.* (e.g. see predators No 2 and 12).
- Assuming constant fractionation simply results in a 'mirror' of the observed predator data (shifted down by 3.4‰ and across by 1‰); the model of Olive *et al.* results in a dramatically different distribution of predicted diets.
- The model of Olive *et al.* results in predictions which are much more consistent with stomach-content data compared to simply assuming constant-fractionation; e.g. the three sparids (*Diplodus sargus*, *D. annularis* and *Oblada melanura* - 19, 5, 7) cluster around benthic amphipods and sea urchins (green B & G); the planktivorous fish *Chromis chromis* (3), *Spicara maena* (4) and *Boops boops* (14) cluster with zooplankton (copepods - green L) rather than benthic amphipods (green G).
- The per-trophic level fractionation factor can vary markedly, and in terms of $\delta^{15}\text{N}$ can be much greater than 3.4‰ (e.g. No 12 *Mullus surmuletus*, or No 5 *Oblada melanura*, predicted fractionation factor $>6\%$) depending on the feeding rate, the absorption efficiency and the food quality (N or C content).
- The per-trophic level fractionation factor estimated for the herbivorous fish *Sarpa salpa* (No 2) was 5.1‰ for $\delta^{15}\text{N}$ and 10.9‰ for $\delta^{13}\text{C}$. This suggests that the species consumes a mixture of algal food sources including the nutritious red alga *Sphaerococcus coronopifolius* (blue - E) as well as the predominant species at the site, *Cystoseira balearica* (A) and *Halopteris scoparia* (C). This is in stark contrast to our previous interpretation (assuming a constant fractionation) where we had suggested that *S. salpa* must obtain most of its nitrogen from animal sources.

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References

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