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Advancing Dynamic Isotopic Mixing Models: A Comprehensive approach with a dynamic indicator for decision-making model selection

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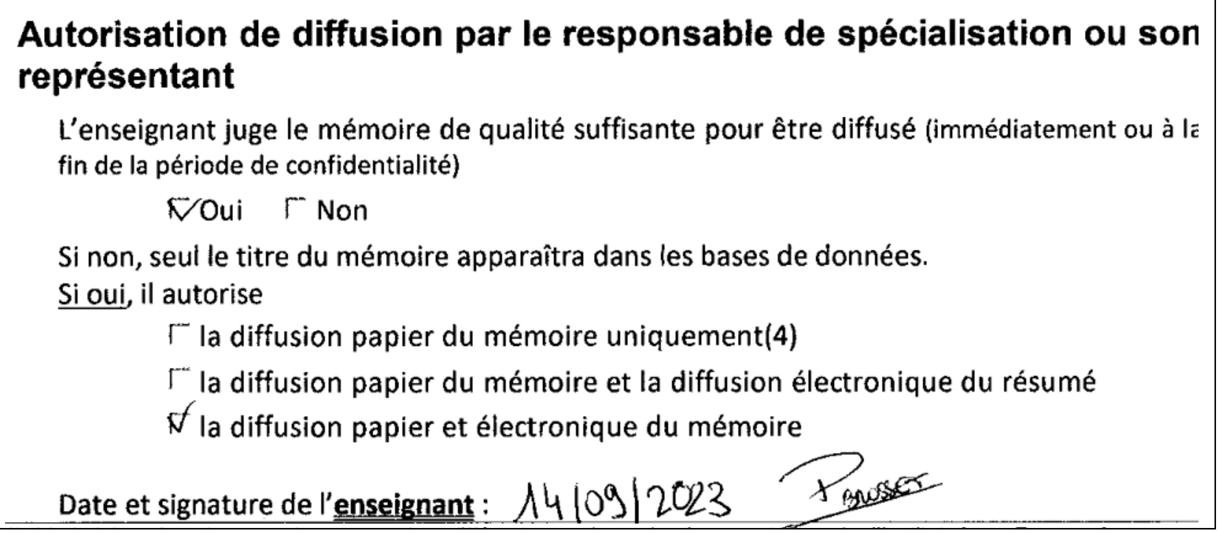

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Glossaire et liste des abréviations

SMM	Static stable isotope mixing model
SMM_delta	Integrated SMM
DMM	Dynamic stable isotope mixing model
DI	Dynamic indicator
λ	Isotopic turnover rate/ isotopic incorporation rate (d^{-1})
$t_{1/2}$	Half-life (d)
$\delta X_t, \delta X_0, \delta X_\infty$	Isotopic ratio (X represents an element) of the consumer (‰) Respectively at a time t, the initial value and at the equilibrium
δX_{obs}	Observed isotopic ratio of the consumer for a given element X (‰)
δX_{pred}	Isotopic ratio of the consumer (‰) predicted by the models for a given element X
δX_s	Isotopic ratio (X represents an element) of the source *s* (‰)
δX_d	Isotopic ratio (X represents an element) of the diet (‰)
ΔX_s	Trophic enrichment factor (‰) for the source s
qX_s	Concentration of the element X in the source *s*
p_s	Proportion of the source *s* in the diet
S	Number of sources
I	Number of isotopes
Δt	Time window of isotopic integration
T	Experiment time (d)
$\Delta_{sources}$	The difference between the values of the two most contrasted sources of the isotopic space (‰)
ω	Frequency of diet switch (d)

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1. Introduction

Some elements, like Carbon, Nitrogen or Oxygen, have different stable forms that differ by their number of neutrons in the core. They are named isotopes. The form with more neutrons is called the heavy form, in opposition to the light form. The ratios of these isotopes, corrected by a standard ratio, can be measured in different organism's tissues (*Garvey & Whiles, 2017*). Stable isotope ratios (also called isotopic signatures) are commonly used in ecology as ecological tracers (*West et al., 2006*). The isotopic ratios of Carbon ($\delta^{13}C$) rather reflects the origin of carbon sources, while Nitrogen ($\delta^{15}N$) is used as a proxy of trophic level. In combination, both ratios can be used to estimate the diet (different food sources with distinct isotopic signatures) of a consumer (*Fry, 2006*), to study its isotopic niche (proxy of the trophic niche, *Shipley & Matich, 2020*), and, in general, provide information about trophic interactions and food-web structure (*Potapov et al., 2019*). Studying diets have several uses, the first being collecting data about feeding behaviour for predators. A change in a consumer diet can be an indicator of ontogenetic change (*Knoff et al., 2007*), drastic changes in feeding habits, resulting in a significative change of the consumer isotopic signature. Studying diets can also help detect migrations (*Hobson, 1999*). Indeed, a change in foraging place can lead to changes in available preys and/or a change of the baseline isotopic signature that varies significantly along environmental gradients (*Savoye et al., 2003)* leading to changes in the isotopic signature of the consumer.

The isotopic signature of a predator (δX) depends on the signature of the different preys that constitute its diet (δX_d, *DeNiro & Epstein, 1978)* giving rise to the famous quote « *you are what you eat (plus a few ‰)* ». The « *plus a few ‰* » part comes from an enrichment of the heavy isotope due to the trophic enrichment factor (ΔX_s, also called trophic discrimination factor). Indeed, the observed isotopic signature of a consumer is higher than the observed value of its prey. The reason is a preferential incorporation of the heavier isotope during the assimilation of elements (*Fry, 2006*) leading to a higher relative concentration of the heavier element in the consumer tissues (thus a higher ratio) than in the diet. This enrichment factor can vary depending on factors like the predator-prey relationship, the type of diet, and the specific isotopes being studied (*McCutchan et al., 2003).* In addition, ΔX_s is crucial for diet reconstruction using mixing models (*Bond & Diamond., 2011*) and requires particular experimental conditions (e.g. diet-switch experiments) to be estimated (*McCutchan et al., 2003).* Another key element to consider in stable isotopes ecology is the isotopic turnover rate (λ). λ refers to the rate at which an organism's isotopic composition changes over time in response to changes in its diet or environment. It is a measure of the turnover or replacement of isotopes within the organism's tissues due to metabolic processes and assimilation of new isotopes from the diet (*Carter el al., 2019*). λ is influenced by various factors, and it varies among different organisms, tissues, and environmental conditions as it depends of the physiological state of the consumer (*Vander Zanden et al., 2015*). λ (and 1/λ which is the retention time in an animal tissue) is linked to the half-life of an element in a tissue, $t_{1/2}$, that is expressed as $t_{1/2} = \frac{\ln(2)}{\lambda}$. As λ express the speed a tissue renews itself, it takes really different values depending the studied tissue, it has really high value in tissue with a low half life such as the blood or plasma and lower values in tissues such as muscle or organs (*Vander Zanden et al., 2015).*λ is proportional to the growth rate of the consumer, when the consumer is at an early stage (larvae), the growth rate is high, and then, its value decreases with time and is inversely proportional to the age and body mass of the consumer (*Vander Zanden et al., 2015; Thomas and Crowther, 2015; Carleton & Martínez del Rio, 2005*). At the same time, λ is related to the parameter *k* and body mass in a Von Bertalanffy growth equation. λ can vary seasonally as well, especially for ectothermic species whose growth depends on the temperature (e.g. bivalves, *Marín Leal et al., 2008*).

Stable isotopes mixing models are quantitative tools used in trophic ecology to estimate the contribution of different food sources to a consumer's diet. Different models have been developed over time, each with its own assumptions, mathematical framework, and applications, allowing researchers to tailor their approach to the specific ecological questions at hand. Static Linear Mixing Models (here after referred to as SMM, *Schwarcz, 1999*) are classical models that remain widely used nowadays. SMM estimates diet contributions by comparing the instantaneous isotopic ratios of a consumer and its prey. SMM was subsequently improved to reduce its bias (*Philips & Gregg, 2003*) by taking others factors into consideration, for example, the concentration-dependence setting (*Philips & Koch, 2002*) which considers the varying contributions of different elements in the diet based on their relative concentrations. Improvements helped as well to broaden the cases where mixing models could be used by adapting the equations and solutions to various number of studied isotopes and food sources (*Philips & Gregg, 2003).* More recently, Bayesian approaches of SMM have been developed *(Stock et al., 2018, Parnell et al., 2010).* The Bayesian framework helped having robust analysis by implementing complex statistical distributions, sources of uncertainties and prior knowledge into SMM. The challenge with SMM models is that they operate under the assumption of isotopic equilibrium, where the isotopic ratios of all three of the consumer, sources and diet remain constant over time. However, this assumption is not always valid, and the model can struggle in providing accurate results when the isotopic ratios undergo changes over time. To reduce this bias, *Philips et al. (2014)* advised the use of averaged values of the source ratios over the incorporation time (instead of only instantaneous values) to consider the source dynamics. The time period on which to average sources should be linked to the half life $t_{1/2}$ (and therefore linked to λ) to reduce this bias. *Ballutaud et al*. (2022) estimated the best time period to be twice the half-life. This model is called the integrated mixing model, (here after refer to as SMM_delta) and although is not a properly dynamic model, provides a good alternative to consider some of the variation of ratios over time.

A proper dynamic mixing model (DMM), was recently developed by *Ballutaud et al. (2022*) with the intent to consider the different dynamics that can influence stable isotopes and mixing models. First, it considers λ as a parameter that can be variable. Similarly, the isotopic signatures of sources and consumers and the diet can vary over time. For now, the DMM model only works for systems studying one single isotope and two sources, does not have a concentration-dependence setting and was not developed in a bayesian framework. The DMM includes the calculation of an indicator to quantify the difference between models (i.e., SMM, SMM_Delta and DMM) but this indicator can only be used in *in-silico* experiments and is not adapted to real datasets making it impossible to use on field studies. Furthermore, in general, all the three models (SMM, SMM_delta and DMM) only work for constrained systems where the models give one unique solution but cannot deal with underdetermined systems (where several solutions exist). Furthermore, in *Ballutaud et al*. (2022), the bias between models was studied as a function of the frequency of diet switches (ω) divided by λ. This ratio was used as an indicator because it was hypothesized to be a factor influencing the variability of results among the models. However, relying solely on this indicator for case studies became challenging. Furthermore, this indicator takes in account the frequency of switch and λ, but other factors of variability that have been observed were not considered such as the variability of the consumer and the sources signatures.

The principal aim of this study was to create a generalized version of the DMM that can be used regardless of the number of isotopes and sources considered, and that can account for concentration-dependent scenarios. This enhanced DMM operates similarly to the previous version, but with the added capability to handle a broader range of experimental settings including underdetermined cases. To assess its performance, it is necessary to develop static mixing models (SMM, SMM_delta) that can be applied under the same conditions. A second objective to introduce a new indicator linked to the bias between models and allowing to elucidate differences in outputs between the mixing models and to help identify contributing factors and variables. This indicator should also be applicable in real-world study cases using authentic datasets and help in the decision making regarding the kind of mixing model to use in different circumstances.

2. Materials and Methods

2.1 The model of isotopic incorporation

This section is described for one isotope, but can be extended to as many isotopes as needed.

The dynamic of the isotopic value of a consumer over time (for a specific isotope δX in ‰) in a static framework is often expressed by the following equation (Eq. 1), known as the first order kinetic one-compartment time model (*Hesslein et al.,1993, Martínez del Rio et al., 2009*).

$$\delta X_t = \delta X_\infty + (\delta X_0 - \delta X_\infty)\exp(-\lambda t) \qquad \text{(Eq. 1)}$$

With δX_∞ the isotopic value of the consumer at the asymptote, where isotopic equilibrium is reached. When this equation is used on a static framework, $\delta X_\infty$and λ are assumed constant over time. As a reminder, λ (in d^{-1}) denotes the isotopic incorporation rate, controlling the time interval between an element being consumed by a predator and its complete incorporation and mixing into the predator's tissues. Its value is considered as varying in a range from 10^{-3} d^{-1} to 10^{-1} d^{-1} (*Thomas & Crowther, 2015*). The half-life ($t_{1/2}$) is expressed in days and represents the time required for a tissue to change half of its elemental composition, with $t_{1/2} = \ln(2)/\lambda$. This parameter is considered in both the DMM and the SMM_delta, differentiating them from the SMM. λ is influenced by the consumer's growth rate and catabolic turnover rate, leading to changes over time. Hence, a dynamic setting is crucial in the mixing models to account for such variations. In a dynamic framework, different sources of variability can influence the isotopic value of the consumer (λ and δX_∞ in particular) and Eq. 1 could not be used anymore. The DMM is rather based on the use of the following differential equation (Eq. 2), which is the derivative form of Eq. 1:

$$\frac{d\delta X}{dt} = \lambda(t)(\delta X_\infty(t) - \delta X) \qquad \text{(Eq. 2)}$$

2.2 The mixing models

Dynamic mixing model

At equilibrium, the isotopic value of a consumer depends on the value of the diet $\delta X_d(t)$ (which is the mixture of the isotopic values of the sources, δX_s, wheighted by their proportion in the diet of the predator, p_s), the associated trophic discrimination factors ΔX_s (as formulated in the mass equation by *Schwarcz (1999), Philips (2001)*), and the concentration of elements in the sources, qX_s (*Parnell et al., 2010, Philips & Koch 2002).* Indeed, two different sources consumed in the same quantity will provide a different number of elements depending of their concentrations in this element. The concentration-dependent linear mixing model takes these former parameters into account. The model assumes that all food sources are known, and the sum of all the proportions equals 1, with $\sum_{i=1}^{s} p_{s(i)} = 1$. The concentration-dependency setting is particularly useful in cases where the diet includes both animal and vegetal sources with different concentrations of elements (e.g., Carbon and Nitrogen) (*Philips & Koch,2002*). Here an example for the diet value δX_d from a two-isotopes (X, Y) and three-sources (A, B, C) case:

$$\begin{cases} \delta X_d = \dfrac{\delta X_A * p_A * qX_A + \delta X_B * p_B * qX_B + \delta X_C * p_C * qX_C}{p_A * qX_A + p_B * qX_B + p_C * qX_C} \\ \delta Y_d = \dfrac{\delta Y_A * p_A * qY_A + \delta Y_B * p_B * qY_B + \delta Y_C * p_C * qY_C}{p_A * qY_A + p_B * qY_B + p_C * qY_C} \\ 1 = p_A + p_B + p_C \end{cases} \qquad \text{(Eq. 3)}$$

By adding the trophic discrimination factors ΔX_s and generalising Eq.3, we obtain a concentration-dependent linear mixing model (Eq.4). This model, is usually used in a static case supposing that on a period of time δX_d is constant and time independant and $\delta X_d = \delta X_\infty$. That supposition is made by assuming that the model is at the isotopic equilibrium.

$$\delta X_\infty(t) = \frac{\sum_{i=1}^{S} p_{s(i)} * qX_{s(i)} * (\delta X_{s(i)} + \Delta X_{s(i)})}{\sum_{i=1}^{S} p_{s(i)} * qX_{s(i)}} \qquad \text{(Eq. 4)}$$

The dynamic mixing model (DMM) uses the linear mixing model (Eq.4) inside the time model (Eq. 2). In this case, λ, $p_{s(i)}$ and $\delta X_{s(i)}$ can vary over time but $qX_{s(i)}$ and $\Delta X_{s(i)}$ are assumed constant. Eq.2 is a first order ordinary differential equation (ODE) with no analytical solution but a numerical solver can be used to solve it. The *lsoda* function which is included in the deSolve package (*Soetaert et al., 2010)* on Rstudio was used for this purpose. Finally, DMM can be expressed in the following way:

$$\frac{d\delta X_t}{dt} = \lambda(t) * (\left(\frac{\sum_{i=1}^{S} p_{s(i)} * qX_{s(i)} * (\delta X_{s(i)} + \Delta X_{s(i)})}{\sum_{i=1}^{S} p_{s(i)} * qX_{s(i)}}\right) - \delta X) \qquad \text{(Eq. 5)}$$

SMM and SMM Delta

SMM is a special case of DMM when λ is infinite and the other forcing variables are assumed constant so that $\delta X(t) = \delta X_\infty$ (according to Eq.1). Both, SMM and SMM_delta use the concentration-dependent linear mixing model (Eq.4) but differ in how they consider the source isotopic values. The SMM uses the actual $\delta X_{s(i)}(t)$ instantaneous values to compute $\delta X_d(t)$, while SMM_delta uses an average value of the $\delta X_{s(i)}$ on a time-window that is equal to twice the half-life value (*Ballutaud et al., 2022*).

2.3 Solving the mixing models

The DMM works as follows. The type of dataset required is an observation of the consumer's signature at t, $\delta X_{obs}(t)$, and at t+Δt, $\delta X_{obs}(\mathrm{t} + \Delta\mathrm{t})$. In this time window (Δt), the dataset must provide a continuous estimation of the source isotopic signatures at any time and an estimation of λ, supposed constant in this window. Then, the proportions of contribution for each food sources can be estimated. These observations are needed for each studied isotope. When the system includes n isotopes for n+1 different food sources, the system is constrained. Then, the DMM has one unique solution that can be resolved using the linear mixing model (Eq.3). But, when copying with multiple sources of uncertainty, where the number of sources exceeds the number of isotopes by one, there is no unique solution to the linear mixing model.

The solution proposed by *Philips & Gregg (2003)* was adopted to generalise the model. First, the model generates various combinations of proportions based on the number of sources present in the dataset. For instance, if the data set includes three different sources, the model will create all possible combinations of three numbers that sum up to one, with a certain level of precision. The precision can be changed to obtain more precise results but this may lead to longer computing times. As the model becomes more precise, it tests a greater number of combinations. Next, the dynamic mixing model (Eq.5) is applied to each combinations of proportions to predict the isotopic value of the consumer $\delta X_{pred}(t + \Delta\mathrm{t})$ and the trajectory between two observations for all the studied isotopes. Then, the model compares the distance between the predicted isotopic value of the consumer $\delta X_{pred}(t + \Delta\mathrm{t})$ and the observed value at the same time $\delta X_{obs}(t + \Delta\mathrm{t})$ following the equation proposed by *Marques et al. (2019*):

$$distance = \sqrt{\sum_{i=1}^{I} (\frac{\delta X_{obs} - \delta X_{pred}}{\delta X_{obs}})^2} \qquad \text{(Eq.6)}$$

With *I* the number of isotopes in the system. The distance decreases when $\delta X_{pred}(t+\Delta\text{t})$ approaches $\delta X_{obs}(t+\Delta\text{t})$. In the cases where the system is well constrained, there is an unique solution so there should be a value of $\delta X_{pred}(t+\Delta\text{t})$ where the associated distance equals zero. But in cases where the system is under-determined, there is no unique solution for which the distance equals zero. So, as the DMM is generalised to work for various states of systems (in terms of studied isotopes and number of sources), a distribution of solutions will be kept instead of only one solution. The model keeps the « best » solutions which are the ones minimising the distance. It is possible to choose how many solutions are to be kept. In the end, the DMM outputs the combinations of proportions for each source that predict the closest consumer signatures to the observed ones. That means the solutions that have the lowest associated distance. It is possible to select the number of solutions that are to be kept (usually the 1% of the solutions minimising the distance).

The SMM and the SMM_delta work in a similar way. The model generates various combinations of proportions. Then, the static mixing model (Eq.4) is applied to each combinations of proportions to predict the isotopic value of the consumer $\delta X_{pred}(t)$ and finally, the model compares the distance between the predicted isotopic value of the consumer $\delta X_{pred}(t)$ and the observed value at the same time $\delta X_{obs}(t)$ only to keep some of them (Figure 1).

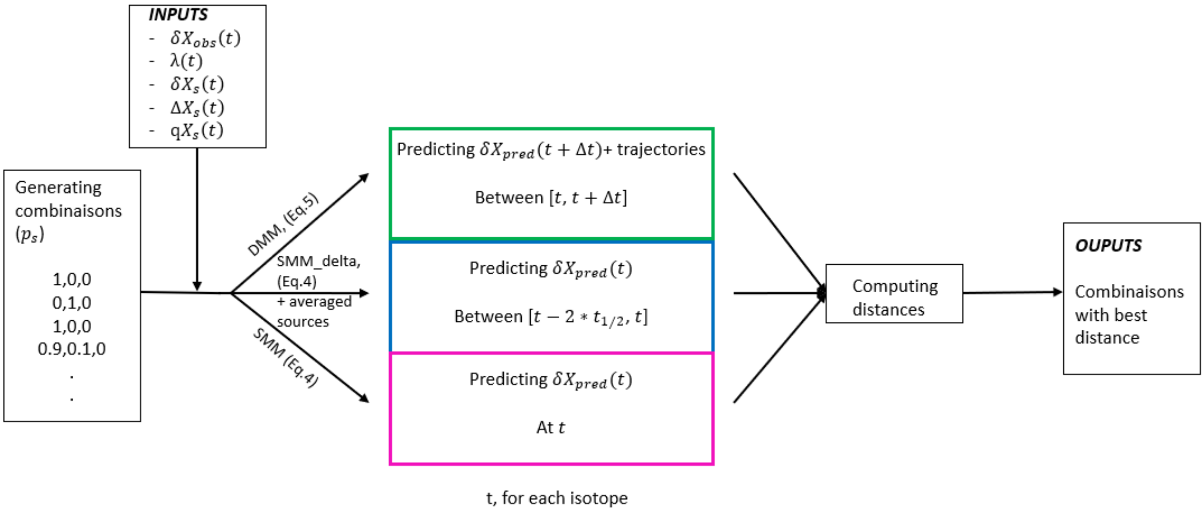


***Figure 1**: Overall framework of the three mixing models, the DMM (green), the SMM_delta (blue) and the SMM (pink).*

The difference between the models stands in the isotopic mixing. SMM uses the instantaneous values of the sources $\delta X_s(t)$ in the mixing model, while SMM_delta uses an averaged value $\overline{\delta X_s(\Delta t)}$ on a period Δt that equals twice the half-life. The three models work on different time scales as well, and provide outputs in terms of distance and proportions of sources for a chosen number of solutions. However, each model offers a distinct estimation approach for a specific time frame. SMM provides a snapshot estimation for a given time, reflecting the current state of affairs. In contrast, SMM_delta integrates estimations over time, equivalent to twice the half-life. Finally, DMM gives an estimation on a period between two observations of consumer isotopic values. When comparing the three models, it is important to keep in mind that they do not predict outputs on similar time scales.

2.4 *In-silico* experiments, bias and the dynamic indicator (DI)

Now that the three models have been generalised and are ready for use, *in-silico* frameworks were implemented to test their behaviour and to investigate why and when the models produce different results. Two different frameworks have been set up: one with one isotope and two different sources and another one with two isotopes and three sources. The first framework aims to compare the results of this study with the results found in *Ballutaud et al.* (2022). This comparison allows for a validation of the results in a comparable setting. The second framework was set up to determine if the differences among models depend on the number of isotopes and/or the number of food sources and to have a framework corresponding to the real case study datasets. It is important to note that both frameworks use well constrained systems (cases with n isotopes and n+1 sources). To compare the three models, DMM is used as a reference. The outputs of DMM, represented by δX_{pred}, serve as the input data to compute SMM and SMM_delta. For each source, the average bias is calculated as the mean difference between the predicted proportion of SMM or SMM_delta and the observed proportion (δX_{pred}) from DMM during the experiment period (Figure 2). The average bias is expressed as a percentage. A higher bias value indicates a greater disparity in the outcomes between the models.

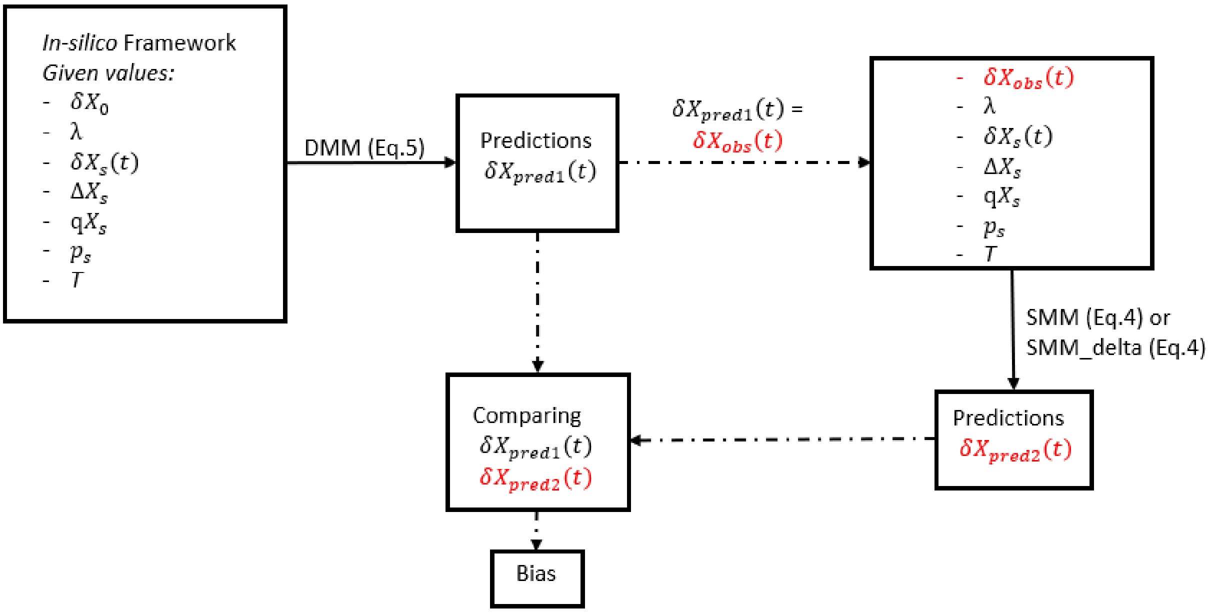


Figure 2: Process to compute the bias. The full line represents the use of one of the mixing models. The dashed lines represent the use of output values for the next step.

It is expected that the models' outputs are particularly different when the ratios of the consumer (δX_{pred}) vary greatly over the time which can happens when there is a diet change, a source signatures variation or a λ change. One way to quantify this change is to study the value of the derivative ($\frac{d\delta X_{pred}}{dt}$) over time. Eq.2 gives an expression of the value of the derivative and shows the different factors of variability affecting the derivative value. First, λ is a clear factor of variability, if its value doubles, the derivative value doubles as well. The other factor is the difference between $\delta X_{\infty}(t)$ and δX, which depends of the initial isotopic value of the consumer δX_0, the diet switch (a change in what percentage of each source the consumer consumes) and the covariation of the isotopic value of the sources. If two sources have very different isotopic values, the values of ($\delta X_{\infty}(t) - \delta X$), as well as the derivative values, will be higher than if two sources had very close isotopic values. However, it is important to note that the difference in calculated derivative values only indicates a relative distinction between the derivative values in the isotopic space. This has been checked in Appendix I. In the 1-isotope framework, tests were made with constant sources having different values and otherwise similar conditions. Regardless of the isotopic values of the sources, it was observed that the average derivative value divided by the difference in isotopic values of the sources followed the same trajectory. To facilitate results comparison, the derivative value at time « *t* » is always divided by the maximum difference of the isotopic values of the sources at that time ($\Delta_{sources}$). Likewise, as the derivative value change proportionally with λ, dividing the derivative value by λ results in the bias following the same trajectory regardless of the specific λ value. The only difference is the maximum derivative value according to λ, see Appendix II.

An indicator has been created in this study, called the Dynamic Indicator (DI) to indicate the extent to which the isotopic value of the consumer changes over time. It is calculated by summing all the derivative values ${}^{d\delta X_{pred}}/_{dt}$ of the consumer, divided by the experimental period (T), and dividing it by λ and the maximum difference in isotopic values between sources. This indicator has no units and is expressed in the following way:

$$DI = \frac{\sum_{i=1}^{T} \frac{{}^{d\delta X_i}/_{dt}}{T}}{\Delta sources * \lambda} \qquad \text{(Eq.7)}$$

Now that the DI indicator is set, the different sources of variability on the $(\delta X_{\infty}(t) - \delta X)$ component were studied. Specifically, we investigated the effects of the initial value, the intensity of diet switch, and the covariation of sources. It quickly became apparent that the initial value and the diet switch were in fact the same source of variability and exhibited similar patterns so they were tested on a same experiment as one factor (Appendix IIIa). Three experiments were then conducted, each testing one parameter at once, using the 1-isotope and 2-isotope frameworks. The experiments were as follows:

- **Experiment 1**: Variation of the initial value/ switch intensity with constant sources.
- **Experiment 2**: Only for the 2-isotope framework. Switching from source 2 to source 3 or from source 2 to source 1 or from source 2 to 50% of each other two sources, with constant sources values (see Figure 3 for the sources values).
- **Experiment 3**: Different covariations of the sources with constant initial value and switch and effect of the time period on these results.

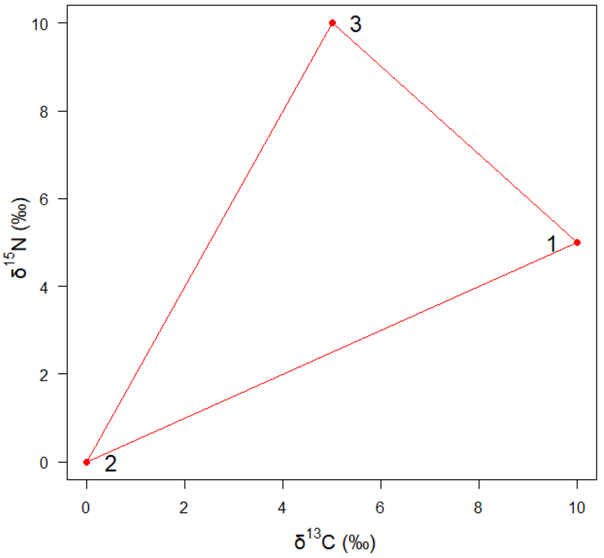


Figure 3: Dual isotope plot with the polygon of food sources (1, 2, 3).

The experiments 1 and 2 have the following settings:

- In the 1-Isotope framework: The two sources have constant ratios over time and equal 0‰ and 10‰. The enrichment factor ΔX_s=1‰ for both sources. At the begining of the experiment, the consumer switched from 100% of source 2 to 100% of source 1.
- In the 2-Isotope framework: The three sources have constant ratios over time for both isotopes. Values are those illustrated in Figure3. The signatures of the sources are very distinct which is not necessarily realistic but highlights the different tested effects. The enrichment factors, ΔX_s equals 1‰ for both isotopes and the three sources. The consumer diet switched from 100% of source 2 to 100% of source 3 in experiment 1. In experiment 2, the bias from all the three sources were measured switching from 100% of source 2 to 100% of source 3, then 100% of source 2 to 100% of source 1 and 100% of source 2 to 50% of source 1 and 50% of source 3.

For the experiment 3, the following parameters have been implemented:

- The consumer switch from 100% of source 2 to 100% of source 3. The initial consumer value is always the same (the initial values of the Source 2). The enrichment factor, ΔX_s, equals 1‰ for all sources in both isotopes.
- Five different linear evolutions of source patterns over time were studied. Source patterns for the 2-isotope framework are detailed in Figure 4. The 1-isotope patterns are detailed in Appendix IV:
    - Pattern (a). **The sources 1, 2 and 3 are constant over time for both isotopes**. This setting is the reference one.
    - Pattern (b). **The sources are getting closer over time with a negative covariance for the first isotope**. For the second isotope, all the sources are constant (same value than the pattern a).
    - Pattern (c). **The sources are getting further over time with a negative covariance for the first isotope**. For the second isotope, all the sources are constant (same value than the pattern a).
    - Pattern (d). **The sources have a positive covariance and are increasing over time for the first isotope.** For the second isotope, all the sources are constant (same value than the pattern a).
    - Pattern (e). **The sources have a positive covariance and are decreasing over time for the first isotope.** For the second isotope, all the sources are constant (same value than the pattern a).

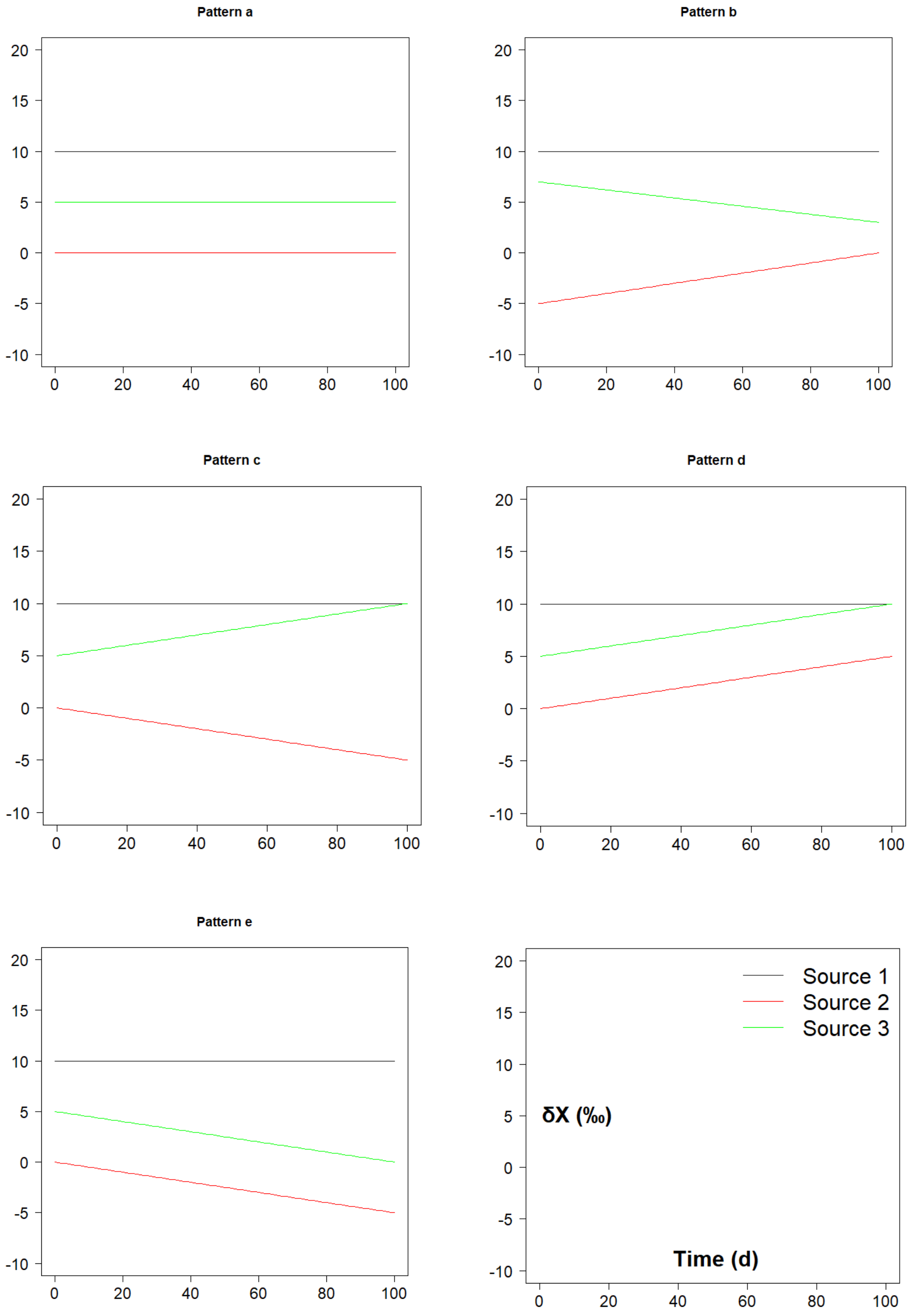


***Figure 4*: Dynamic patterns of source isotopic values for the first isotope tested in experiment 3. For the second isotope, for each pattern tested, the sources are constant over time and have similar values as the pattern a).**

2.5 Case studies: Testing the three models on real case study datasets

In order to test the models and create graphs and other outputs, the three mixing models were applied to several real datasets. The first dataset, from *Marín Leal et al. (2008)*, studied the evolution of the isotopic value of oysters (*Crassostrea gigas*) for two isotopes ($\delta^{13}C, \delta^{15}N$) during one year between May 2004 and May 2005 in the Baie Des Veys site in Normandie every two months. During this period, the consumer food sources isotopic values were collected. There were three different food sources, one is the *a priori* aggregation of Microphytobenthos and ulvas ($\delta^{13}C_{MPBxU}, \delta^{15}N_{MPBxU}$) (because they have a really close isotopic signatures for the two isotopes), the second one is phytoplankton ($\delta^{13}C_{phyto}, \delta^{15}N_{phyto}$) and the last one is terrestrial organic matter ($\delta^{13}C_{TOM}, \delta^{15}N_{TOM}$). At each sampling time, the λ associated to the oysters were estimated using a DEB (Dynamic Energy Budget) model for the oyster (*Pouvreau et al., 2006).* The $\Delta^{13}C$ and $\Delta^{15}N$ values used were the ones proposed by *Mc Cutchan et al. (2003)*, as for all the studied dataset except the next one.

The second dataset, comes from a diet switch experiment from *Jomori et al. (2008)*. In this study, the types of food consumed by pacu (*Piaractus mesopotamicus*) larvae were studied using stable isotopes during a controlled experiment. The isotopic signature of fish larvae for the carbon and nitrogen were measured every three days for forty-five days starting from their first feeding. Different experiments were made giving alternatively dry food or artemia (*Artemia nauplii*), for which the isotopic signatures were known, to the larvae. Here, as it is a diet switch experiment, the ΔX_s were estimated using the dataset. For this dataset, since there were only two food sources studied and the λ associated to the nitrogen could not be estimated, only the data from the carbon isotopic signature were used for the mixing models. Therefore, we have a well constrained system with one isotope and two sources. The λ value was estimated using the time model (Eq.1) on a diet switch between two monospecific diets.

The third dataset is from *Mascart et al. (2018)*, in which four species of benthic copepods were studied. Their isotopic signatures for C and N were observed during one year (between 2011 and 2012) at each season at different sites in Corsica. Three sources were observed as well, epiphytes, macrophytodetritus (MPD), and suspended particles organic matter (SPOM) once per season for their carbon and nitrogen signatures. λ values were estimated using the secondary production observed in *Danavaro et al. (2002)* with the production over biomass (P/B) ratio.

Finally, the last dataset is from *Varin (2014)* and is about common cockle (*Cerastoderma edule*) studied during a year (between 2012 and 2013) with observations each season in the baie des Veys in Normandie. Their isotopic signatures were observed for Carbon and Nitrogen. The same were done for the three sources which are the plankton within the water column, the organic matter coming from rivers, and the microphytobenthos (MPB). The λ were computed with data of cockle body mass, provided with the isotopic data as well as body temperature data using the equations from *Vander Zanden et al. (2015)*.

3. Results

3.1 Bias, the Dynamic indicator (DI) and the different sources of variability

Overview

The observations made from the *in-silico* experiments showed that when the isotopic values ($\delta^{13}C$ and $\delta^{15}N$) of the consumer stays inside of the polygon of the isotopic values of the sources (called the source polygon from now on), there is a positive linear link between the bias and the DI. Except when specified, all experiments and resulting observations were carried out in these conditions. There is a maximum bias value that can be reached which is equal to 50% for SMM and 20% for SMM_delta, regardless of the values of the model parameters (i.e., predator initial value, diet, λ, trend in sources isotopic values) and the studied framework (the maximal values are the same for both frameworks). Similarly, there is also a maximal value reached by the DI which depends on the chosen λ and the time period for the experiment. Overall, when switching between two sources in a 2-isotope framework, there is a linear relation between the DI and the bias for each model (SMM, SMM_delta) that is unique regardless of λ and period value (Eq.8). For the 1-isotope framework, the maximal value of the DI is 0.43, and for the 2-isotope framework, the maximal value for DI is 0.86. Then, the higher the value of λ compared to the experiment time, the lower the maximum value of DI. Only the results for the 2-isotope and three sources framework results will be presented as this is the most common set-up found in isotopic studies including the case studies used here. The outputs of the 1-isotope framework can be found in Appendix III.

$$\begin{gathered} for\ DI\ \in [0, 0.86] \\ Bias\ (SMM) = 67.6 * DI \qquad \text{(Eq.8)} \\ Bias\ (SMM_{delta}) = 28.9 * DI \end{gathered}$$

In-silico experimental outputs:

In **Experiment 1**(Variation of the initial value/ switch with constant sources), the equilibrium is reached when the consumer's isotopic ratios have the same value than the source 2 plus the ΔX_s value. The furthest the initial value is from the equilibrium, the greatest the DI and the bias will be (Figure 5). The outputs of this experience follow the linear equation (Eq.8). Note that the maximum value of DI is dependent on the surface of the polygon of food sources isotopic values (Figure 3). When the surface is small in the case where the consumer stays inside the polygon, it has less possibility to move leading to a low value of DI and inversely for a large surface. In this section, we present the bias related to only one of three sources that were studied. In this case DI and bias are presented for source 2.

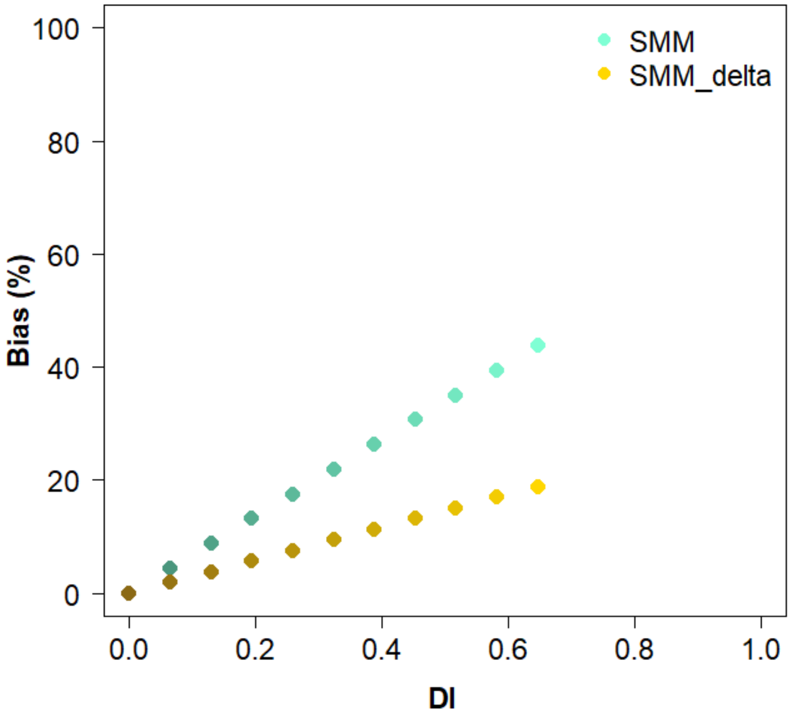


Figure 5: Effect of changing the consumer initial value (Experiment 1) on the DI and bias for SMM and SMM_delta on the 2-isotope framework. The lighter the colour, the further the initial value is from the equilibrium (source 3).

The **Experiment2** shows that the bias of source 2 (used in all the results) is independent of the switch done on the experiment (that means to which source the consumer switches to or if it is a partial or a complete switch). Figure 6a) shows very similar results than in Figure 5, except that the bias associated with all the three sources are displayed instead of one (and only the SMM outputs are shown). Similarly, the Figure 6b) and c) show the bias associated to the three sources but doing switch to different sources (either source 1 or a mix of source 1 and 3). When switching from one source to another (Figure 6), the bias is the same for these two sources and the bias of the third source equals 0. In the third case (Figure 6c), the bias of the source 2 is the same than in the two others experiments. That means that when studying diet switches between two sources, focusing on the bias of one source or the other is equivalent. In any case, observing the bias of the source consumed before the switch (source 2) seems to give the same values regardless of the kind of switch.

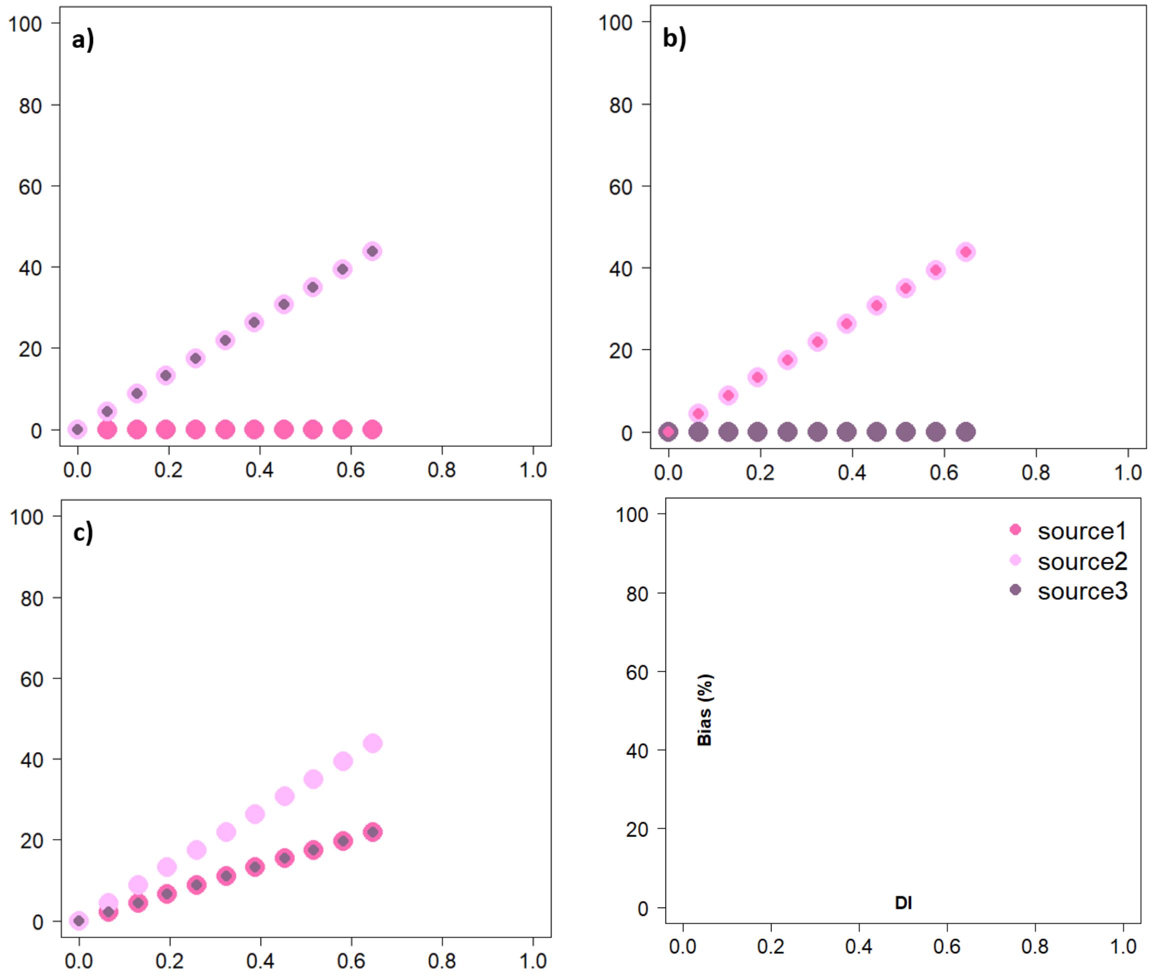


Figure 6: The consumer initial value experiment but with all sources on display (Experiment 2). a) switching from source 2 to source 3, b) switching from source 2 to source 1, c) switching from source 2 to 50% of source 3 and 50% of source 1. For this experiment, λ=0.02d^{-1} and the period is T=100d.Here, only the bias associated to SMM is presented but the results are similar for SMM_delta results, see Appendix V.

Experiment3. The relative variation of the sources (patterns a to e Figure 4) have an effect on the DI and the bias (Figure 7). Most of the points followed the previously presented linear relation (Eq.8), while some were a bit off the line (pattern c in Figure 7a). This indicates that the source relative variations can have a noise effect on the relationship. The pattern d) is the only one giving a higher bias than the reference (pattern a) and appeared as the maximum bias value. This can be explained by the fact that with this source pattern, the sources polygon shrinked more rapidly than allowed by lambda resulting in the consumer ratios splitting out of the polygon. Then, when a consumer is out of the source polygon, the bias could be higher than it should be. Furthermore, a variation of the δX_s leads to a change of the source polygon and therefore, a change of the space for the consumer's ratios to change. In a case where 1 isotope is studied, the effects of the source relative variations are rather easy to observe because the isotopic space is in two dimensions (the ratio of the isotope and the time). When two isotopes are studied, the isotopic space is in three dimensions (the two isotopes ratios and the time) and there are more possibilities of change over time than the one presented here.

About the effect of different sources covariation, another parameter to consider, is the period of the experiment compared to twice the half-life ($\frac{T}{2t_{1/2}}$). Some covariation patterns (b) and d)) seem to have the same effect regardless of $\frac{T}{2t_{1/2}}$. The pattern b) seems to reduce the bias compared to the reference (pattern a).

In this scenario, the polygon is getting smaller meaning that the consumer has less space to evolve and get more easily at equilibrium. On the other hand, the pattern d) seems to always create an out-of-polygon situation by, increasing the bias. For this pattern, the source polygon moves but its surface does not decerase and the consumer cannot reach equilibrium.

Other patterns have various reactions linked to the $\frac{T}{2t_{1/2}}$ value. The pattern c) has a lower DI than the reference when $T = 2t_{1/2}$or $T = 4t_{1/2}$ but give a similar response when $T \geq 3 * 2t_{1/2}$. The pattern e) gives the same kind of results than the c). To sum up, when $\frac{T}{2t_{1/2}}$ is low, the sources covariations have different effects on the bias but are less and less marked as $\frac{T}{2t_{1/2}}$ increases. As a matter of fact, for a same λ, changing the time of the experiment changes the effect of the different patterns. Indeed, the λ value affects the minimum time needed by the consumer to reach its isotopic equilibrium regardless of the other parameters. λ is an indicator of the maximum speed the consumer isotopic ratios can change. If an experiment time is much longer than the needed time for the consumer to reach the equilibrium, then all the bias will be lower than if the model has just the time to reach the equilibrium or if the equilibrium is unreached.

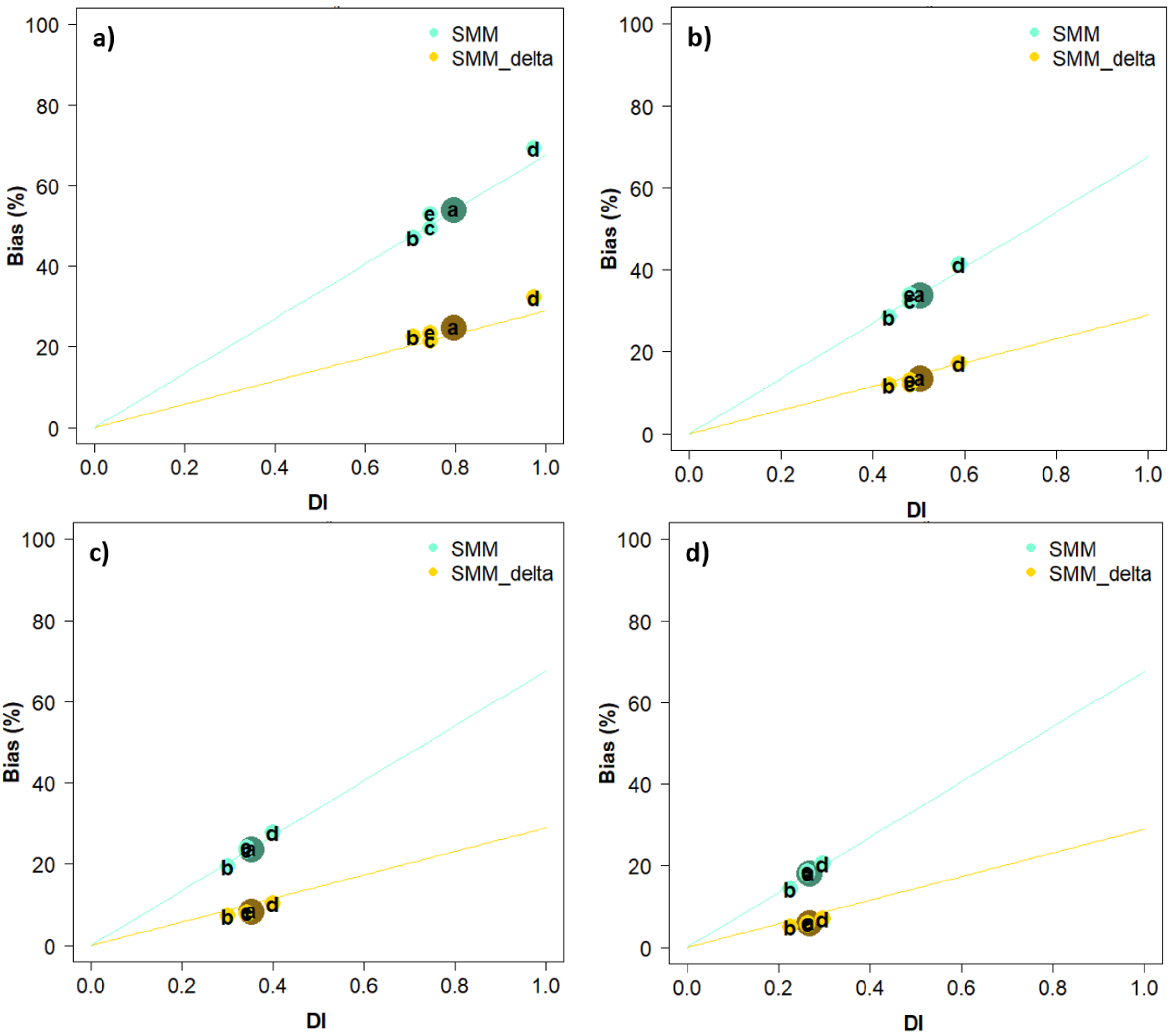


Figure 7: Effect of different source variation patterns (Experiment 3). The dots labelled « a » correspond to the pattern a) of source variation, is the reference and is represented in a darker colour. The lines correspond to the linear relation between DI and the bias (Eq.8). Each graph corresponds to a different experiment time (T) where a) $T = 2t_{1/2}$, b) $T = 4t_{1/2}$, c) $T = 6t_{1/2}$ and d) $T = 8t_{1/2}$.

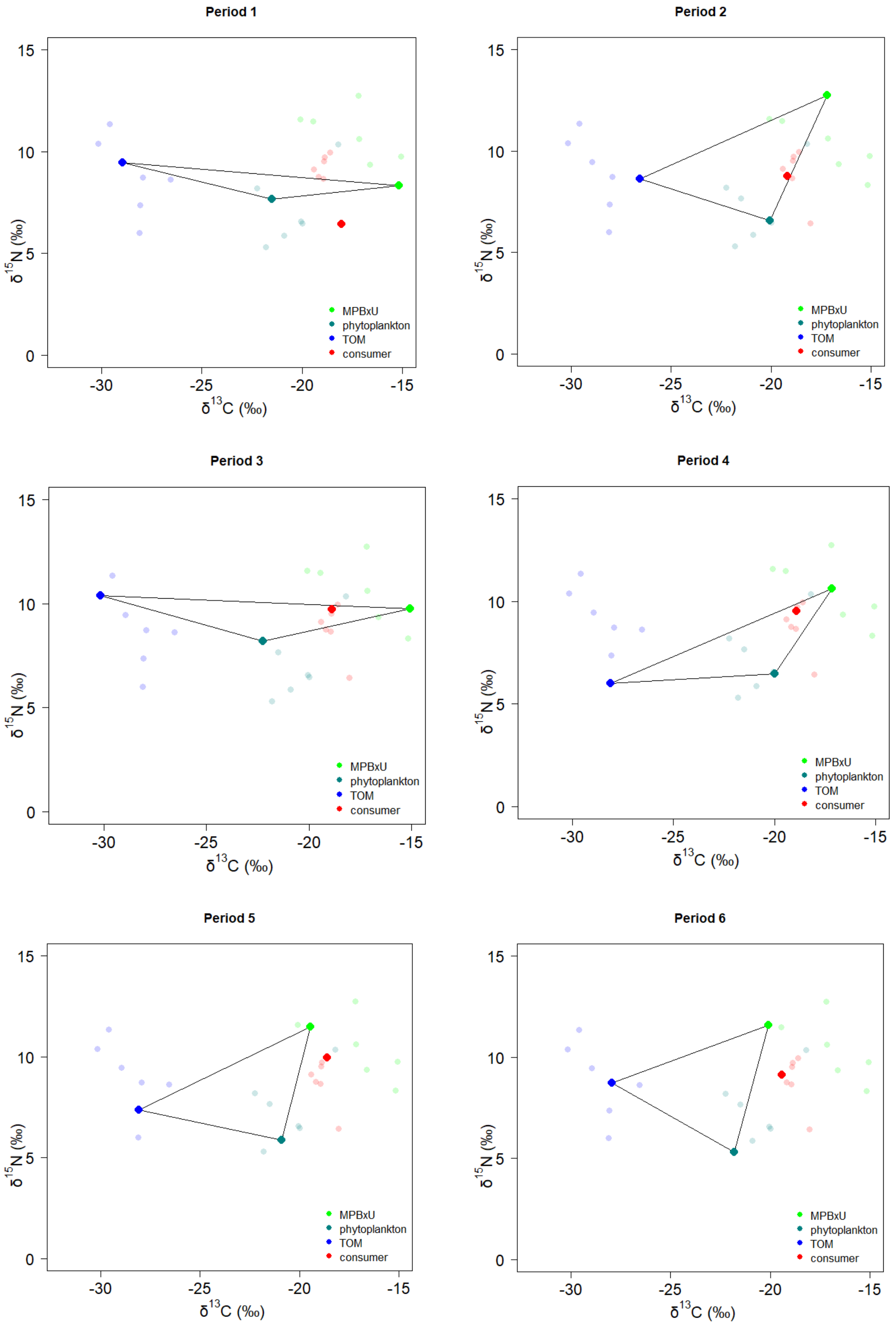
3.2 Outputs of the different models from case studies

All the models ran using the datasets had a precision of 0.01 resulting in the models testing 5151 solutions of combinations. Keeping the 50 best implies keeping the 1% best solution and similarly, keeping 10 solutions means keeping the 0.1% best solutions. Only the results retrieved from the oysters (Marín Leal et al., 2008) and a part of the fish larvae (Jomori et al., 2008) datasets will be presented. These two datasets are complementary because they represent both ends between controlled conditions and field data leading to different knowledge and control over the results. Complementary results from these datasets and results of the other datasets can be found in Appendix VI to IX.

The Oyster dataset

Raw data and seasonal variations

The $\delta^{13}C$ values of oysters showed minimal changes over time, with only a variation of approximately 2‰. On the other hand, $\delta^{15}N$ values exhibited significant changes during the initial period of observation, with a variation of around 4‰ over time. However, $\delta^{15}N$ values remained relatively stable thereafter, showing little variation. The source values ranged between 6.42‰ and 9.96‰ for $\delta^{15}N_s$ and between -18.05‰ and -19.43‰ for $\delta^{13}C_s$. Each of the source values changed throughout the year and for the terrestrial organic matter (TOM) a seasonal pattern was observed (Appendix VII). The λ value was estimated at each observation, so its value changed over the year. As the λ is linked to the oyster's growth, it is the lowest in winter (0.004 d^{-1} in January) and at its maximum in late spring (0.027 d^{-1} in May). Furthermore, one crucial consideration when using mixing models is to examine the polygon formed by the various sources corrected by the ΔX_s values on the dual-isotope plot and verify that the consumer isotopic value falls within this polygon. If the consumer's isotopic value lies outside the polygon, then at least the results of the SMM and SMM_delta are bound to be biased. However, in certain cases, the DMM can still provide valuable estimates (Figure 13). Figure 8 is useful to see that the consumer is only within the source polygon for the periods 2,3 and 4.



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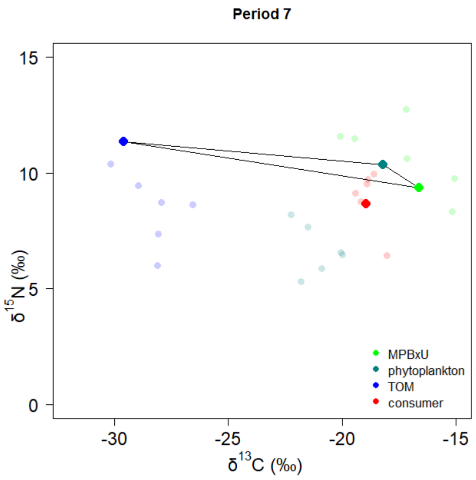

Figure 8: Biplot representing the source polygon (blue and green dots and triangle) for the three sources Microphytobenthos and Ulva (MBPxU), phytoplankton and terrestrial organic matter (TOM) plus the consumer (red) ratios at each period of the experiment. The three sources ratios were corrected by the ΔX_s values. Each graph represents the measured signatures of the sources (corrected by the ΔX_s values) forming the source polygon and the measured consumer signature during the data collection time (day 0, day 60, day 120, day 180, day 240, day 300 and day 360).

DMM specificities

One difference between DMM and the two static mixing models is that the isotopic trajectories of the consumer are estimated $(\delta X(t))$ between two observations (Figure 9). Moreover, the slope of the isotopic trajectories between two observations within a period, indicates when the isotopic equilibrium was reached. It is the case when the slope decreases towards zero and the consumer’s ratio for one isotope becomes constant. This pattern is observed in the nitrogen plot (Figure 9b) between 60 and 120 days. Likewise, when the slope does not change over time (the trajectories are like a line) or if the slope does not equal zero, then the isotopic equilibrium could not be reached during that time period. This pattern can be observed for example on the carbon graph (Figure 9a) between the days 240 to 300 and on the nitrogen graph between 0 and 60 days. These « disequilibrium periods » correspond to either a wide consumer changing period (nitrogen day 0 to 60) or a wide source evolving period (carbon day 240 to 300).

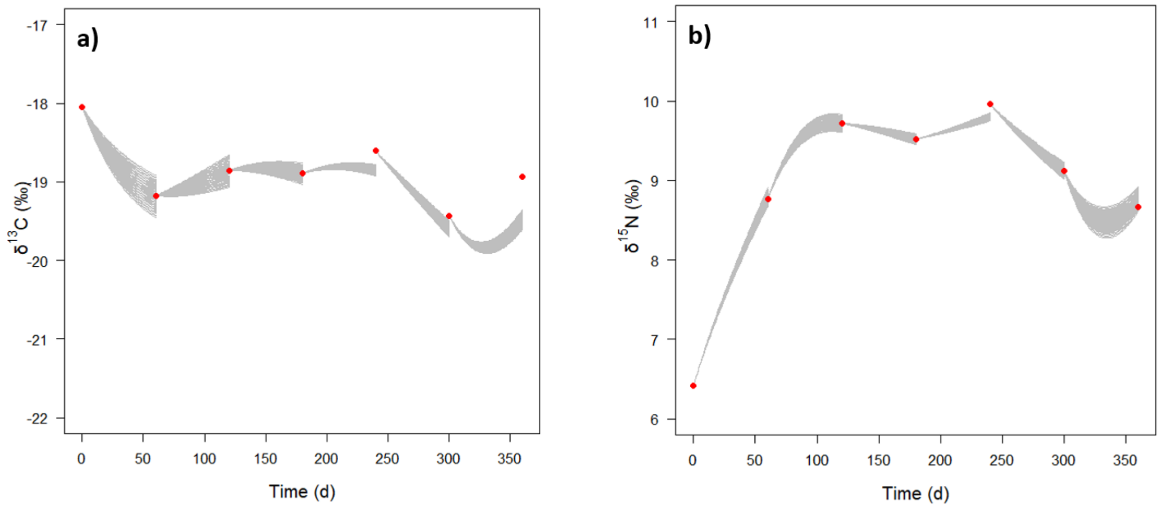

Figure 9: Evolution of the isotopic ratio for the two studied isotopes over time, a) Carbon, b) Nitrogen. The red dots are the observed values and the grey lines are the predicted trajectories by the DMM using the best 50 diet solutions.

Additionally, it is important to note that there may be periods where the model cannot accurately predict the next value of the consumer ratio so that $\delta_{c\_obs}(t+\Delta t)$ and $\delta_{c\_pred}(t+\Delta t)$ are different (Figure 9). For example, in the case of δ^{13}C, there is a deviation in the last period where the DMM fails to find the exact value. However, overall, the predictions are generally accurate for both isotopes.

Comparing the three models

The dynamics of the contribution of each source between the three models are illustrated on Figure 10, which are the main outputs for the mixing models. Focusing on the source MPB (in blue), the models estimated different contributions, but the trajectory over time is consistent between DMM, SMM_delta and SMM until 300 days when the contribution increases for the SMM but decrease for the other two. At the beginning of the time series (which is t=60 days, when the three models give a value), MPB represents around 50% of the diet (for SMM_delta and DMM) and its contribution increases until reaching a peak around day 240. Afterward, the contribution starts to decline. However, this decrease is not clearly visible when examining the outputs of SMM. In terms of absolute values or estimates, there are differences observed in the contributions of the MPB source among the three models (Figure 10). For example, at the day 240, the average value of the contribution distribution for MPB is close to 100% for the DMM while it is closer to 75% for SMM_delta and SMM. The difference is also well illustrated on the last time period, where the average contribution for DMM is around 10% where SMM_delta average estimation is a little inferior to 50% and the SMM one is higher than 75%. This is a period when the consumer is out of the source polygon and the results of all three models are biased (see Figure 9 for DMM). It is a good reminder that one should pay attention to this because the models will give results even at configurations where it is biased. Finally, there is no value at the time 0 days for DMM and SMM_delta because these models work on a time period rather than on a snapshot like SMM (see Figure 11). Then, the first results from DMM (at *t*=60 days) are the results between 0 and 60 days and similarly, the first results from the SMM_delta are the integrated results between day 9 (60 minus twice the half-life) and 60.

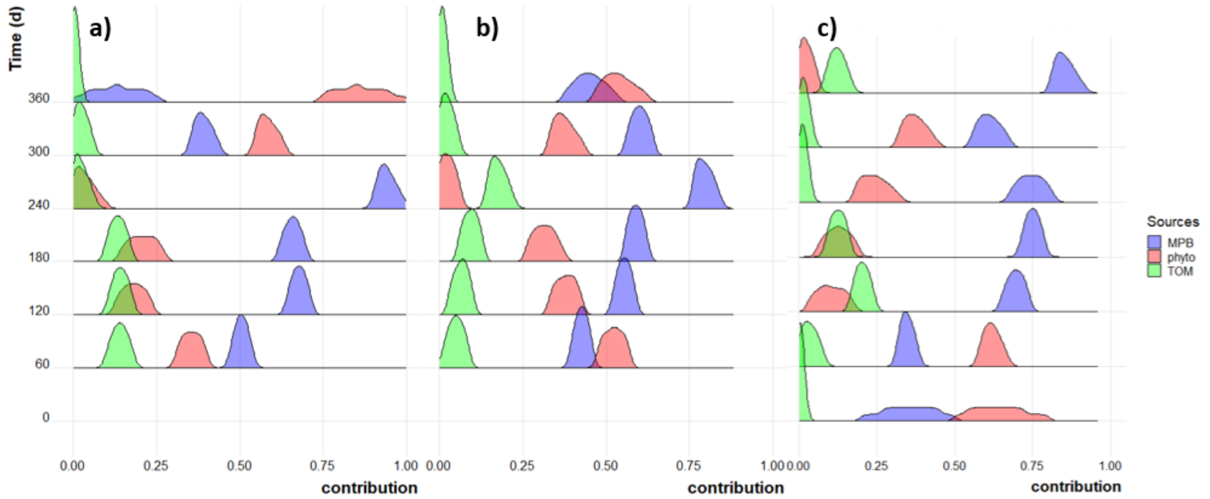

Figure 10: Outputs of the mixing models: estimated contributions of each source from the three models a) DMM, b) SMM_delta and c) SMM for the contributions of the three sources (Microphytobenthos MPB, Phytoplankton, Terrestrial Organic Matter TOM) over time. The densities are created with the best 50 solutions for each model.

The difference in time scales and contributions to the diet (%) for the three models is illustrated in Figure 11. This figure provides another way to observe the outputs presented in Figure 10 while also highlighting the different integration times used by the models. We can observe that some integration windows for SMM_delta are overlapping. For example, the estimation of the SMM_delta on day 240 uses the sources values averaged over a time window covering all the samplings until this point. This complexifies the diet estimations between the previous estimation (day 180) and the current one.

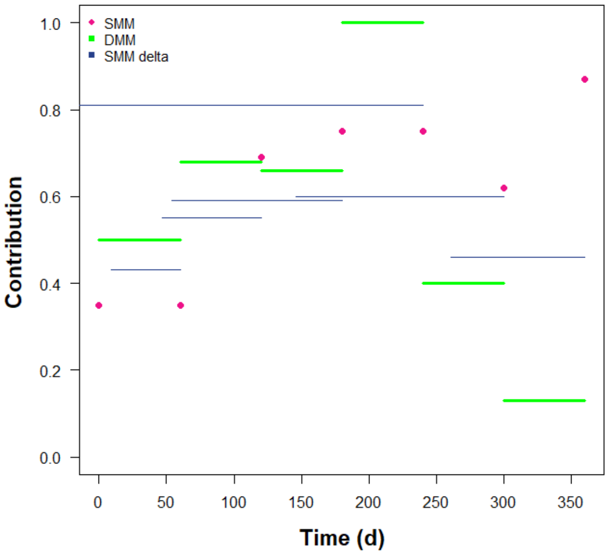

Figure 11: Comparison of the estimated contribution of the source Microphytobenthos and Ulva to the diet over time for the three models and their integration in time. Here, only the best solution is kept for each model. SMM works as a snapshot, so there are estimations only when there are observations (red dots). For SMM_delta, the sources ratios are averaged on a time window depending of λ, which varies over time here explaining non-constant time windows (blue lines). DMM works between two observations and as the data were collected every 60 days in this case the time windows are constant (green lines).

The dynamic indicator (DI) can be used as a tool for explaining the differences observed among the three models (Figure 12). In this case, the DI has been calculated and estimated for each time period between two observations. The observed differences in DI between the periods can be attributed to specific factors within the experiment. At the beginning of the experiment (between 0 and 60 days), there is a substantial variation of the sources, as demonstrated in Appendix VII. This indicates that the system is far from reaching equilibrium, which explains the higher DI value (0.81) during this period. During the experiment, DI and bias are getting smaller as the system is getting closer to equilibrium. The smallest predicted bias is on the period between 120 and 180 days (DI=0.28). For the associated bias, there is a difference between the estimation and the calculation except in the period [60,120]. For the others, there is a difference of about 10% for the period in the beginning and middle of experiment (periods [0,60], [120,180] and [180,240]) and a difference around 20% for the two last periods ([240,300], [300,360]).

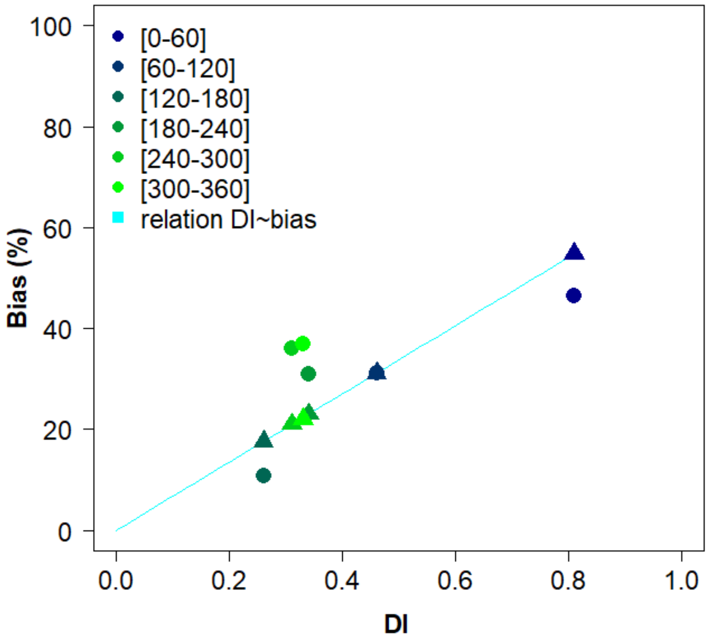

Figure 12: Estimated and computed bias values for the SMM compared to the DMM. Each point represents a period of time in the dataset (first period between 0 and 60 days in dark blue, last period from 300 to 360 days in light green). The blue line represents the linear relation between DI et Bias for the SMM (see Eq. 8). The triangles on the line are the estimated bias using the linear relation and the other dots are the computed bias calculated for the in-silico experiments.

Another tool to compare the three models is to assess the accuracy of estimations. This can be achieved by calculating the *distance* using Eq.6. The closest the averaged distance is to 0, the best a model can estimate the proportion of each source consumed by the consumer. In Figure 13, we can see that most of the time DMM and SMM_delta give similar values of distance, except for the last observation (*t*=360 days) where the results kept by DMM are closer to the observed data than the one given by SMM_delta. For SMM, on the first part of the observation, the distance value is equivalent to the two others, but on the second part (after day 200), the values given by the SMM are more biased than the ones given by the two others. When focusing on the last observation (*t*=360), a period where the consumer is out of the source polygon, we can see that the less biased model was the DMM, followed by the SMM_delta and then the SMM. This observation can also be made in other datasets where the consumer is often out of the polygon (Appendix XIII, the copepod dataset for the first two periods and Appendix IX, all the periods of the cockle dataset are out the source polygon). However, the distance can not be used alone to measure the accuracy of the models because it estimate the precision of the consumer signature prediction but that does not mean that the associated contribution of each source will be accurate.

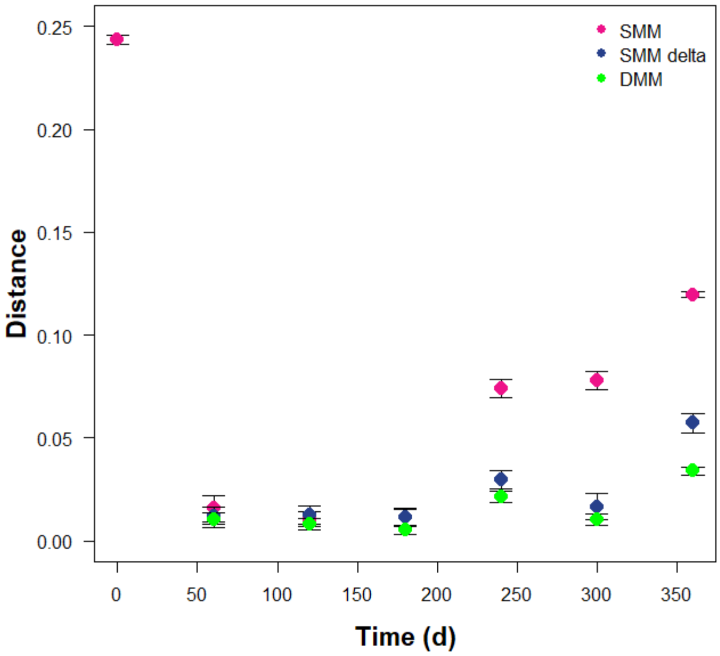

Figure 13: Average distance (and standard deviation) for the 50 best solutions for each model.

The fish larvae dataset

Here, only the results coming from an experiment where the larvae were fed on the first 12 days with Artemia and then fed with dry food are presented.

In this dataset, the source ratios are constant over time and $\delta^{13}C_s$ equals -14‰ and -23‰ (corrected by the $\Delta C_{Artemia/dry\ food}$value). However, the fish δ^{13}C values exhibited significant variation over time (9‰ of variation) with values ranging between the signatures of the two sources. As the growth of the larvae was exponential during the experiment, the estimated λ were considered as constant. The consumer was always situated within the source polygon. The observed distances are very alike for all the models except for the last period (Figure 14b)) but the DMM cannot predict the last three observed values (Figure 14a)). Also, as SMM and SMM_delta give the same results, in Figure 14b) the circles are overlaying.

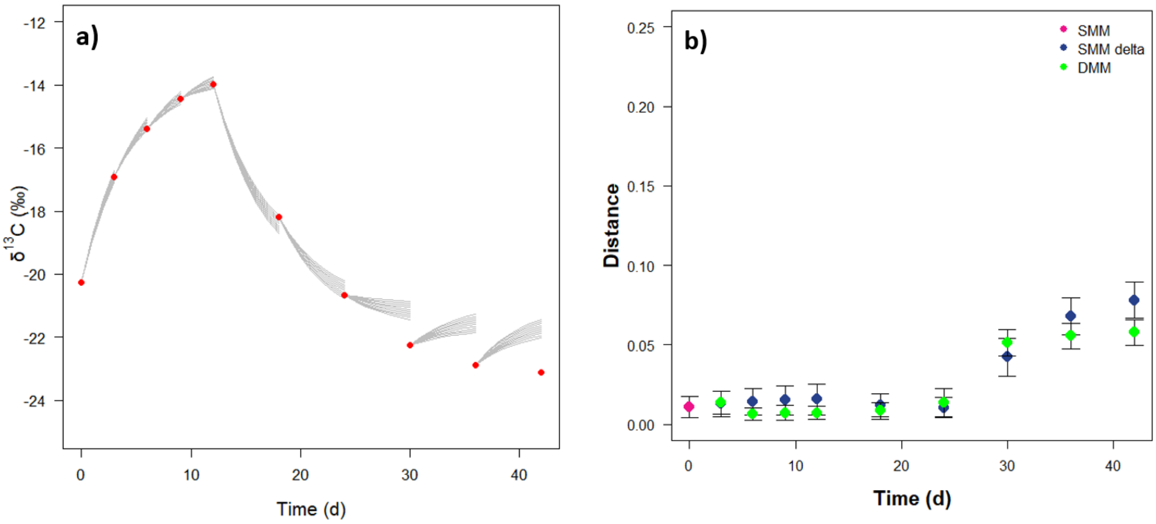

Figure 14: General information about the models with a) the trajectories predicted by the DMM for the 10 best solutions and b) the observed distance (and standard variation) for the three models.

The solutions of relative contribution to the diet of the sources using SMM and DMM are shown in Figure 15 where large differences in the diet contributions are observed between day 3 and 30. The diet switch is clearly visible with DMM while it is more blurred with SMM. For SMM, two periods are visible, one between 0 and 18 days where the diet seems to be slowly changing from Dry food to Artemia, and then on the day 18 there is another change to dry food. From DMM outputs, on the first period the larvae appear to be only eating Artemia until the day 18 and then they are only eating dry food. The DMM gives results that are much closer to the reality than the SMM in this case.

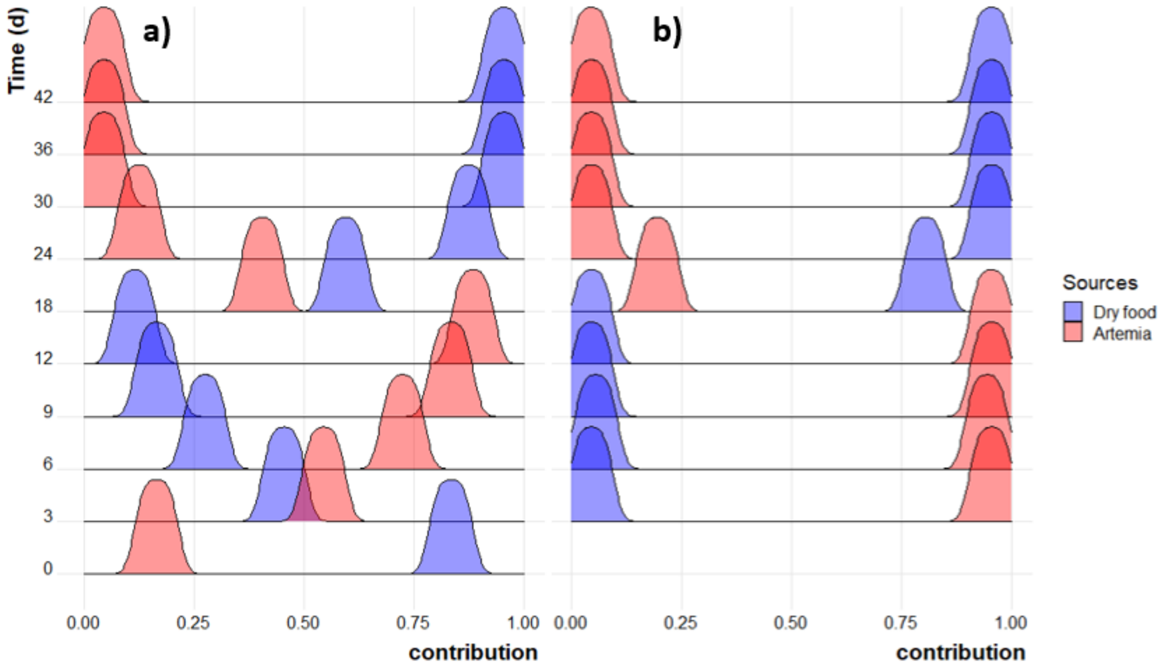

Figure 15: Contribution of the two food sources over time from the a) SMM and the b) DMM. Here, during the first 12 days, the larvae are only fed with Artemia and on day 12 to the end, they are only fed with dry food. Here, the SMM and SMM_delta give similar results and only the 10 best solution were kept.

4.Discussion

A generalised DMM

The DMM is a mixing model that allows the consideration of various isotopic associated dynamics. It can surpass some classical static mixing model limits outlined by *Philips et al. (2014)* like the seasonal variation in isotopic ratios (for the consumer and its sources), and the incorporation rate (λ). The latest version of DMM has also the possibility to be concentration-dependent and can consider seasonal variation of element concentration in the sources. Furthermore, when studying seasonal variations for species with a very low λ, DMM gives more precise results than SMM_delta, as the integration time needed for SMM_delta would be very long and sometimes encompassing several seasons, making it difficult to analyse the outputs. In addition, although sometimes DMM fails to predict exact predator's value (Figure 9, Figure 14a, outputs from the others dataset in Appendix VI to IX), the solutions estimated (Figure 13, outputs from the others dataset in Appendix VI to IX) are often similar or better than SMM_delta and most of the time better than SMM. Therefore, DMM seems to be the model giving the most precise solutions out of the three models. Moreover, the periods where the DMM cannot exactly predict the next value often corresponds to moments where the consumer is out of the source polygon so where the application conditions of the mixing models are not fulfilled (*Philips et al., 2014*). In addition, this DMM version is generalised and can be used in any case regardless of the isotopes studied and the number of sources. It should work as well for under-determined systems by giving distribution of possible solutions, although it has not been tested yet. Hence, it can be used on a much larger panel of dataset than the previous DMM. This version makes it possible to use for a two isotopes and three sources dataset, that are very common studied systems (*Marín Leal et al., 2008, Mascart et al., 2018, Szepanski et al., 1999*). However, the use of DMM (and the SMM_delta) requires λ values and so additional data are required for its estimation (e.g., growth, secondary production…). This can make the data collection process more resource-intensive compared to using the SMM.

Different context of use for the three models

The DMM gives coherent estimations to diet proportions in various cases, but it necessitates more data than the others mixing models. Then, depending on the context, it is still possible to use static mixing models and to have accurate estimations of the diet. When studying the diet of a consumer on a short time period (for one season for example) where the food sources and consumer ratios do not change much, static mixing models should give suitable results. The risk of bias in these cases resides in missing a food source during the sampling. Likewise, when the studied consumer has a rapid growth (for example copepods, *Mascart et al., 2018* dataset), so high λ values, even when there is a variation in the food sources or a diet switch, the consumer will quickly be back at equilibrium. Then, the bias between the models are quite low and the three models give relatively similar trends. Differences rely when precise results are expected. For example, in the *Jomori at al. (2008)* dataset, the two static models give relatively similar results but DMM is much more precise. Baseline (primary producers) isotopic values change along the seasons (*Woodland et al., 2012, Solomon et al., 2008*). When λ has very low values (for example bivalves, *Marín Leal et al., 2008* and cockle dataset *Varin, 2014*), the integration time for SMM_delta can be longer than just one season and cause imprecision because seasonal changes are blurred in averaged values. So, when studying seasonal variations in cases of low-growth species, SMM_delta model is not necessarily appropriate. It actually depends on the source values changing speed compared to λ and the needed time to reach equilibrium.

The intensity of the diet switch is another important factor to determine if a consumer is at equilibrium. It depends on the feeding behaviour of the consumer, if it is a dietary-specialist species (always consuming the same preys) or a generalist species (consuming different kind of preys, often making diet switch). In addition of the behaviour, it depends as well on the general variation of isotopic ratios for the consumer according to its trophic level. Isotopic values at the baseline change along the seasons (*Woodland et al., 2012, Solomon et al., 2008*) and this change is also visible going up the food web, although less pronounced in higher trophic levels (*Harvey et al., 2002*). So, isotopic ratios of dietary-specialist species in high trophic levels do not change much over time and are adapted to the use of SMM_delta. If the time integration window is right for its preys, SMM_delta is also adapted to high trophic levels generalist species like omnivorous fish (*Grønkjær et al., 2020*). However, for migrating species, DMM will be more precise to pinpoint the time where the migration happens (*Gelpi et al., 2013*). On the other hand, for low trophic level generalist species, the isotopic values of the consumer and sources are more variable over time, especially for species foraging in large areas or migratory species (*Bearhop et al., 2004*). The variability in isotopic values is also higher in low trophic levels dietary-specialist species. In this case, DMM is the most adequate model to consider all of the dynamics.

Importance of λ and its estimation

The λ parameter has a significant impact on the mixing models (*Philips et al., 2014, Carter et al., 2019)* as it reflects the time needed for a consumed element to become a part of the predator's tissues, and can lead to very distinct results between SMM, SMM_delta and DMM. Surprisingly, the turnover rate is still overlooked in trophic ecology while it exists various methods to estimate it. In controlled or semi-controlled conditions such as diet switch experiments, the isotopic turn-over rate can be estimated using the time model (Eq.1) (*Jomori et al., 2008, Madigan et al., 2021, Vander Zanden et al., 2015*). Then, the studied species are limited by their aptitude to be kept in captivity, the authorization to be experimented on, and having λ values high enough to allow for a decent experiment time. A DEB model (*Koojiman, 2000*) can also be used to estimate λ (*Marín Leal et al., 2008)* requiring data such as length (or weight), water temperature, and an existing DEB model associated with the studied species. Additionally, λ can also be estimated using body mass and temperature (*Thomas & Crowther ,2015*). All those different methods can sometimes be used simultaneously and estimate very different λ values for the same dataset, especially when working on field data (cockle dataset, *Varin, 2014*). However, this can sometimes lead to very different estimation according to the method used. Therefore, it is even more important to continue developing ways to estimate λ for mixing models, especially under field conditions. This will enable us to use accurate λ values and consider the specific complexities of real-world environments. Furthermore, λ has also a key role in the estimation of the DI. Indeed, it is a direct factor of variation of the DI and dictates its maximal value. It also indicates how fast the consumer reacts to a change in diet to return to equilibrium that has a direct impact on DI as well as DI depends on the $\frac{T}{2t_{1/2}}$ value. When the experiment time is shorter compared to $2t_{1/2}$, the DI and the associated bias are higher than when the experiment time is longer. This relation between experiment time and λ needs to be explored more to rigorously quantify this link.

Contribution to different sources of variation to the bias

Regarding the Dynamic Indicator (DI), further tests are needed to fully understand its sources of variation and its impacts on bias. Combined effects of the different variation factors (λ, switch, source covariation) could be tested in the future to identify possible predominant factors of variation. The relation between bias and isotopic equilibrium could be studied in more detail, as it seems to have a significant impact on the bias between models. Nevertheless, the DI considers several sources that affect the bias between models, helping to explain the differences observed between them. More than that, it helps visualize what parameters influence the models and to highlight their effect. It can be used as a tool on real datasets as a preliminary analysis. Indeed, by studying the DI on a dataset, it would be possible to predict the bias between the three models and choose which model to use. Furthermore, it would be possible to test different study-cases and pinpoint which cases will need a DMM or if a static model could be satisfactory. Then, the choice of the model could be made by balancing the convenience of a model with the bias it creates.

For now, the bias was only tested on constrained systems (one isotope and two sources or two isotopes and three sources) but all mixing models coded in this study (SMM, SMM_delta and DMM) are made to give results also for under-determined systems. It is then interesting to study the bias associated with those cases that are common in ecological studies. Our hypothesis is that the relationship between the DI and the bias will be different, leading to higher bias than in well-determined cases. This is due to the fact that the mass equation (Eq. 3) has several solutions instead of one, leading to a wider range of feasible solutions for the three mixing models. Consequently, these wider ranges of solution would exhibit more differences than in well-determined systems, thereby explaining the higher bias values. It is important to estimate the potential bias of static mixing models on underdetermined systems because experiments working with a wide number of preys are less likely to have the capacity to estimate λ values for each source and consumer, and they would probably resort to using static mixing models (SMM). Having an estimated value of the bias could aid in correcting the results. It is interesting to use the DMM as a reference because, with the trajectory's estimations, it is possible to easily observe how well this model fits the data and the overall level of bias in the outputs.

However, the DI as it stands works for environment where the consumer evolves within the polygon and its value exceeds the range of possible value when the consumer wanders off the polygon. Then, the DI may not be completely suited to use in study-cases where the mixing models are applied while the consumer is out polygon (Appendix VIII and IX, Figure 8). The Figure 12 shows that the computed bias for SMM is different from the estimated one when the consumer is off polygon showing a need for adjustment. To adapt the DI to out-polygon scenarios, the $\Delta_{sources}$ in Eq.7 may be changed by including the consumer ratios in it too. If it stands inside of the polygon, the DI is computed exactly the same way and if not, the consumer ratios participate to the estimation of DI. In addition, the DI can also be viewed as an estimation on how far the system is from equilibrium and that depends on the experiment time compared to λ (Figure 7). The experiment time should be included in the DI calculation as well. Furthermore, the averaged bias computed to estimate the difference between models might not be the best way to represent it. Estimating the bias only at the end of an experiment period could be a good estimator as well rather than an averaged value on all the period.

The trophic enrichment factor ΔX_s

The values of ΔX_s are important in the mixing models as they control the relative position of the consumer inside the source polygon. Changing its value, change the relative proximity of each source to the consumer and thus change the results computed by the mixing models.

The enrichment factor changes from one species to another and the conditions needed to estimate it may be hard to realise (switch experiment, see *Jomori et al., 2008*). For a single species, it can also change according to the food quality (*Oelbermann & Scheu, 2002*). ΔX_s is linked to the physiology of the consumer as it increases with nutritional stress and disease and decreases with growth (*Remien, 2015, Lefebvre & Dubois, 2016*). Then, this parameter changes alongside λ during the life of the consumer (*Lefebvre & Dubois, 2016*). However, it is often complex to estimate the enrichment rate, especially when working with field collected data. Therefore, some reference values exist in the literature (*McCutchan et al., 2003, Fry, 2006*) and are often used in stable isotope studies, as in this study. It is possible to use existing values from another group with a similar trophic position (*Marín Leal et al., 2008*) as well. But these values can be imprecise or even incoherent, so it could be useful to analyse the sensibility of the mixing models to the enrichment rate and quantify the possible bias induced by a poor choice of ΔX_s.

Towards a bayesian SMM_delta, DMM

MixSIAR *(Stock et al., 2018)* is an R package and application that uses a Bayesian framework to resolve static mixing models. In MixSIAR, JAGS (Just Another Gibbs Sampler) is used as the underlying computational engine for Bayesian inference using Markov chain Monte Carlo (MCMC) methods. Because of its Bayesian framework, it allows for a more robust and flexible analysis, incorporating prior knowledge, uncertainties, and complex statistical distributions. MixSIAR has been widely used in studies involving mixing models (e.g., *Giraldo et al., 2017*). However, despite including different sources of variability (e.g., uncertainty in source signatures, variable isotopic fractionation and covariables), it is not dynamic (the predator is assumed to be at equilibrium) and does not consider λ.

In this section we explore what would be needed to develop a Bayesian version of the DMM and the integrated SMM_delta. The current limitation in creating a dynamic Bayesian model is that the dynamic model is based on an ordinary differential equation (ODE) for the likelihood (Eq. 4). While JAGS is a powerful tool for conducting Bayesian analyses, it has limitations when it comes to solving ODEs because in many cases, the solutions to ODEs do not have simple analytical expressions that can be directly used in the MCMC algorithm. However, an analytical solution could be found as shown in Eq.1. The issue is that this solution assumes a constant λ over time, which does not match the purpose of the DMM. Alternatively, it is also possible to use Bayesian R packages resolving ODEs without using JAGS, such as deBInfer (*Boersch-Supan et al., 2017*). *Yeakel et al. (2016)* proposed an analytical solution for Eq.2 when λ is constant. Then, another track to follow is reasoning by time-window where λ can be considered as constant.

Another challenge is generating solutions following a Dirichlet distribution, where the sum of all the proportion equals 1, for different number of food sources. A first step could be to create a model that works on 2-isotope-3-sources cases. For a Bayesian SMM_delta, the likelihood is not a problem since it uses the classical mixing model (Eq. 4). The question would be how to average the sources' values (data) depending on λ (prior), which itself depends of time (since λ changes over time). This could be handled by using hyperparameters to control the sources' mean value, which would depend of time. The models would also work using time-windows dictated by λ. There are less changes to make in the use of a SMM_delta in a Bayesian approach than for DMM.

5. Conclusion

There are different dynamics that affect stable isotope signatures and their uses in trophic ecology. The static mixing models only partially take these parameters (e.g., $\delta X, \delta X_s$and λ) into account (a first consideration of the δX_s dynamics linked to λ for SMM_delta), but DMM is the only one that is able to consider all of the dynamics at once. To have accurate results SMM should only be used in conditions where the equilibrium is reached and the dynamics effects can be neglected (for example the copepod dataset, see Appendix VIII). What is more, using a correct time window (Δt) adapted to the λ value allows SMM_delta to give correct estimations most of the time. However, SMM_delta only gives an estimation of an averaged diet and still requires a good estimation of λ to work. Our results show that DMM gives accurate results under most conditions but requires additional data and sampling efforts. After this study, DMM can now be used in a wider range of datasets and could help reduce the bias caused by an imprecise diet estimation.

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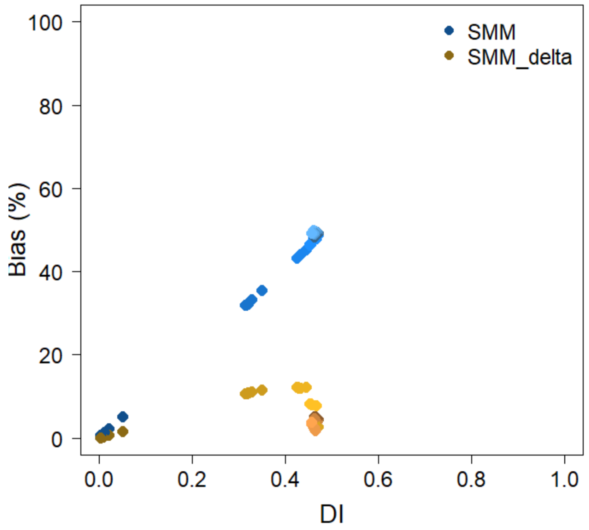
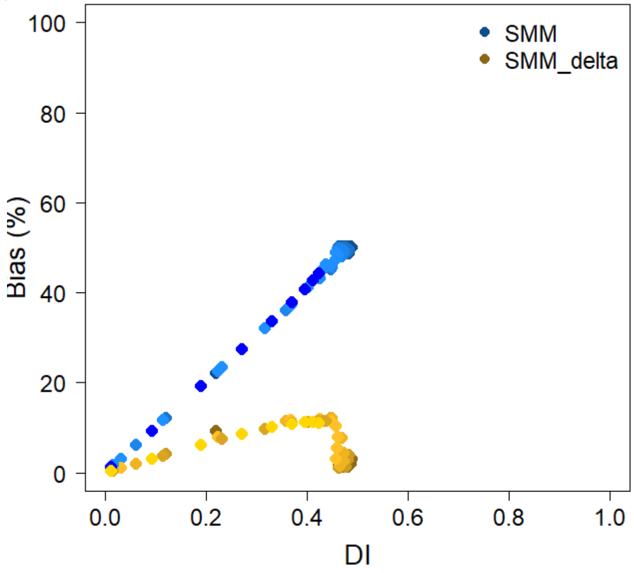
Appendix

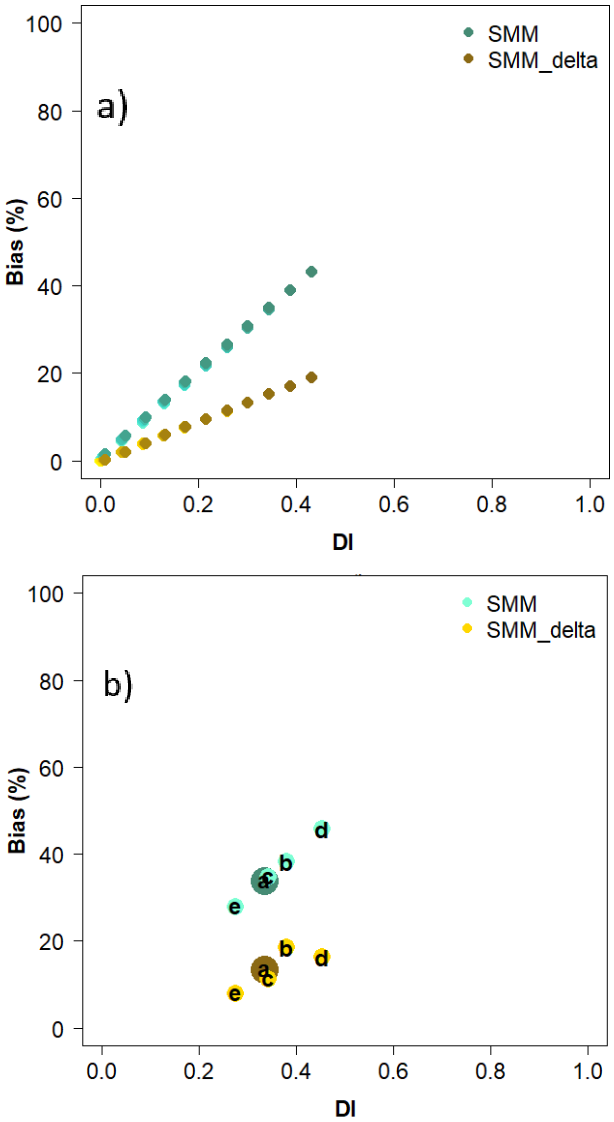
Appendix I: Dynamic indicator (DI) and bias for different source isotopic values. On the 1-isotope framework, 10 set of constant sources values were tested going from the couple with very alike isotopic values (0‰,2‰) to different isotopic values (0‰,250‰). Each color represents the results of one of the source set. Most of the dots are superimposed.



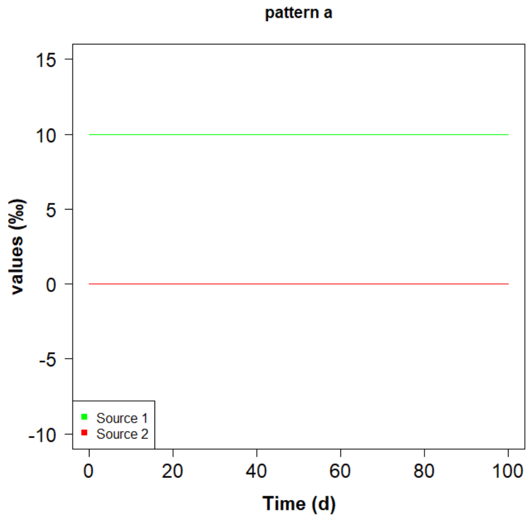
Appendix II: Dynamic indicator (DI) and bias for different λ values. On the 1-isotope framework, 6 different λ ranging from (0.005 d-1 to 0.1 d-1) were tested, all other conditions being equal (sources, initial value…),. Each color represents the results coming from one λ.

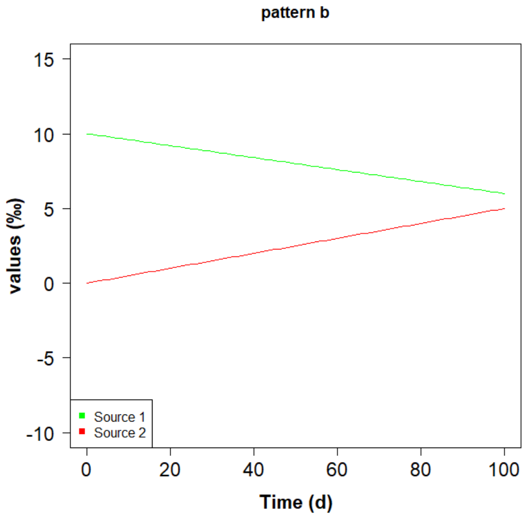


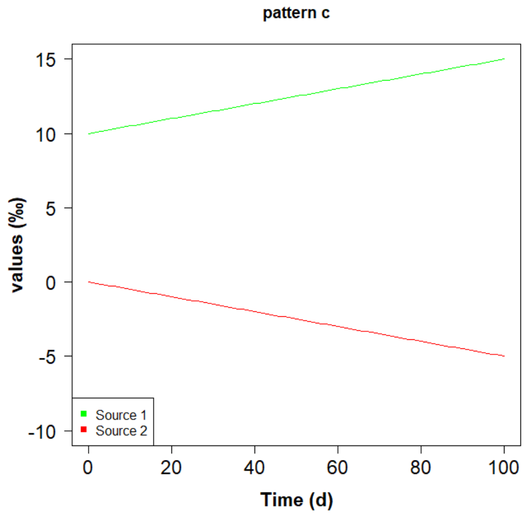
Appendix III: Outputs from the 1-isotope framework, here $\lambda=0.02d^{-1}$: a), the combined effect of changing initial value and the switch value in otherwise same conditions on the 1-isotope framework. Each color corresponds to one kind of switch (complete shift from a source to another or different partial shifts) associated with one initial value. All the switch and initial value are tested. b), the results from the experiment 3 aka the effect of different source variation for a period of 140d. The pattern a) is the reference and is presented in a darker color

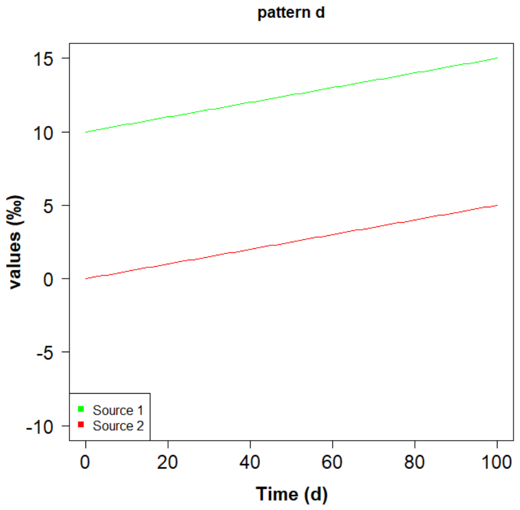


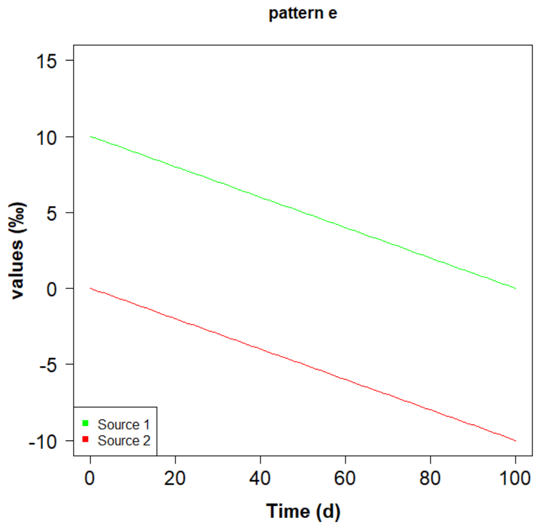
Appendix IV: Source variation patterns for the 1-isotope framework



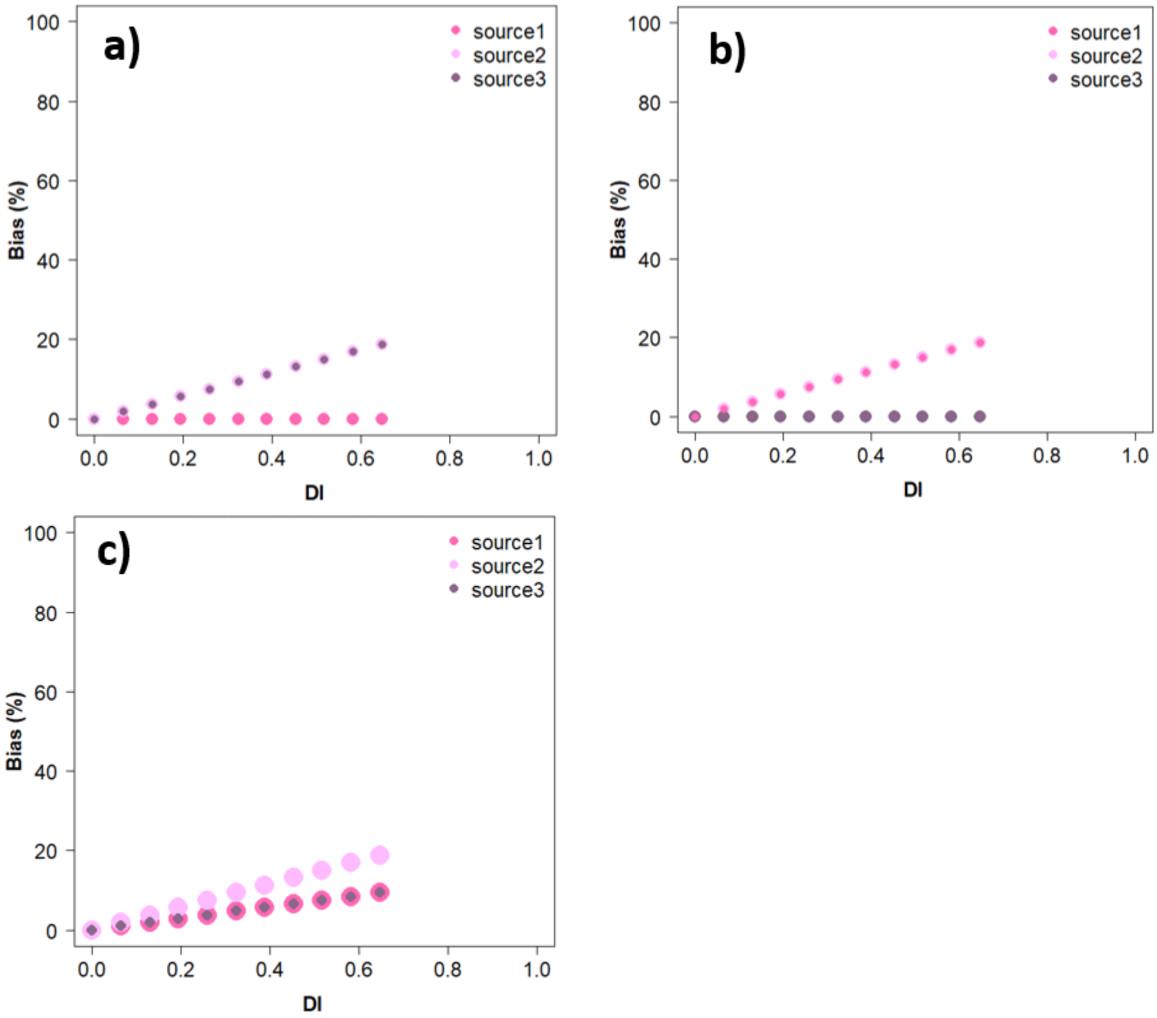








Appendix V: Outputs from the 2-isotope framework. The consumer initial value in in silico-experiment but with all sources on display. a) switching from source 2 to source 3, b) switching from source 2 to source1, c) switching from source 2 to 50% of source 3 and 50% of source 1. Only the SMM_delta results are represented.

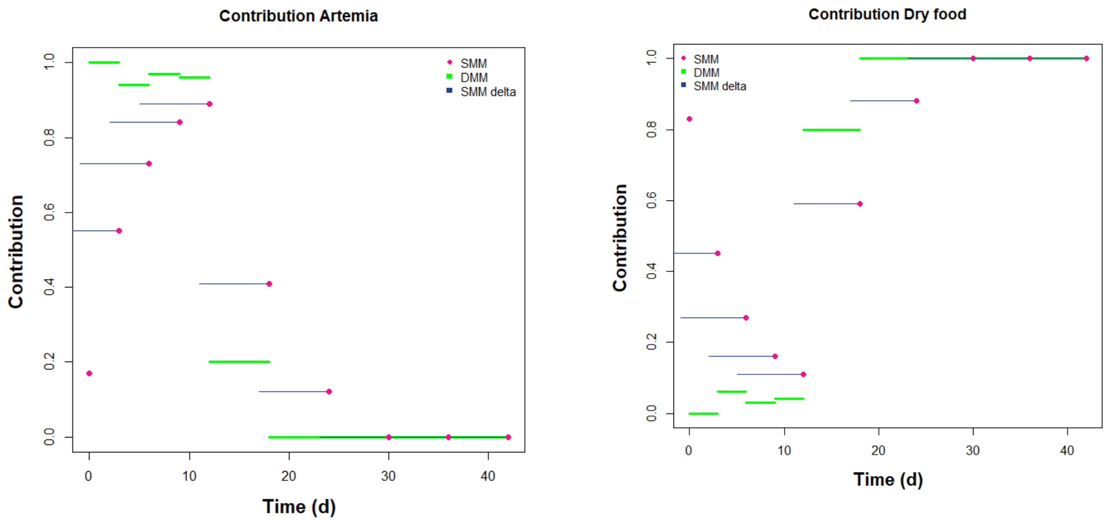


Appendix VI: Outputs from the Fish Larvae dataset (*Jomori et al.,2008*)

For The two experiments the food sources ratios are constant for the Carbon with $\delta^{13}C_{Dry\ food} = -24.2‰$ and $\delta^{13}C_{Artemia} = -15.5‰$ and the fish larvae ratios are always within the source polygon. The $\Delta C_{Dry\ food/Artemia} = 2.29‰$ have been estimated.

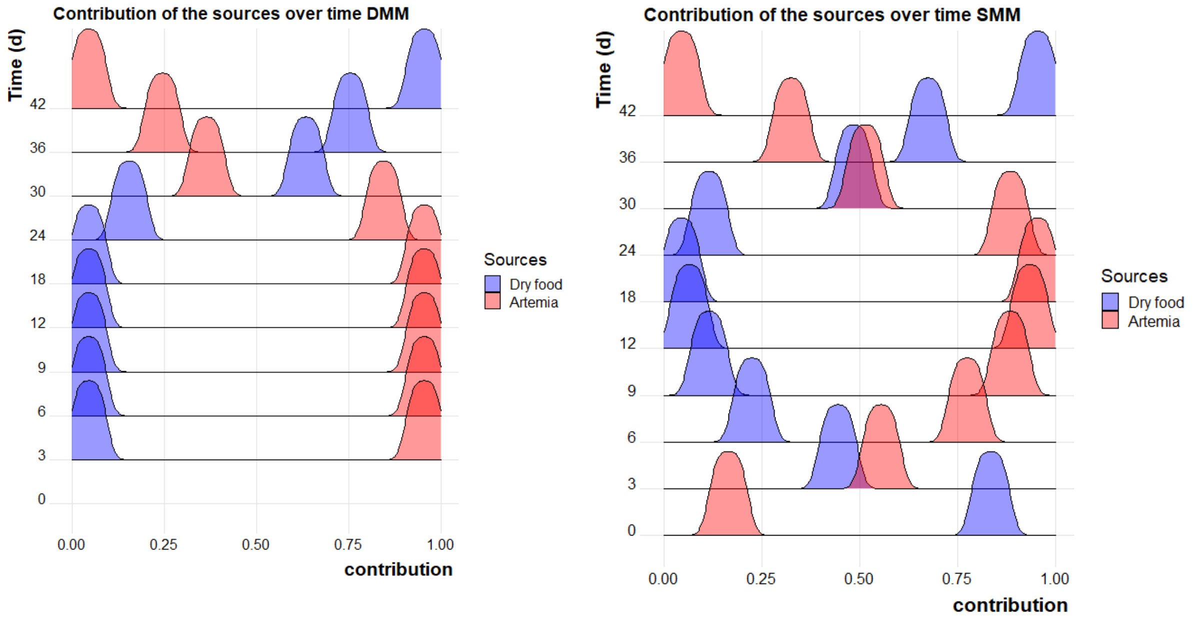
Experiment 1: Fish fed with Artemia for the first 12 days and then fed with Dry food.

Comparison of the predicted contribution of the sources Artemia and Dry food to the diet over time for the three models and their integration in time. SMM outputs are instantaneous and are represented as red dots while SMM_delta takes averaged sources value on a time-windows that equals twice the half-life. In this experiment the λ was considered as constant explaining the constant time-window. For DMM the integration of the dynamics is made between two observations so each prediction period is 3-days long.

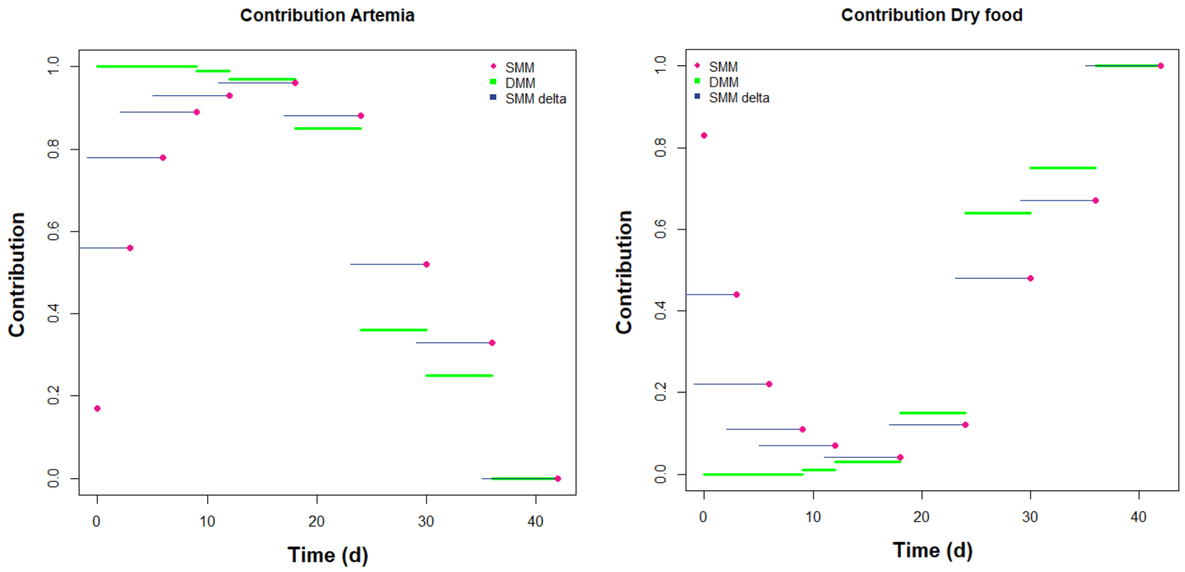


Experiment 2: Fish with both Artemia and Dry food in the environment all the experiment

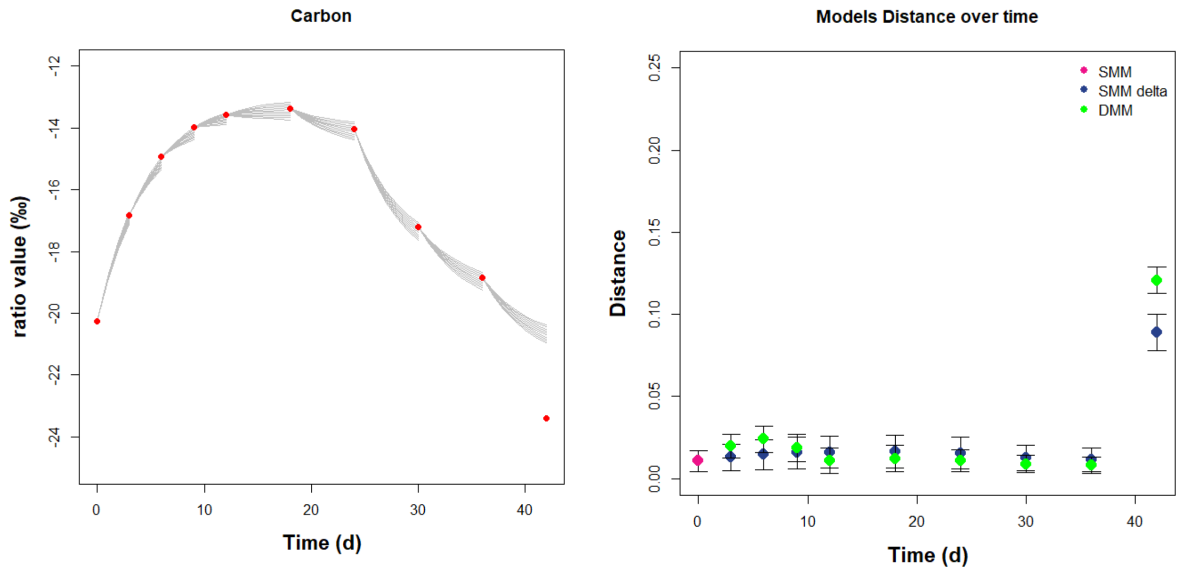
Contribution of the sources to the diet over time according to the DMM and SMM. Here the two aliments are present in the environment and a switch of diet from Artemia to dry food is visible between day 24 and 30.



Comparison of the predicted contribution of the sources Artemia and Dry food to the diet over time for the three models and their integration in time. SMM outputs are instantaneous and are represented as red dots while SMM_delta takes averaged sources value on a time-windows that equals twice the half-life. In this experiment the λ was considered as constant explaining the constant time-window. For DMM the integration of the dynamics are made between two observations so each prediction period is 3-days long.

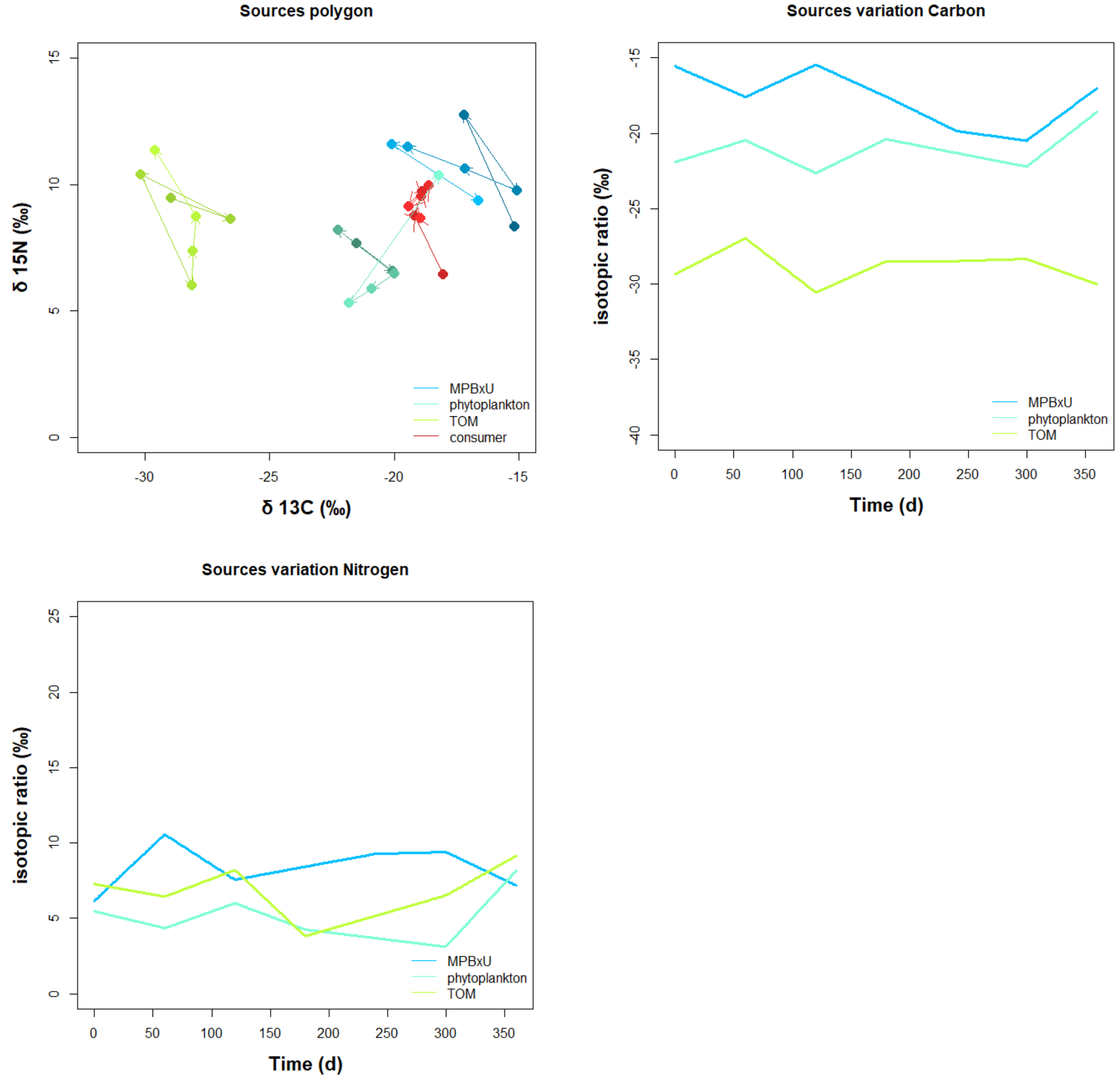


Trajectories predicted of the Carbon ratio of the consumer by the DMM for the 10 best solutions. The red dots are the observed ratio values for the carbon and the grey lines represent the predicted trajectories. The second graph represent the averaged distance for the 10-best solutions of all the models. The results of SMM and SMM_delta are the same so the dots are overlaying.



Appendix VII: Outputs from the Oyster dataset (*Marín Leal et al.,2008*)

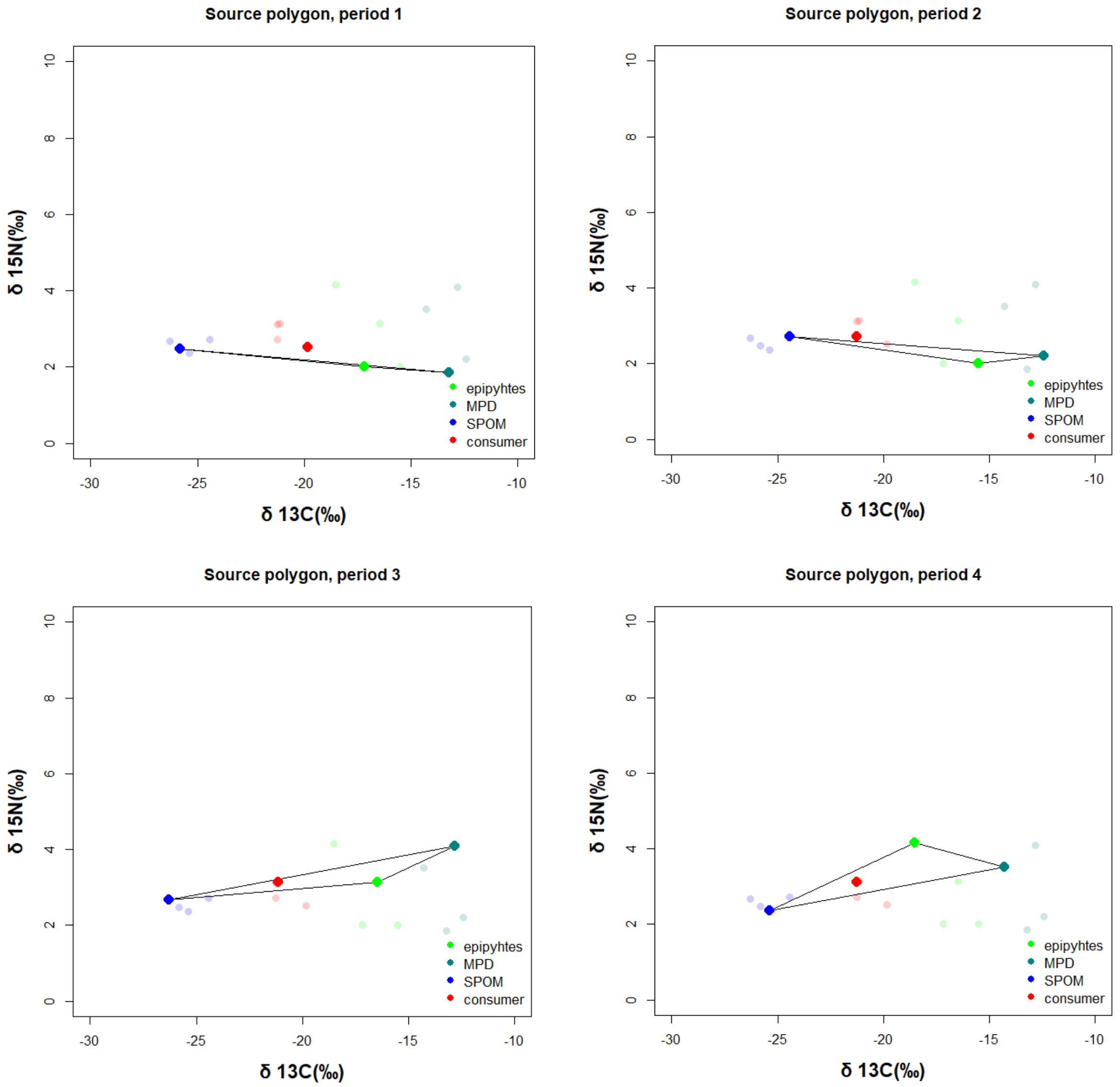
Biplot of the source (corrected by the ΔX_s *values) and consumer values and their evolution along the year for the oyster dataset, the darkest colors are the data at the beginning of the series and the lightest at the end, and source variation along the experiment time. The other two graphs represent the source ratio of Carbon and Nitrogen of each source and its evolution along the year.*



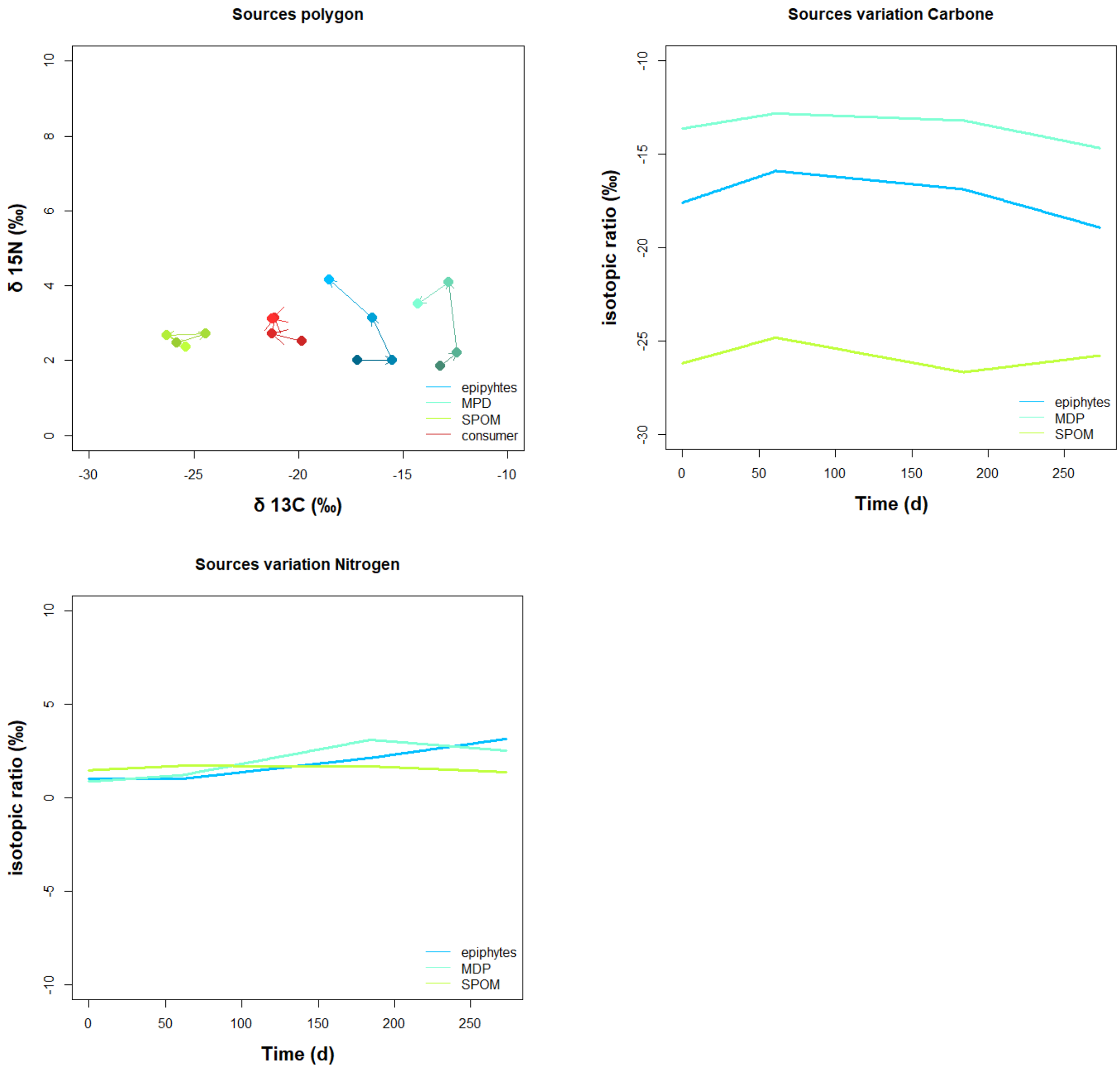
Appendix VIII: Outputs from the Copepod dataset (*Mascart et al.,2018*)

The results shown are for the copepod *Clausocalanus arcuicornis*.

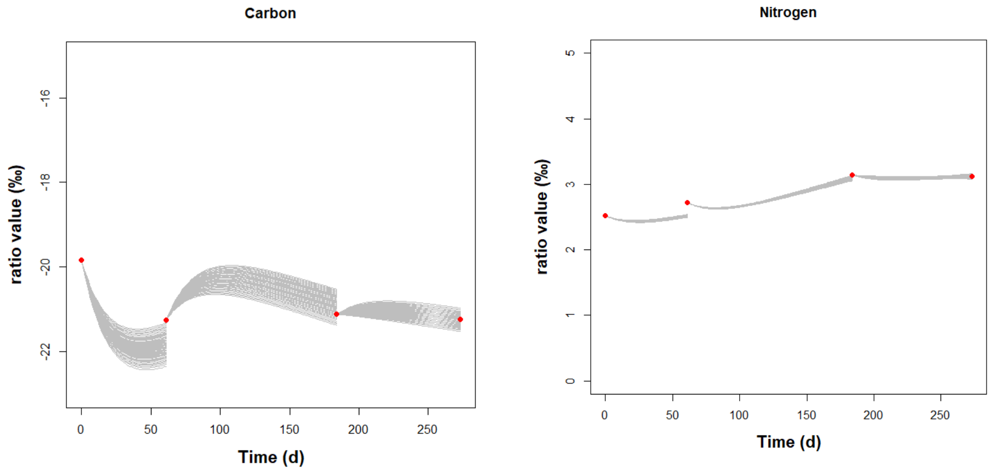
Evolution of the source polygon (corrected by the ΔX_S values) formed by epihpytes, Microhytodetritus (MDP) and suspended particular organic matter (SPOM). The consumer isotopic signature is represented in red. If the consumer is outside the source polygon, the condition of application for mixing models are not respected.



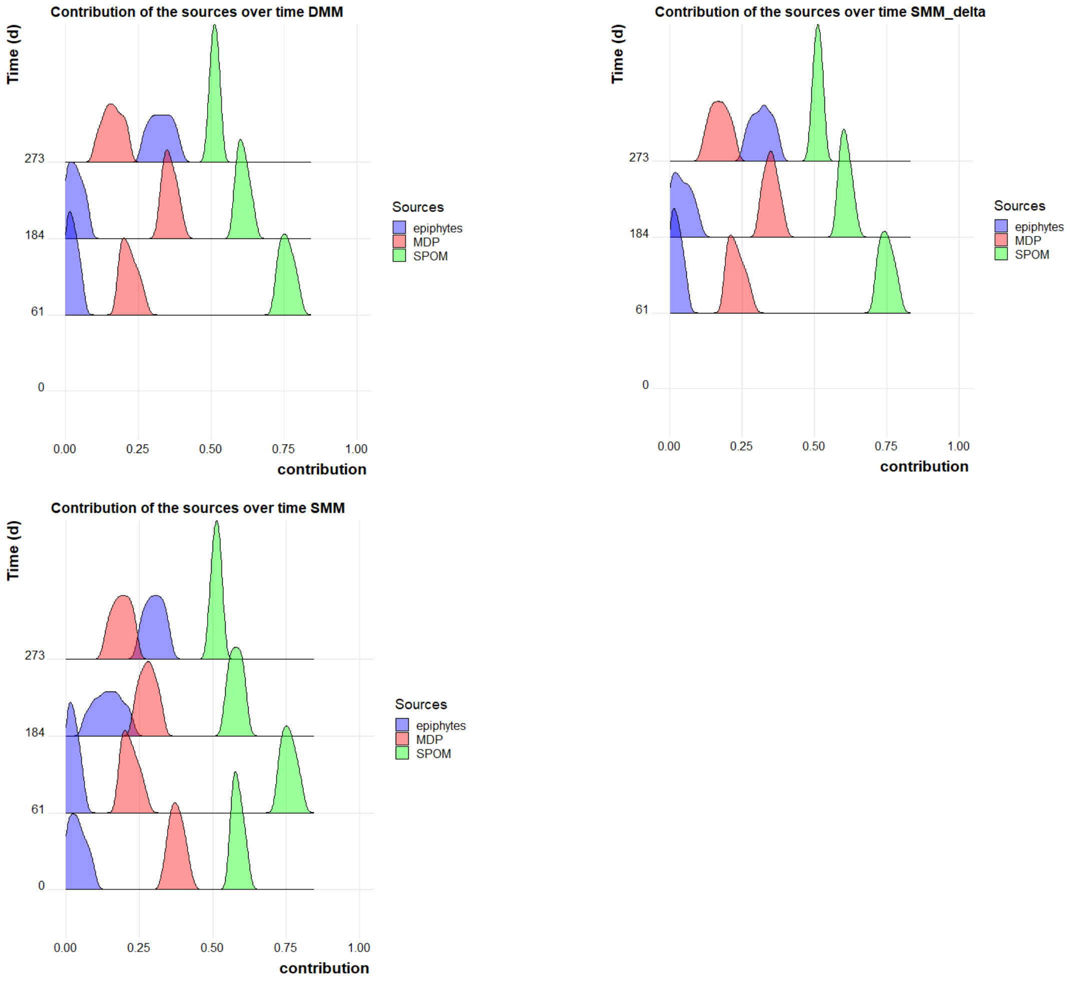
Biplot of the source (corrected by the ΔX_s values) and consumer values and their evolution along the year for the oyster dataset, the darkest colors are the data at the beginning of the series and the lightest at the end, and source variation along the experiment time. The other two graphs represent the source ratio of Carbon and Nitrogen of each source and its evolution along the year.



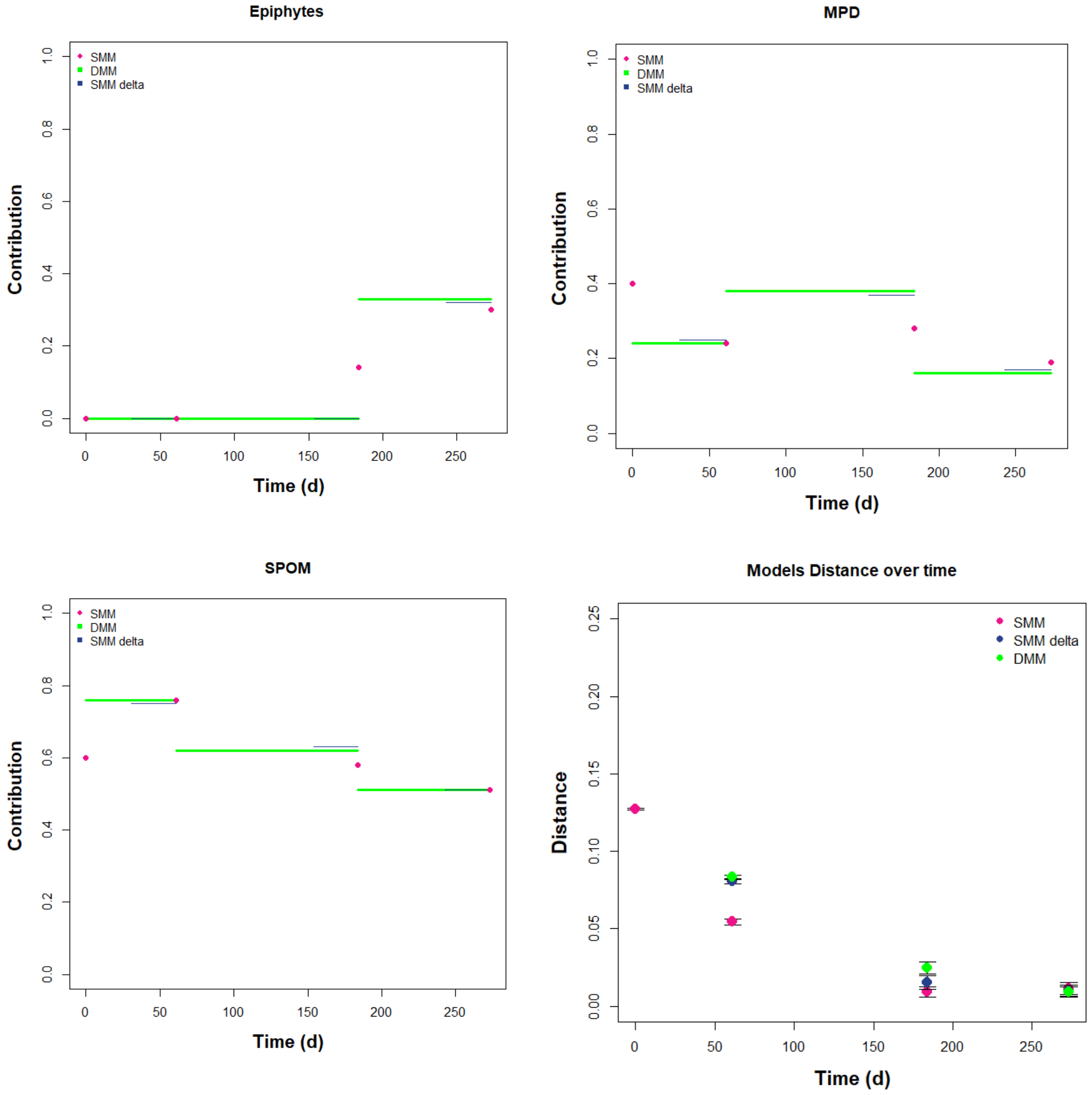
Predicted trajectories for the DMM of the 50 best solutions for Carbon and Nitrogen. The red dots are the observed ratio values for the carbon and the grey lines represent the predicted trajectories.



Distribution of the different solutions for the estimated contributions of each source (epiphytes, microphytodetritus and suspended particular organic matter) along the experiment time for DMM, SMM_delta and SMM.

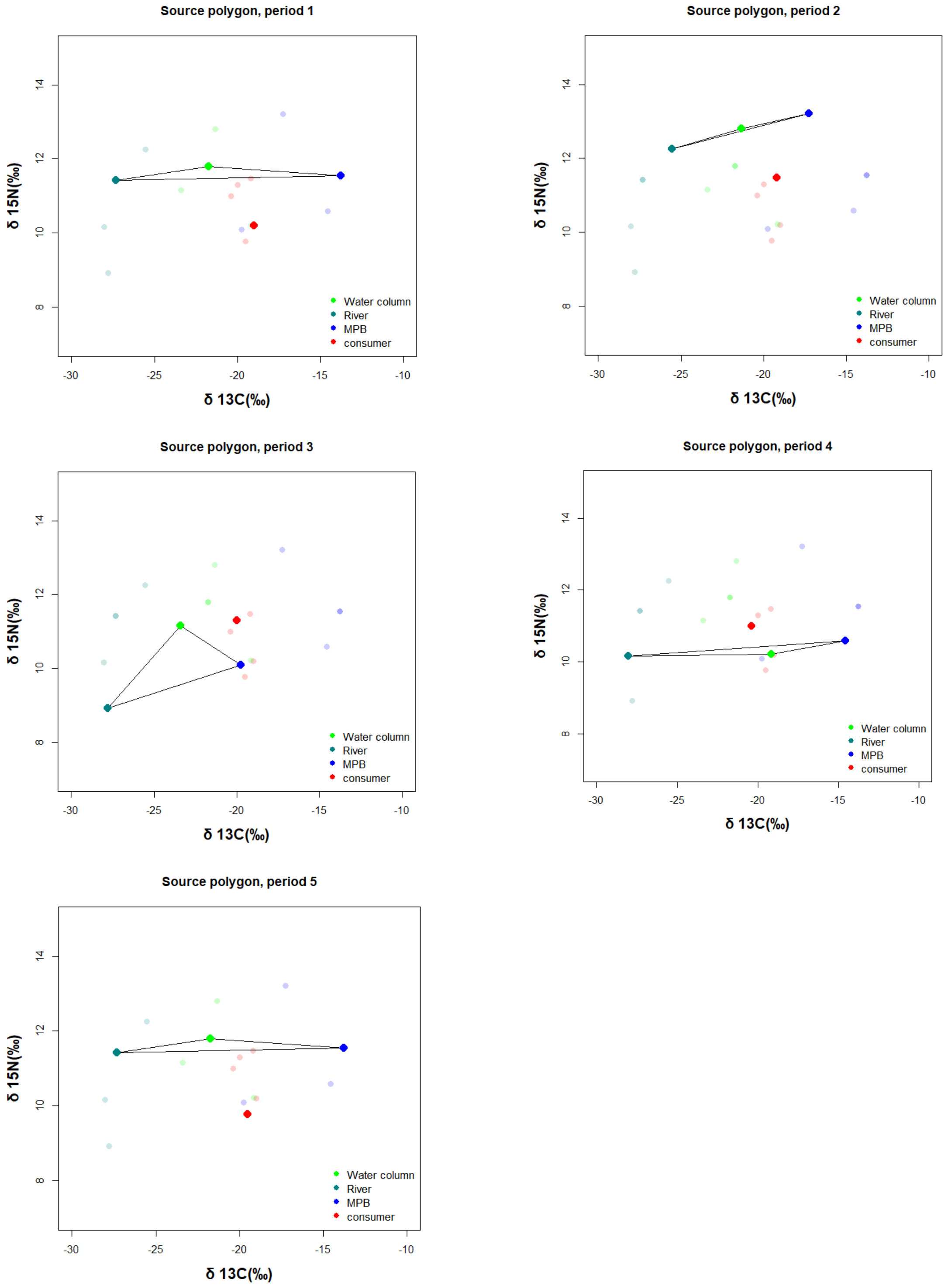


Contribution of each source (Epiphytes, microphytodetritus (MDP) and suspended particular organic matter (SPOM)) according to the three models. SMM outputs are instantaneous and are represented as red dots while SMM_delta takes averaged sources value on a time-windows that equals twice the half-life. In this experiment the λ was considered as constant explaining the constant time-window. For DMM the integration of the dynamics are made between two observations so each prediction period is 3-days long. The last graph represent the averaged distance of the solution for the three models with standard deviation.

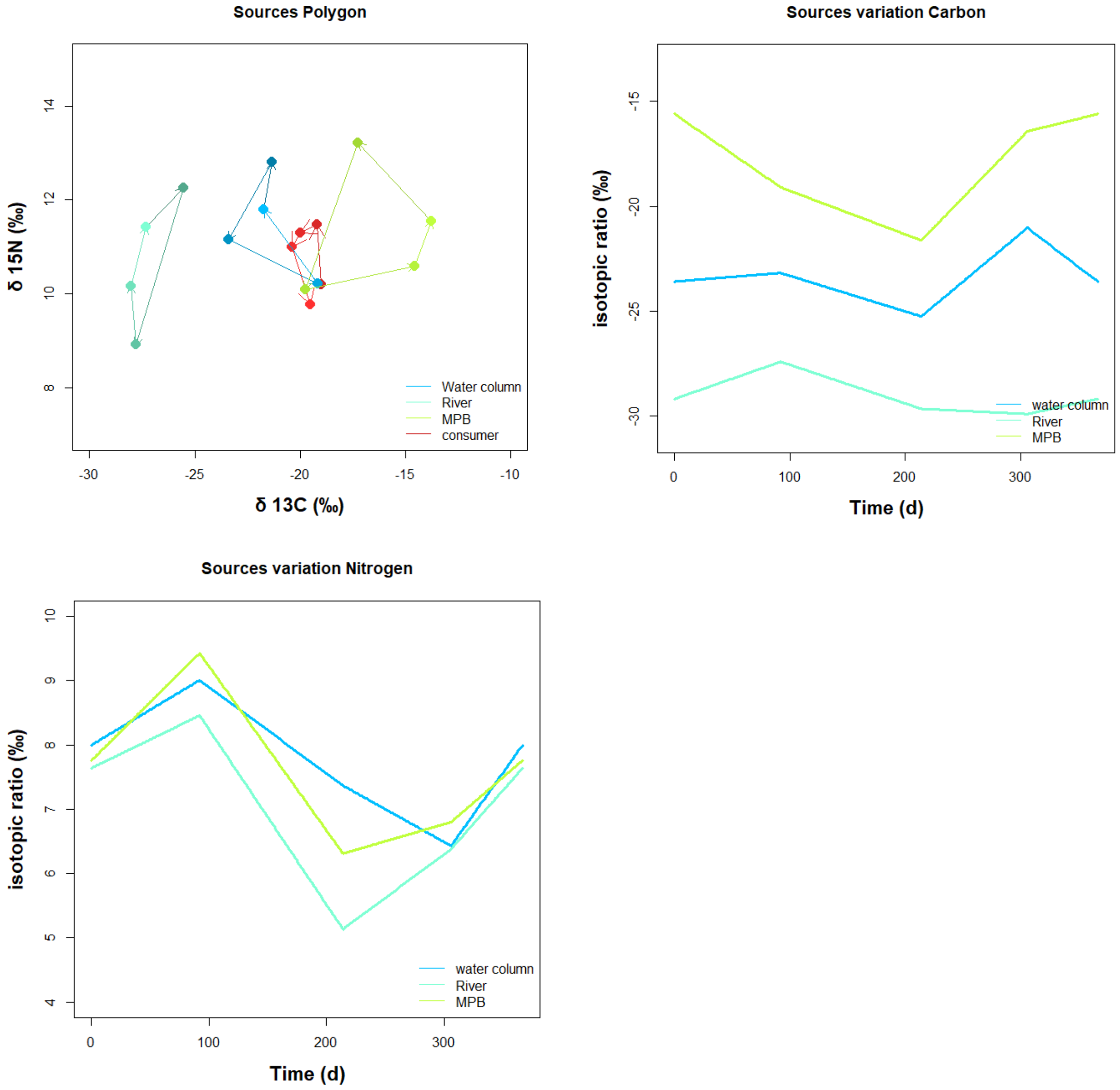


Appendix IX: Outputs from the Cockle dataset (*Varin,2014*)

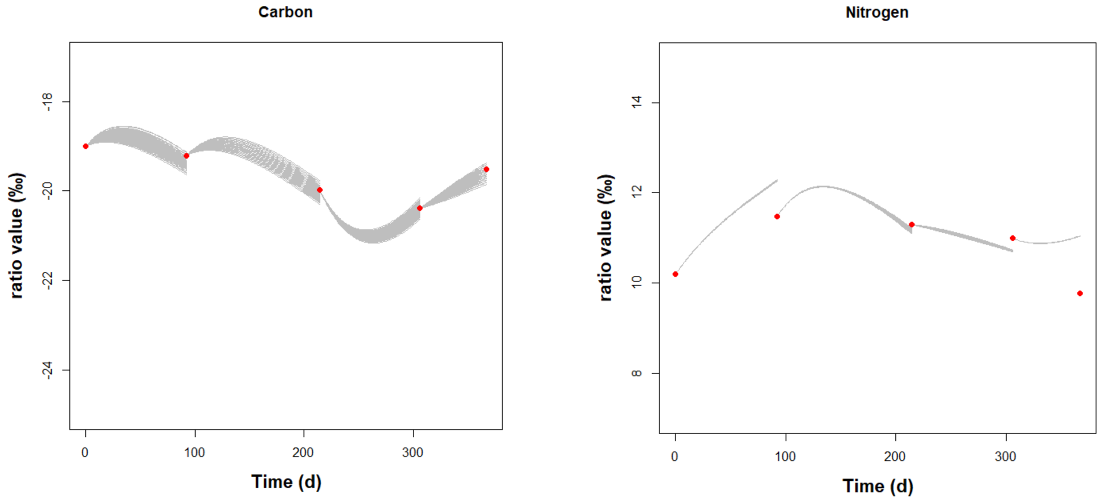
Evolution of the source polygon (corrected by ΔX_Svalues) formed by the Water column, the river and microphytobenthos (MPB). The consumer isotopic signature is represented in red. If the consumer is outside the source polygon, the condition of application for mixing models are not respected.



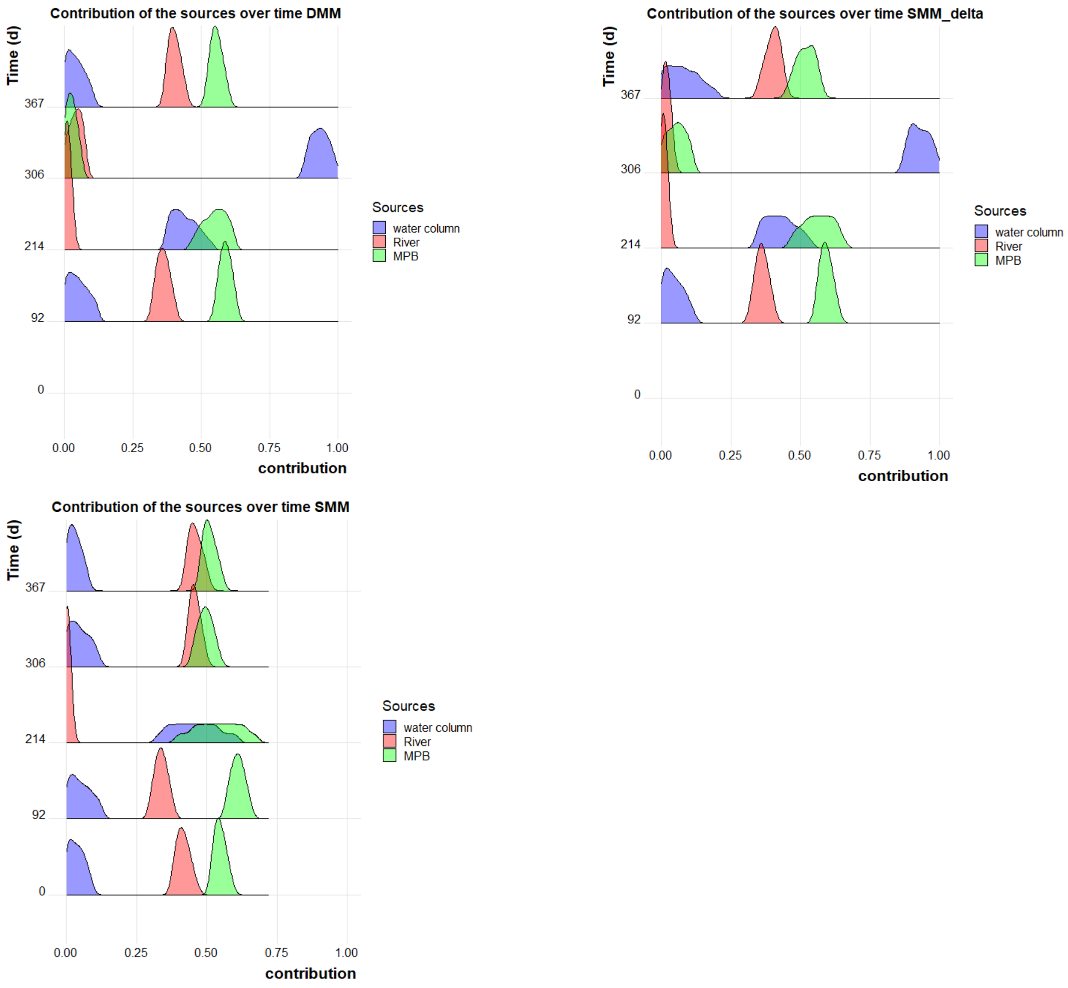
Biplot of the sources and consumer values and their evolution along the year for the cockle dataset the darkest colors are the data at the beginning of the series and the lightest at the end, and source variation along the experiment time. The other two graphs represent the source ratio of Carbon and Nitrogen of each source and its evolution along the year.



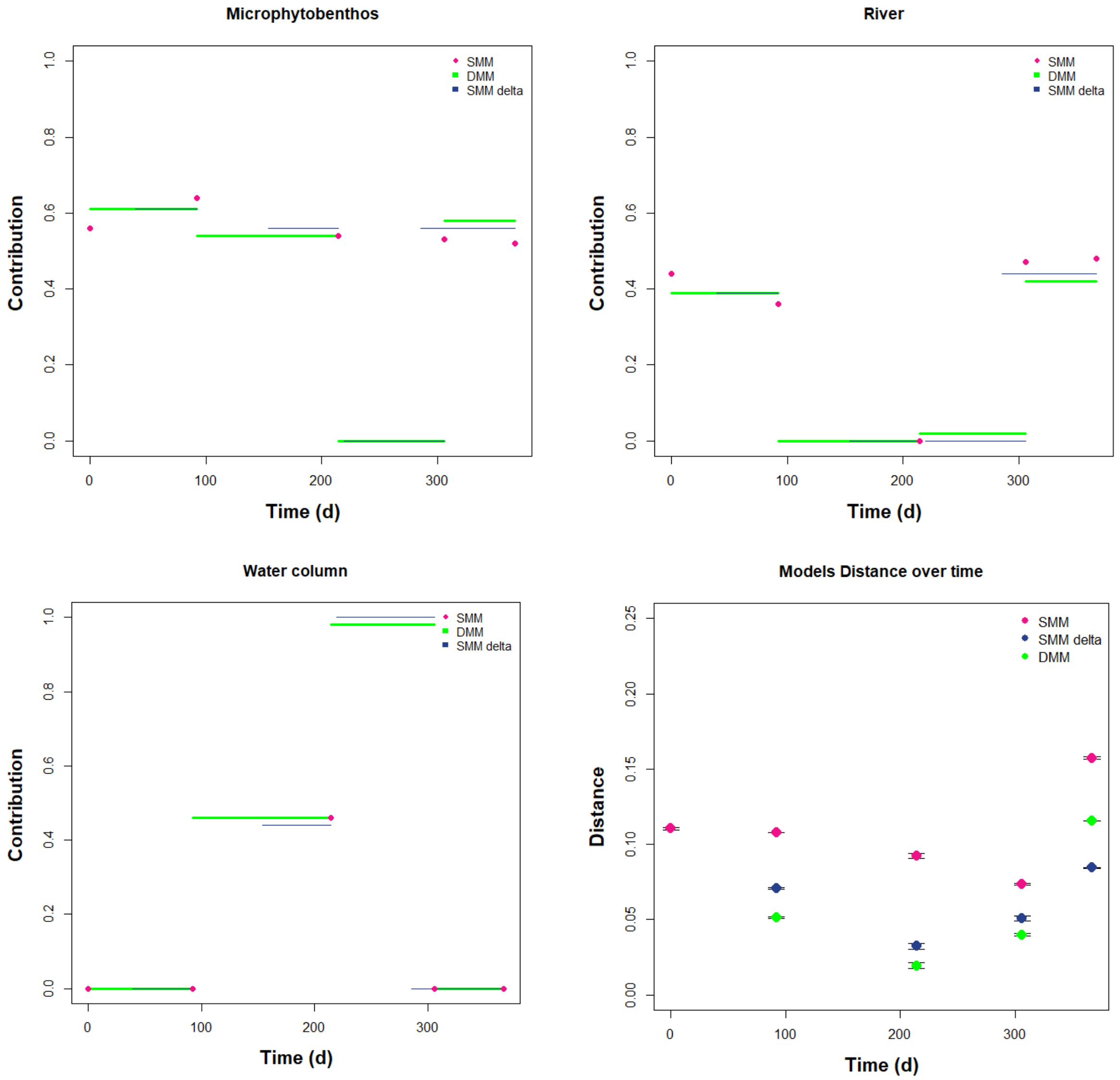
Predicted trajectories for the DMM of the 50 best solutions for Carbon and Nitrogen. The red dots are the observed ratio values for the carbon and the grey lines represent the predicted trajectories.



Distribution of the different solutions for the estimated contributions of each source (epiphytes, microphytodetritus and suspended particular organic matter) along the experiment time for DMM, SMM_delta and SMM.



Contribution of each source (microphytobenthos, River and Water column) according to the three models. SMM outputs are instantaneous and are represented as red dots while SMM_delta takes averaged sources value on a time-windows that equals twice the half-life. In this experiment the λ was considered as constant explaining the constant time-window. For DMM the integration of the dynamics are made between two observations so each prediction period is 3-days long. The last graph represents the averaged distance of the solution for the three models with standard deviation.



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Titre français : Faire progresser les modèles de mélange isotopique dynamiques : Une approche globale avec un indicateur dynamique pour une aide à la sélection de modèles

Titre anglais : Advancing Dynamic Isotopic Mixing Models: A Comprehensive approach with a dynamic indicator for decision-making model selection

Résumé : Les rapports des isotopes stables du carbone et de l'azote peuvent être utilisées pour estimer les régimes alimentaires. Cette estimation dépend de différents paramètres dynamiques au cours du temps qui ne sont pas considérés comme tels dans le cadre d'utilisation des modèles de mélanges isotopiques statiques classiques (SMM). D'autres modèles prennent en compte partiellement (SMM_delta) ou complètement (DMM) ces dynamiques, mais ne sont adaptés que pour fonctionner avec des systèmes précis comportant un nombre limité d'isotopes étudiés et de sources de nourriture. Les trois modèles ont été généralisés pour fonctionner dans toutes les situations et montrer l'importance de la prise en compte de ces dynamiques dans les modèles de mélange et quantifier le biais généré le cas échéant. Un indicateur de dynamique (DI) a été mis en place pour estimer les biais entre modèles et aider au choix du modèle le plus adapté en fonction des données disponibles. Le DMM donne en général de meilleurs résultats, le SMM_delta et le SMM peuvent générer des biais cumulant à 20% et à 50% respectivement. Le taux d'incorporation isotopique (λ en j^{-1}) est particulièrement impliqué dans la dynamique car il contrôle la vitesse de réaction d'un consommateur pour retourner à un état d'équilibre isotopique lors d'un changement de diète ou d'une variation de signature des sources. C'est aussi un facteur déterminant la valeur du DI.

Abstract: Stable isotope ratios of Carbon and Nitrogen are used to estimate animals' diet over time. This estimation depends on several parameters that can change dynamically over time and are not accounted for in a conventional static mixing model framework (SMM). Some models partially consider these dynamics, as in the case of the integrated static model (SMM_delta), or completely for the dynamic mixing model (DMM). However, these models were only adapted to work with specific systems with a predetermined number of studied isotopes and food sources. We have generalized the three models to be used in any situation, and show the importance of incorporating dynamics features in mixing models by highlighting the resulting bias when they are omitted. For this purpose, a dynamic indicator (DI) was created to estimate the bias between models in real datasets and help to identify the best model to use according to the available data. DMM usually gives more accurate solutions and the bias between it and other models can reach up to 20% for SMM_delta and 50% for SMM. One of the dynamic parameters, the isotopic incorporation rate (λ in d^{-1}) is particularly important. It controls the speed a consumer reacts to a change in diet (or source isotopic ratios) to go back to equilibrium, and is also a parameter impacting the DI value.

Mots-clés : isotopes stables, dynamiques, modèles de mélange isotopique, comparaison de l'efficacité des modèles de mélange

Key Words: Stable isotopes, dynamics, isotopic mixing models, comparing mixing model efficacity