

Quantifying the multiple facets of isotopic diversity: New metrics for stable isotope ecology



Julien Cucherousset^{a,*}, Sébastien Villéger^b

^a CNRS, Université Toulouse III Paul Sabatier, ENFA; UMR5174 EDB, Laboratoire Évolution & Diversité Biologique, 118 route de Narbonne, F-31062 Toulouse, France

^b Laboratoire Biodiversité marine et ses usages (MARBEC), UMR 9190 CNRS-IRD-UM-IFREMER, Université de Montpellier, CC 093, 34 095 Montpellier Cedex 5, France

ARTICLE INFO

Article history:

Received 31 October 2014

Received in revised form 25 February 2015

Accepted 25 March 2015

Keywords:

Diversity indices
Trophic diversity
Food web
Stable isotope analyses
Communities
Populations
Biodiversity
Bioindication

ABSTRACT

Stable isotope analyses have emerged as an insightful tool for ecologists, with quantitative methods being developed to analyse data at the population, community and food web levels. In parallel, functional ecologists have developed metrics to quantify the multiple facets of functional diversity in a n-dimensional space based on functional traits. Here, we transferred and adapted metrics developed by functional ecologists into a set of four isotopic diversity metrics (isotopic divergence, dispersion, evenness and uniqueness) complementary to the existing metrics. Specifically, these new metrics are mathematically independent of the number of organisms analysed and account for the abundance of organisms. They can also be calculated with more than two stable isotopes. In addition, we also provide a procedure for calculating the levels of isotopic overlap (similarity and turnover) between two groups of organisms. These metrics have been implemented into new functions in *R* made freely available to users and we illustrated their application using stable isotope values from a freshwater fish community. Transferring the framework developed initially for measuring functional diversity to stable isotope ecology will allow more efficient assessments of changes in the multiple facets of isotopic diversity following anthropogenic disturbances.

© 2015 Elsevier Ltd. All rights reserved.

1. Introduction

During the last three decades, stable isotope analyses have been widely used by ecologists as an integrative tool in food web ecology (Parnell et al., 2010; Phillips and Gregg, 2003). Stable isotope analyses provide a time-integrated measurement of the relationship between consumers and their resources and they have been increasingly used to quantify the trophic implications of a wide range of ecological processes (Fry, 2006; Layman et al., 2012), including individual specialization (Araújo et al., 2007; Cucherousset et al., 2011; Vander Zanden et al., 2010) and trophic subsidies (Cole et al., 2006; Larson et al., 2011; Solomon et al., 2011). Moreover, stable isotope analyses have widely been used to quantify the ecological consequences of human-induced disturbances (e.g. Cucherousset et al., 2012a; Gratton and Denno, 2006; Vander Zanden et al., 1999).

Stable isotope analyses are powerful to quantify the interactions between organisms and the fluxes of energy in terrestrial, marine or freshwater ecosystems (Fry, 2006). Several tools have been developed to analyse stable isotope data (reviewed in Layman et al., 2012), including mixing models to quantify the relative contribution of different prey to the diet of a consumer (Hopkins and Ferguson, 2012; Parnell et al., 2010; Phillips and Gregg, 2003), circular statistics to quantify temporal and spatial changes in stable isotope values (Schmidt et al., 2007) and metrics to quantify the isotopic structure of a group of organisms (Layman et al., 2007a). Specifically, the six metrics developed by Layman et al. (2007a) were based on the position of organisms in a 2-D space (i.e. carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) stable isotopes). These metrics included, for instance, the convex hull area (TA: the smallest area filled by all organisms), the ranges of isotope values (CR and NR representing the ranges of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) or the mean nearest neighbour distance (NND) (Layman et al., 2007a). While some authors (e.g. Hoenighaus and Zeug, 2008) have argued that, under certain circumstances (e.g. comparison among sites with variable ranges of stable isotope values of their putative resources), some metrics might suffer from bias in the estimation of the trophic structure

* Corresponding author. Tel.: +33 5 61 55 84 61.

E-mail address: julien.cucherousset@univ-tlse3.fr (J. Cucherousset).

of community, these metrics have been successfully used to quantify, for instance, the impacts of habitat fragmentation (Layman et al., 2007b), eutrophication (Rawcliffe et al., 2010) or the effects of non-native species (Sagouis et al., 2015). Additional metrics have subsequently been developed based on Bayesian approaches to account for sample size effects and provide confidence intervals of metrics quantifying the isotopic niche size and the level of overlap between groups of organisms (e.g. Jackson et al., 2011; Swanson et al., 2015). Despite these important advancements, methods to quantitatively assess multiple aspects of isotopic diversity, notably by including the abundance of organisms in the food webs, are still lacking while being needed for a more integrative assessment of isotopic diversity. Indeed, isotopic diversity indices calculated based only on stable isotope values of organisms make the implicit assumption that all organisms have the same contribution to isotopic diversity, which is unrealistic in natural ecological systems to provide a comprehensive assessment of the multifaceted isotopic diversity.

Methods developed by functional ecologists (Mouchet et al., 2010; Villéger et al., 2008) to quantify functional diversity could be adapted for stable isotope ecology. Indeed, functional diversity is measured in a multidimensional space where axes represent functional traits, i.e. complementary variables that describe the ecological strategy of an organism (Mouillot et al., 2013). The functional diversity of a community is the distribution of its organisms and of their relative dominance (density or biomass) in this multidimensional functional space (Villéger et al., 2008). Several metrics have been developed to quantify the complementary facets of functional diversity (Mouillot et al., 2013). In addition, metrics to measure the functional similarity between several communities (i.e. overlap in the functional space) have been proposed (Mason et al., 2008; Villéger et al., 2008). All these functional diversity metrics require identifying an organism coordinates in multidimensional functional space and, when possible, weighting each point based on biomass, abundance, or other estimates of organism's importance relevant to the question under investigation.

Here, we repurpose some functional diversity metrics based on organisms functional traits into a set of isotopic diversity metrics based on stable isotope values of organisms that complements the framework developed by Layman et al. (2007a). These new metrics are mathematically independent of the number of organisms used in the calculation and, importantly, they can account for the abundance or biomass of organisms. They can be calculated with more than two stable isotopes, potentially adding ecological insights when elements such as, but not exclusively, hydrogen (δD), sulfur ($\delta^{34}S$) or oxygen ($\delta^{18}O$) are used in conjunction with carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) (Fry, 2006; Grey, 2006; Newsome et al., 2007; Soto et al., 2013). In addition, we provide a procedure for scaling stable isotope axes when quantifying isotopic diversity and for quantifying the levels of isotopic overlap between two groups of organisms.

2. Quantifying the complementary facets of isotopic diversity

In this section, we present a set of metrics (Table 1 and Fig. 1) to quantify different facets of isotopic diversity for a group of organisms. The generic term “organisms” here refers to various levels of biological organization following the question addressed; mainly species within a community or individuals within a population. Organisms are represented by points and their positions in the stable isotope space correspond to their stable isotope values for several elements (e.g. C, N, H, O, S). A weight could be added to each of the points according to an organism abundance (or biomass) in a population or a community. Organism weights can be

measured as the body mass of an individual compared to the total biomass of a population, or as the relative number of individuals (or relative biomass) of a species within a community. Here, isotopic metrics are illustrated using stable isotope values ($\delta^{13}C$ and $\delta^{15}N$) from a freshwater fish community sampled in a lake located in South-western France and composed of 14 species (Cucherousset, unpublished data). Abundance data used here are the relative biomass of each species within the community (Fig. 1 and SOM 1).

2.1. Isotopic divergence

Convex hull area (Layman et al., 2007a) measures only one facet of the isotopic diversity (isotopic richness), i.e. the amount of isotopic space filled by a group of organisms. Therefore this index does not take into account the distribution of points within the convex hull and their weight. For instance, for a given amount of isotopic space filled, most of the points (or most of their weight) could be distributed near the borders of the convex hull or, at the opposite, at its centre. Isotopic divergence *IDiv* could be measured based on the functional divergence index (Villéger et al., 2008). *IDiv* is calculated using the following formula:

$$IDiv = \frac{\Delta d + \overline{dG}}{\Delta |d| + \overline{dG}}$$

where

$$\Delta d = \sum_{i=1}^N w_i \times (dG_i - \overline{dG}),$$

$$\Delta |d| = \sum_{i=1}^N w_i \times |dG_i - \overline{dG}|, \quad \overline{dG} = \frac{1}{N} \sum_{i=1}^N dG_i$$

and where

$$dG_i = \sqrt{\sum_{k=1}^{SI} \left(\delta k_i - \frac{1}{V} \sum_{i=1}^V \delta k_i \right)^2}$$

with, for each organism i [1,N], δk_{ik} is its value for stable isotope k [1,SI] and w_i is its weight (by default $1/N$). V organisms are vertices of the convex hull. In other words, *IDiv* is computed according to the sum of deviances (Δd) and absolute abundance-weighted deviances ($\Delta |d|$) of distances between all organisms and the centre of gravity of convex hull vertices.

IDiv is minimal (i.e. tends to 0) when most of the points (or most of their weight) are close to the centre of gravity of the convex hull, i.e. when organisms with the most extreme stable isotope value(s) (e.g. primary producers and/or top-predators) in a community are rare. *IDiv* tends to 1 when all the points (or their weight) are located on the edges of the convex hull, i.e. when organisms with the most extreme stable isotope value(s) dominate the food web (Fig. 1c). *IDiv* is mathematically independent from the convex hull area (Villéger et al., 2008). This was not the case of the previous metrics proposed to measure divergence, such as the ‘mean nearest neighbor distance—NND’ (Layman et al., 2007a), that are based on distances between points and are thus positively correlated to isotopic richness (convex hull area). The introduction of large-bodied invasive species with extreme trophic position (Cucherousset et al., 2012b) is predicted to increase *IDiv*.

2.2. Isotopic dispersion

We propose an index of isotopic dispersion *IDis*, based on *FDis* index (Laliberté and Legendre, 2010). This index measures the

Table 1

Details on the stable isotope diversity and isotopic overlap (similarity and nestedness) metrics.

Name	Code	Unit [range]	R function	Adapted from
Isotopic divergence	IDiv	Unitless [0; 1]	IDiversity	Villéger et al. (2008)
Isotopic dispersion	IDis	As axes [0; +∞] [*]	IDiversity	Laliberté and Legendre (2010), Layman et al. (2007a)
Isotopic evenness	IEve	Unitless [0; 1]	IDiversity	Villéger et al. (2008)
Isotopic uniqueness	IUni	Unitless [0; 1]	IDiversity	Mouillot et al. (2013)
Isotopic similarity	ISim	Unitless [0; 1]	IOverlap	Villéger et al. (2011)
Isotopic nestedness	ITurn	Unitless [0; 1]	IOverlap	Villéger et al. (2013)

[§] Since stable isotope values are usually expressed in ‰, this metric is expressed in ‰.^{*} Note that when axes are scaled between 0 and 1, isotopic dispersion is unitless and ranges from 0 to 1.

weighted-deviation to the average position of points in the stable isotope space divided by the maximal distance to the centre of gravity. *IDis* is calculated using the following formula:

$$IDis = \sum_{i=1}^N w_i \times dO_i / \max(dO_1, \dots, dO_i, \dots, dO_N)$$

with

$$dO_i = \sqrt{\sum_{k=1}^{SI} \left(\delta k_i - \frac{1}{N} \sum_{i=1}^N \delta k_i \right)^2}$$

IDis could be seen as a scaled multidimensional variance accounting for both the convex hull area and the isotopic divergence. It is a generalization of the ‘centroid distance—CD’ metric proposed by Layman et al. (2007a), which however did not account for organisms weight, was not scaled between 0 and 1 and was thus correlated to isotopic richness. *IDis* equals 0 when all organisms have the same stable isotope values and it increases to 1 when most of the points (or their weight) are far from the centre of gravity of the group of points, i.e. when organisms tend to have contrasted stable isotope values (e.g. primary consumers and top-predators with similar abundances in a community). Trophic downgrading, i.e. the loss of large apex predatory species driven by human-activities (Estes et al., 2011) that could cascade into an increased biomass of herbivorous species, is for instance predicted to decrease *IDis*.

2.3. Isotopic evenness

Isotopic divergence *IDiv* and isotopic dispersion *IDis* do not consider the distance between all organisms, while assessing the regularity in the filling of the convex hull by organisms and their weight could be informative. We propose as an index of isotopic evenness, *IEve*, that is derived from *FEve* (Villéger et al., 2008). It quantifies the regularity in the distribution of organisms and of their weight along the shortest tree that links all the points. *IEve* is calculated using the following formula:

$$IEve = \sum_{l=1}^{N-1} \min \left(\frac{EW_l}{\sum_{l=1}^{S-1} EW_l}, \frac{1}{N-1} \right) - \frac{1}{N-1} \Bigg/ 1 - \frac{1}{N-1}$$

with

$$EW_{l(i,j)} = \frac{\sqrt{\sum_{k=1}^{SI} (\delta k_i - \delta k_j)^2}}{w_i + w_j}$$

and with *l* being a branch of the minimum spanning tree linking organisms in the isotopic space (Fig. 1e).

IEve tends to 0 when most of organisms (or their weight) are packed within a small region of the stable isotope space while a few others are far from this cluster (e.g. most of species are strictly

herbivorous and there are only few predators in a community). *IEve* tends to 1 when organisms are evenly distributed in the stable isotope space. *IEve* has some similarities with the ‘standard deviation of the nearest neighbor distance—SDNND’ (Layman et al., 2007a). However, compared to SDNND, *IEve* is mathematically independent from both the convex hull area and the isotopic divergence while it accounts for organism weight. *IEve* is predicted to increase when large predators are removed from ecosystems (e.g. trophic downgrading; Estes et al., 2011), because the decrease of predator relative biomass and the increase abundance of herbivorous species because of predation release should increase the proportion of biomass at low trophic levels.

2.4. Isotopic uniqueness

We define isotopic uniqueness *IUni* as the inverse of the average isotopic redundancy. Isotopic redundancy reflects the average closeness of organisms in the stable isotope space. For each organism, the distance to its nearest neighbour could be computed and we propose as an index of isotopic uniqueness *IUni*, i.e. the weighted-average of these distances divided by the maximal distance between two nearest neighbours. *IUni* is calculated using the following formula:

$$IUni = \sum_{i=1}^N w_i \times NND_i / \max(NND_1, \dots, NND_i, \dots, NND_N)$$

with

$$NND_i = \min_{i \neq j} \left(\sqrt{\sum_{k=1}^{SI} (\delta k_i - \delta k_j)^2} \right)$$

This index equals 0 when each organism has at least one organism with the same position in the stable isotope space (e.g. communities made of species pairs with similar diets) or when most of the weight belongs to organisms that are isotopically similar. The index tends to 1 when most of the organisms (or organisms with the highest abundance) are isolated in the stable isotope space, i.e. their stable isotope values are very different from all other species (e.g. a freshwater invertebrate community with the most abundant species being the only detritivorous species that consume allochthonous inputs such as terrestrial litter). Invasions of freshwater invertebrates communities by crayfish, a species much larger than most native invertebrates and that can consume terrestrial leaf litter (Larson et al., 2011) is predicted to increase *IUni* in invaded invertebrates communities.

3. Measuring stable isotope overlap among groups of consumers

Some ecological questions require comparing the position and size of the isotopic niche between groups of organisms, i.e. their

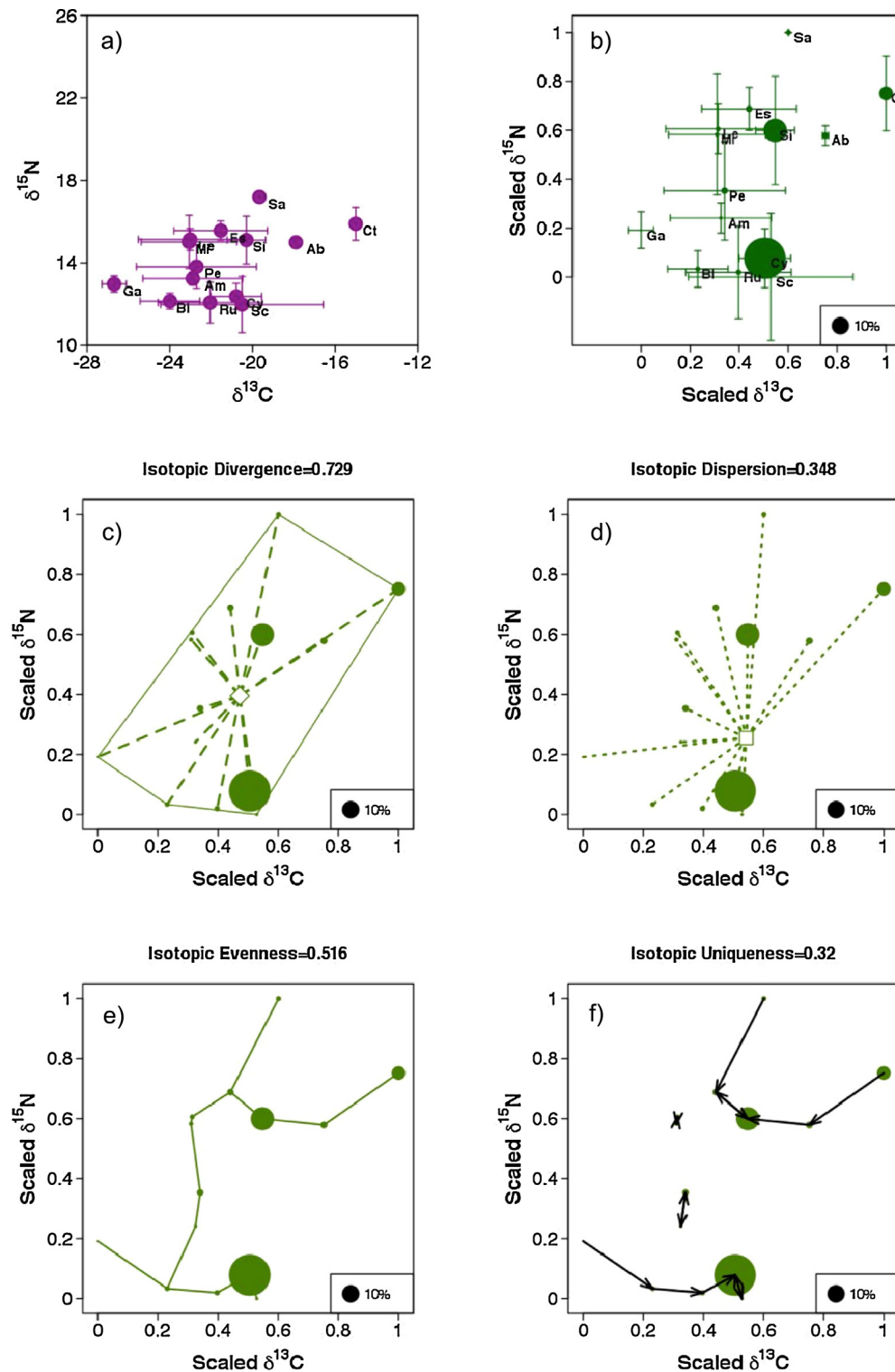


Fig. 1. Stable isotope values of freshwater fish species and illustration of the isotopic diversity metrics. (a) mean (\pm SD) of $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values of each fish species. The two axes have the same range of values to illustrate that $\delta^{13}\text{C}$ range is greater than $\delta^{15}\text{N}$ range. (b) species position in the isotopic space after scaling each stable isotope axis (values ranging between 0 and 1). (c) isotopic divergence (IDiv) measures the distribution of organism importance within the border of the convex hull (green polygon) and is computed using the distances between the points and the centre of gravity of the vertices (dashed lines to green diamond). (d) isotopic dispersion (IDis) is the weighted-mean distance to the centre of gravity of all points (green square). (e) isotopic evenness (IEve) is the regularity of points (position and importance) along the shortest tree linking all the points (green dendrogram) and (f) isotopic uniqueness (IUni) is measured as the weighted-mean of distances to nearest neighbour (black arrows). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

overlap in the isotopic space. For instance, what is the isotopic overlap between juveniles and adults within a population or what is the isotopic overlap between two groups of species within a community? Quantifying raw overlap using stable isotope analyses has

already been performed (e.g. Guzzo et al., 2013; Jackson et al., 2012). The simple way to quantify the closeness of two groups of points in a multidimensional space is to computing the distance between their centre of gravity, i.e. the distance between the weighted-average

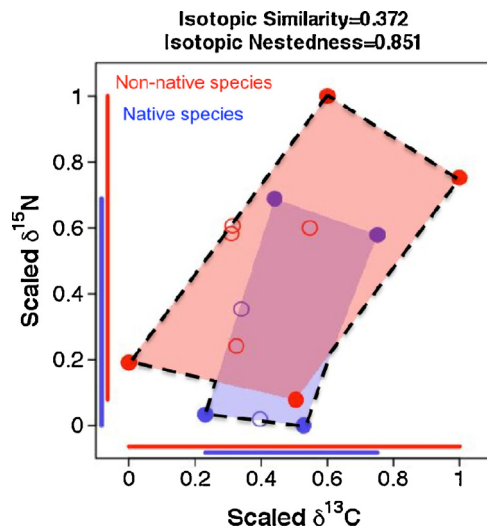


Fig. 2. Isotopic overlap metrics between two groups of organisms (red and blue, respectively) in a two-dimensional isotopic space ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$). Isotopic overlap metrics are measured using the isotopic richness of the two groups (i.e. convex hull volume represented by the red and blue areas, respectively) and the volume of isotopic space they shared (i.e. volume of their intersection, delimited by the purple line). Isotopic similarity is the ratio between the volume shared (purple area) and the volume of the union of the two convex hulls (delimited by the dashed black line). Isotopic nestedness is the ratio between the volume shared and the volume of the smallest convex hull (here in blue). Isotopic overlap on each stable isotope axis is illustrated by the overlap of the colored segments symbolizing range of values for each group. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

position of each group in the stable isotope space. However, both the shape and size of the volume filled by the points influence this estimate and its values may not be directly comparable between studies. Therefore, an informative assessment of isotopic overlap has to be multidimensional and unitless. Here we propose to use two complementary metrics derived from functional ecology that are based on the volume of the intersection between two convex hulls (Villéger et al., 2011, 2013).

The first index, i.e. isotopic similarity (*ISim*), is the ratio between the volume of the intersection and the volume of the union of the two groups of organisms in the stable isotope space (Villéger et al., 2011) (Fig. 2). Isotopic similarity is calculated based on the formula:

$$ISim = \frac{IRic(O_1 \cap O_2)}{IRic(O_1) + IRic(O_2) + IRic(O_1 \cap O_2)}$$

where *IRic* is the total convex hull area filled by organisms from the two groups, *IRic* (O_1) and *IRic* (O_2), and the area of the intersection between the two convex hulls *IRic* ($O_1 \cap O_2$). *ISim* ranges from 0 (when the two groups of organisms fill totally different parts of the stable isotope space, e.g. primary consumers and top-predators) to 1 (when they fill the same portion of the stable isotope space).

ISim is however influenced by the differences in size of the convex hull area of the two groups of organisms. For instance, if one group has a very small convex hull area compared to the area filled by the other group, their overlap will be low both when their intersection is null and when the group with the smallest convex hull area is nested in the convex hull area of the second group. Therefore, we propose a complementary index, isotopic nestedness (*INes*), which is the ratio between the volume of the intersection and the minimal volume filled by a group (Fig. 2). Isotopic nestedness *INes* is calculated using the following formula:

$$INes = \frac{IRic(O_1 \cap O_2)}{\min(IRic(O_1), IRic(O_2))}$$

It ranges from 0 when there is no isotopic overlap (e.g. when comparing juveniles and adults of the same population when the species displays a strong ontogenetic diet shift) to 1 when the group with the lowest isotopic richness fills a subset of the isotopic space filled by the group with the highest isotopic richness (e.g. the stable isotope niche of non-native species in a subset of the stable isotope niche of native species).

Stable isotope overlap metrics (*ISim* and *INes*) can also be quantified on each stable isotope axis separately using the range of observed values for each stable isotope. These one-dimensional metrics could help to further understand the determinants of the multidimensional isotopic overlap. For instance, a null *ISim* between two groups of organisms could result from the absence of overlap on the $\delta^{13}\text{C}$ axis, from the absence of overlap on the $\delta^{15}\text{N}$ axis or from the absence of overlap on both axes. *ISim* and *INes* were developed here using the convex hull area (Layman et al., 2007a) to quantify the isotopic niche size. While the convex hull area quantifies the entire isotopic niche and accounts for the importance of organisms located at the edges of the niche, it can be affected by sample size (e.g. Syväranta et al., 2013) and an approach based on standard ellipse area has been developed to limit this issue (Jackson et al., 2011). We think that these two approaches are in fact complementary, with standard ellipse area focusing on the centre of the trophic niche while the convex hull area fully integrates the importance of organisms located at the edges of the isotopic niche, and that their use in conjunction can provide interesting ecological insights (e.g. Zhao et al., 2014; Sagouis et al., 2015). In addition, it is important to notice that ellipse-based approaches make the assumption that stable isotope values are normally distributed (Jackson et al., 2011; Swanson et al., 2015). While we think that such an assumption is likely to occur when investigating the isotopic niche of individuals within a population, it is less realistic among species within a community, notably because of the potentially restricted number of species. Thus, measuring isotopic overlap based on convex hull area is the most appropriated approach when investigating overlap between two groups of species (e.g. between two families or between native and non-native species).

4. Measuring isotopic diversity in practice

4.1. Building an unbiased stable isotope space

A potential bias that should be accounted for when quantifying isotopic diversity is the difference in the ranges of values between different stable isotopes (Fry, 2006; Hoesinghaus and Zeug, 2008). For instance, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values can range over c. 14‰ and c. 5‰, respectively (Solomon et al., 2011). In the freshwater fish community used as an example here, $\delta^{13}\text{C}$ values ranged over 11.68‰ and $\delta^{15}\text{N}$ values ranged over 5.22‰ (Fig. 1). Moreover, these differences in stable isotope ranges could be more important with the stable isotope of hydrogen for which values often spanned over a range of c. 100‰ (Cole and Solomon, 2012; Cole et al., 2011; Doucett et al., 2007). While these differences are accounted for when computing mixing models or calculating food chain length, they can blur the quantification of isotopic diversity as the axis with the largest range will mathematically have more importance in the calculation of the isotopic diversity metrics. For instance, in our example, $\delta^{13}\text{C}$ will contribute 2.24 (11.68/5.22) times more than $\delta^{15}\text{N}$ to the calculation of the convex hull area (Fig. 3). Therefore, following the approach used by functional ecologists (Villéger et al., 2008), we recommend to compute stable isotope diversity metrics in a standardized multidimensional space where each axis is scaled to have the same range (e.g. 0–1) for the each stable isotope (δk). This can be easily done using the formula:

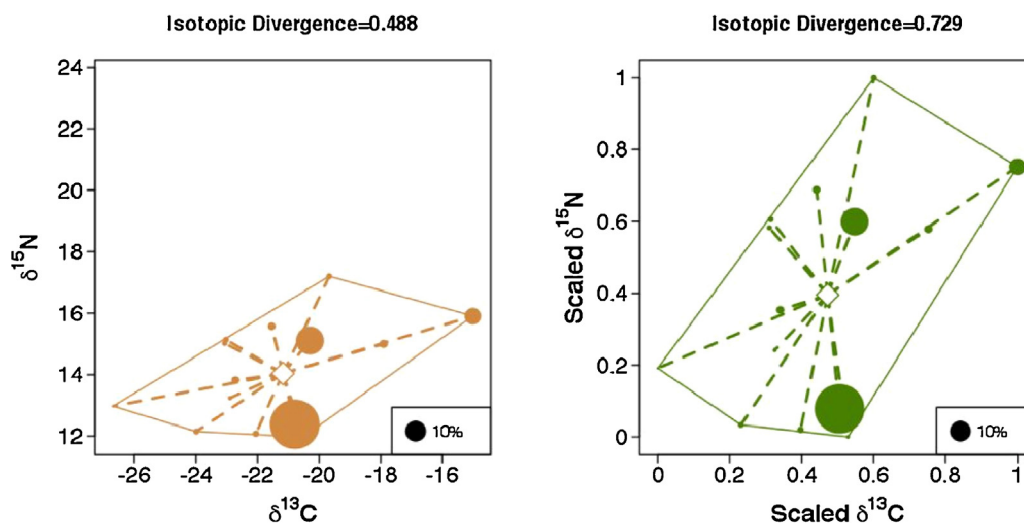


Fig. 3. Effects of scaling stable isotope values on isotopic diversity metrics. Isotopic divergence (IDiv, illustrated as the distribution of points and of their weight within the convex hull) was calculated on the same fish community considering raw stable isotope values (*left*, IDiv = 0.488) and after scaling each stable isotope values between 0 and 1 (*right*, IDiv = 0.729). In this example, IDiv is underestimated by a factor 1.5 when not scaling stable isotope values.

$$\delta k_{st} = \frac{(\delta k - \min(\delta k))}{(\max(\delta k) - \min(\delta k))}$$

which does not affect the distribution of the values but only their unity (Fig. 3 and additional details in SOM 1).

The scaling procedure is intuitive when all the organisms that are being considered for computing diversity indices have the same set of potential trophic resources (e.g. different fish species in a single ecosystem). When the research question aims at comparing stable isotope diversity spatially or temporally, it is frequent that basal resources differ and/or have different stable isotope values. In these cases, we suggest first to pool the stable isotope values of all organisms studied before scaling them to give each isotope the same weight (instead of doing the scaling independently for each ecosystem). Such a procedure will guarantee that the diversity of resources is accounted for in the computation of isotopic diversity. Importantly, depending of course upon the question under investigation, variations in the stable isotope values of basal resources could be perceived as a source of bias when quantifying the trophic diversity of communities based on stable isotope values since variations could be driven by non-trophic variations (Hoeinghaus and Zeug, 2008). In such cases, we recommend to follow, for instance, the procedure used by Quevedo et al. (2009) to transformed raw values of $\delta^{15}\text{N}$ for fish into trophic position according to the $\delta^{15}\text{N}$ of their prey (see also Vander Zanden et al., 1997) and raw values of $\delta^{13}\text{C}$ into a proportion of reliance in littoral carbon based on the difference in stable isotope values between the littoral and pelagic resources. This approach is very similar to the framework developed by Newsome et al. (2007) that consisted in transforming a δ -space into a p -space where the axes are the estimated dietary contribution of different putative prey, although here the two axes are mathematically independent. These standardised axes should eventually be scaled and all the stable isotope diversity metrics could subsequently be computed to compare metrics values across systems. However, we think that environmental effects on stable isotope values of basal resources (assuming that they have been quantified) that subsequently affect the isotopic diversity of consumers and the associated metrics could provide insightful information in some cases, for instance when investigating the impacts of human-induced perturbations (e.g. Layman et al., 2007b; Evangelista et al., 2014).

4.2. Using more than two stable isotopes

A very large majority of studies investigated the trophic ecology of consumers using the stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) jointly (Cucherousset et al., 2012b; Grey, 2006; Layman et al., 2007a; Newsome et al., 2007). However, many other stable isotopes (e.g. hydrogen δD , sulfur $\delta^{34}\text{S}$ or oxygen $\delta^{18}\text{O}$) have an ecological meaning (Fry, 2006; Grey, 2006; Newsome et al., 2007; Soto et al., 2013), such as the quantification of the contribution of terrestrial allochthonous subsidies to aquatic ecosystems (Cole et al., 2011; Doucett et al., 2007; Solomon et al., 2011). Therefore, we think that quantifying the whole breadth of isotopic diversity will benefit from using a trophic space with more than two dimensions (i.e. more than two stable isotope ratios). The isotopic diversity metrics presented here fit this requirement and can be calculated in n -dimensions.

4.3. Accounting for the abundance of organisms

Previous stable isotope metrics do not account for the abundance (or biomass) of individuals in populations or of species in communities and all organisms are assumed to have the same importance (Layman et al., 2007a; Jackson et al., 2011). This is ecologically not realistic and food web ecologists have long recognised that the abundance or biomass of organisms should be accounted for (e.g. Huxel and McCann, 1998). For instance, as an individual increases its body mass and size with ontogeny, energy requirements generally increase and stable isotope niche can change though ontogenetic niche shift (e.g. Post, 2003; Zhao et al., 2014). Therefore, weighting stable isotope values by the abundance or the biomass of individuals in a population appears straightforward. Similarly, in communities, species highly differ in their abundance and these differences are trophically-biased, i.e. species biomass is not distributed similarly/equitably across trophic levels in ecosystems (Trebilco et al., 2013). In addition, the distribution of species across trophic levels could be affected by human activities, such as biological invasions (Cucherousset et al., 2012a) or trophic downgrading (Estes et al., 2011). Therefore, accounting for species biomass/abundance when quantifying isotopic diversity is crucial. Four metrics presented above (namely isotopic evenness, isotopic divergence, isotopic dispersion and isotopic uniqueness) take into account the abundance or biomass of organisms in their

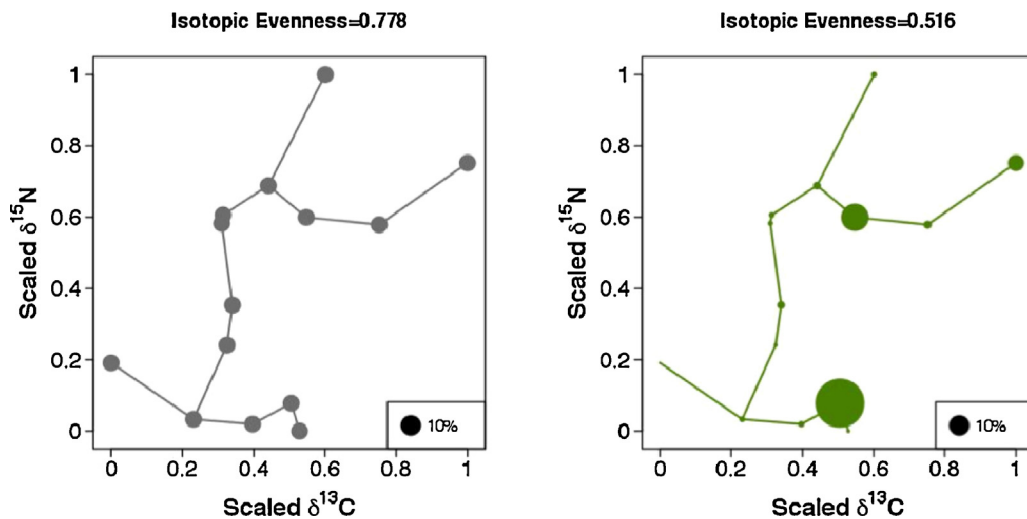


Fig. 4. Effects of accounting for organism abundance on isotopic diversity metrics. Isotopic evenness (IEve, illustrated as the distribution of points on the shortest tree linking them) was calculated on the same fish community without considering species biomass (i.e. all points have the same weight, *left*, IEve = 0.778) and with accounting for their relative importance in the calculation (*right*, IEve = 0.516). In this example, IEve is overestimated by a factor 1.5 when not accounting for the relative biomass of species in the community.

calculations. We thus advise to include the abundance or biomass of organisms when this information is available, for instance when interested in ecological questions related to the fluxes of energy between trophic levels or changes in the structure of communities. When abundance or biomass of organisms has not been estimated, the isotopic metrics could still be computed but they should be interpreted keeping in mind that their values reflect the implicit hypothesis that each species has the same weight in the calculation of the metric. For instance, isotopic evenness will tend to be higher when abundance is unknown because all species will artificially have the same weight (Fig. 4).

4.4. Accounting for intraspecific variability in stable isotope values

Commonly, the quantification of isotopic diversity of communities is computed using mean stable isotope values of each species (e.g. displayed in Fig. 1a). Thus, intraspecific variability (i.e. variance of stable isotope values within each species) is not explicitly accounted for in these analyses. This variance could be driven by natural variability in the stable isotope values of individuals within a population (e.g. trophic specialization, ontogenetic niche shift) and also, generally to a lower extent, by measurement errors and/or contamination during samples analyses. We suggest first to quantify the level of intraspecific variability compared to interspecific variability. If intraspecific variability of stable isotope values is low compared to interspecific differences, computing isotopic diversity using the mean stable isotope values of species is relevant. If intraspecific variability is high for some species (e.g. coefficient of variation >1), we suggest that it should be accounted for and several solutions are available. First, when a sufficient number of individuals have been sampled, the simplest solution is to classify individuals into several “isotopic entities” (e.g. matching age-classes; Zhao et al., 2014), and eventually to compute isotopic diversity independently for each isotopic entity of the different populations in the community. Second, isotope diversity metrics could be computed after bootstrapping individuals within each species (i.e. random selection of a subset of individuals), calculating the mean stable isotope values for each iteration and computing isotopic diversity metrics. This procedure would produce a high number of isotopic diversity values that can eventually be summarized with a confidence interval. This approach is similar to the Bayesian framework proposed by Jackson et al. (2011) that allows,

for instance, computing standard ellipse area (i.e. a type of isotopic richness) and the Layman metrics accounting for variability between individuals (see details in Jackson et al., 2011). Isotopic diversity metrics based on convex hull area (e.g. isotopic richness of a population and isotopic similarity and nestedness between species) are influenced by the number of individuals because of the relationship between the number of points and the probability of having extreme values and therefore higher convex hull area. One should remember this relationship has some ecological meaning and could vary in different contexts, and we also suggest comparing these isotopic diversity metrics using species richness as a covariable in the analyses (Sagouis et al., 2015). The other metrics are based on the distances between organisms in the isotopic space and are a priori independent of the number of organisms (Villéger et al., 2008). In most cases, the number of individuals analyses for stable isotopes per species does not reflect the relative abundance of species in the ecosystem but instead differences in sampling effort and efficiency or financial limitation in the amount of samples analysed, and this notably true for the most rare and most abundant species. Such differences in the number of individuals per species should be corrected before computing isotopic richness of each species. This could be done using a bootstrap procedure for each species, i.e. randomly subsampling a given number of individuals of each species and computing isotopic diversity metrics on these subsets of individuals (e.g. Zhao et al., 2014). An example of such a bootstrapping approach is provided in Appendix S2. Bayesian approach could also be used here (see details Jackson et al., 2011).

5. Discussion

Following the framework initially developed by Layman et al. (2007a), and transferring the multidimensional space framework developed for measuring functional diversity (Mouillot et al., 2013), we propose here additional tools for ecologists interested in stable isotope ecology. As a solution for ecologists interested in computing trophic diversity metrics, we provide a set of R functions to compute these isotopic metrics as Supplementary Online Materials (Table 1, “si_div” R file in SOM 1 and associated example). These functions could be used with multiple types of input data (e.g. raw stable isotope values, transformed data, scaled data). In addition

to isotopic diversity metrics values these, functions also provide graphical outputs (Figs. 1–3).

The new metrics proposed here are complementary to stable isotope metrics previously developed (e.g. isotopic richness), adding multidimensional, abundance-weighted and unitless indices to the toolbox of stable isotope ecologists. Such informative and uncorrelated metrics are needed for a comprehensive description of the multifaceted isotopic diversity and we argue that this is the first step towards a better understanding of the linkage between biodiversity, food-web structure and ecosystem functioning and the ecological consequences of human-induced perturbations. First, these metrics will offer a unique opportunity to make stable isotope more operational as ecological indicators to assess how each facet of isotopic diversity responds to different types of anthropogenic disturbances (e.g. non-random addition or removal of species in communities, changes in resources availability). For instance, the addition of piscivorous fish in a community where native predator are initially absent is predicted to increase isotopic richness, isotopic divergence and isotopic evenness, while the introduction of omnivorous species is predicted to decrease isotopic evenness and divergence. Second, we think that these metrics could subsequently be used to disentangle the relationships between trophic diversity and ecosystem functioning. It should be noticed that the first step towards this achievement is the assessment of the context-dependency of the relationship between isotopic diversity and ecosystem functioning, notably through meta-analyses on large datasets to identify which isotopic diversity facets are the most important for a given ecosystem function (e.g. energy flows, primary production, nutrient cycling) and through the quantification of isotopic diversity in reference ecosystems with good ecological status to serve as a baseline for bioindication.

Acknowledgements

We are grateful to Thomas Pool and three anonymous reviewers for valuable comments on an earlier version of the manuscript. This work was financially supported by the ONEMA (Projet ISOLAC—Convention 13-V5-28) and an “ERG Marie Curiegrant (PERG08-GA-2010-276969) to JC in the lab EDB, part of the French Laboratory of Excellence project “TULIP” (ANR-10-LABX-41; ANR-11-IDEX-0002-02).

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.ecolind.2015.03.032>

References

- Araújo, M.S., Bolnick, D.I., Machado, G., Giarretta, A.A., Reis, S.F., 2007. Using $\delta^{13}\text{C}$ stable isotopes to quantify individual-level diet variation. *Oecologia* 152, 643–654, <http://dx.doi.org/10.1007/s00442-007-0687-1>
- Cole, J.J., Carpenter, S.R., Kitchell, J., Pace, M.L., Solomon, C.T., Weidel, B., 2011. Strong evidence for terrestrial support of zooplankton in small lakes based on stable isotopes of carbon, nitrogen, and hydrogen. *Proc. Natl. Acad. Sci.* 108, 1975–1980, <http://dx.doi.org/10.1073/pnas.1012807108>
- Cole, J.J., Carpenter, S.R., Pace, M.L., Van de Bogert, M.C., Kitchell, J.L., Hodgson, J.R., 2006. Differential support of lake food webs by three types of terrestrial organic carbon. *Ecol. Lett.* 9, 558–568, <http://dx.doi.org/10.1111/j.1461-0248.2006.00898.x>
- Cole, J.J., Solomon, C.T., 2012. Terrestrial support of zebra mussels and the Hudson River food web: a multi-isotope, Bayesian analysis. *Limnol. Oceanogr.* 57, 1802–1815, <http://dx.doi.org/10.4319/lo.2012.57.6.1802>
- Cucherousset, J., Acou, A., Blanchet, S., Britton, J.R., Beaumont, W.R.C., Gozlan, R.E., 2011. Fitness consequences of individual specialisation in resource use and trophic morphology in European eels. *Oecologia* 167, 75–84, <http://dx.doi.org/10.1007/s00442-011-1974-4>
- Cucherousset, J., Blanchet, S., Olden, J.D., 2012a. Non-native species promote trophic dispersion of food webs. *Front. Ecol. Environ.* 10, 406–408, <http://dx.doi.org/10.1890/12.WB.018>
- Cucherousset, J., Bouletreau, S., Martino, A., Roussel, J.-M., Santoul, F., 2012b. Using stable isotope analyses to determine the ecological effects of non-native fishes. *Fish. Manag. Ecol.* 19, 111–119, <http://dx.doi.org/10.1111/j.1365-2400.2011.00824.x>
- Doucett, R.R., Marks, J.C., Blinn, D.W., Caron, M., Hungate, B.A., 2007. Measuring terrestrial subsidies to aquatic food webs using stable isotopes of hydrogen. *Ecology* 88, 1587–1592, <http://dx.doi.org/10.1890/06-1184>
- Estes, J.A., Terborgh, J., Brashares, J.S., Power, M.E., Berger, J., Bond, W.J., Carpenter, S.R., Essington, T.E., Holt, R.D., Jackson, J.B.C., Marquis, R.J., Oksanen, L., Oksanen, T., Paine, R.T., Pikitch, E.K., Ripple, W.J., Sandin, S.A., Scheffer, M., Schoener, T.W., Shurin, J.B., Sinclair, A.R.E., Soule, M.E., Virtanen, R., Wardle, D.A., 2011. Trophic downgrading of planet earth. *Science* 333, 301–306, <http://dx.doi.org/10.1126/science.1205106>
- Evangelista, C., Boiché, A., Lecerf, A., Cucherousset, J., 2014. Ecological opportunities and intraspecific competition alter trophic niche specialization in an opportunistic stream predator. *J. Anim. Ecol.* 83, 1025–1034, <http://dx.doi.org/10.1111/1365-2656.12208>
- Fry, B., 2006. *Stable Isotope Ecology*. Springer, New York, NY.
- Gratton, C., Denno, R.F., 2006. Arthropod food web restoration following removal of an invasive wetland plant. *Ecol. Appl.* 16, 622–631, [http://dx.doi.org/10.1890/1051-0761\(2006\)016\[0622:AFWRFR\]2.0.CO;2](http://dx.doi.org/10.1890/1051-0761(2006)016[0622:AFWRFR]2.0.CO;2)
- Grey, J., 2006. The use of stable isotope analyses in freshwater ecology: current awareness. *Pol. J. Ecol.* 54, 563–584.
- Guzzo, M.M., Haffner, G.D., Legler, N.D., Rush, S.A., Fisk, A.T., 2013. Fifty years later: trophic ecology and niche overlap of a native and non-indigenous fish species in the western basin of Lake Erie. *Biol. Invasions*, <http://dx.doi.org/10.1007/s10530-012-0401-z>
- Hoeinghaus, D.J., Zeug, S.C., 2008. Can stable isotope ratios provide for community-wide measures of trophic diversity? *Comment. Ecology* 89, 2353–2357, <http://dx.doi.org/10.1890/07-1143.10>
- Hopkins, J.B., Ferguson, J.M., 2012. Estimating the diets of animals using stable isotopes and a comprehensive Bayesian mixing model. *PLoS ONE* 7, e28478, <http://dx.doi.org/10.1371/journal.pone.0028478>
- Huxel, G.R., McCann, K., 1998. Food web stability: the influence of trophic flows across habitats. *Am. Nat.* 152, 460–469.
- Jackson, A.L., Inger, R., Parnell, A.C., Bearhop, S., 2011. Comparing isotopic niche widths among and within communities: SIBER—Stable Isotope Bayesian Ellipses in R. *J. Anim. Ecol.* 80, 595–602, <http://dx.doi.org/10.1111/j.1365-2656.2011.01806.x>
- Jackson, M.C., Donohue, I., Jackson, A.L., Britton, J.R., Harper, D.M., Grey, J., 2012. Population-level metrics of trophic structure based on stable isotopes and their application to invasion ecology. *PLoS ONE* 7, e31757, <http://dx.doi.org/10.1371/journal.pone.0031757>
- Laliberté, E., Legendre, P., 2010. A distance-based framework for measuring functional diversity from multiple traits. *Ecology* 91, 299–305.
- Larson, E.R., Olden, J.D., Usio, N., 2011. Shoreline urbanization interrupts allochthonous subsidies to a benthic consumer over a gradient of lake size. *Biol. Lett.* 7, 551–554, <http://dx.doi.org/10.1098/rsbl.2011.0089>
- Layman, C.A., Araújo, M.S., Bouček, R., Hammerschlag-Peyer, C.M., Harrison, E., Jud, Z.R., Matich, P., Rosenblatt, A.E., Vaudo, J.J., Yeager, L.A., Post, D.M., Bearhop, S., 2012. Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biol. Rev.* 87, 545–562, <http://dx.doi.org/10.1111/j.1469-185X.11;2011.00208.x>
- Layman, C.A., Arrington, D.A., Montaña, C.G., Post, D.M., 2007a. Can stable isotope ratios provide for community-wide measures of trophic structure? *Ecology* 88, 42–48, [http://dx.doi.org/10.1890/0012-9658\(2007\)88\[42:CSIRPF\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2007)88[42:CSIRPF]2.0.CO;2)
- Layman, C.A., Quattrochi, J.P., Peyer, C.M., Allgeier, J.E., 2007b. Niche width collapse in a resilient top predator following ecosystem fragmentation. *Ecol. Lett.* 10, 937–944, <http://dx.doi.org/10.1111/j.1461-0248.2007.01087.x>
- Mason, N.W.H., Lanoiselée, C., Mouillot, D., Wilson, J.B., Argillier, C., 2008. Does niche overlap control relative abundance in French lacustrine fish communities? A new method incorporating functional traits. *J. Anim. Ecol.* 77, 661–669, <http://dx.doi.org/10.1111/j.1365-2656.2008.01379.x>
- Mouchet, M.A., Villéger, S., Mason, N.W.H., Mouillot, D., 2010. Functional diversity measures: an overview of their redundancy and their ability to discriminate community assembly rules. *Funct. Ecol.* 24, 867–876, <http://dx.doi.org/10.1111/j.1365-2435.2010.01695.x>
- Mouillot, D., Graham, N.A.J., Villéger, S., Mason, N.W.H., Bellwood, D.R., 2013. A functional approach reveals community responses to disturbances. *Trends Ecol. Evol.* 28, 167–177, <http://dx.doi.org/10.1016/j.tree.2012.10.004>
- Newsome, S.D., Martinez del Rio, C., Bearhop, S., Phillips, D.L., 2007. A niche for isotopic ecology. *Front. Ecol. Environ.* 5, 429–436, <http://dx.doi.org/10.1890/060150.1>
- Parnell, A.C., Inger, R., Bearhop, S., Jackson, A.L., 2010. Source partitioning using stable isotopes: coping with too much variation. *PLoS ONE* 5, e9672, <http://dx.doi.org/10.1371/journal.pone.0009672>
- Phillips, D.L., Gregg, J.W., 2003. Source partitioning using stable isotopes: coping with too many sources. *Oecologia* 136, 261–269, <http://dx.doi.org/10.1007/s00442-003-1218-3>
- Post, D.M., 2003. Individual variation in the timing of ontogenetic niche shifts in largemouth bass. *Ecology* 84, 1298–1310, [http://dx.doi.org/10.1890/0012-9658\(2003\)084\[1298:IVITOT\]2.0.CO;2](http://dx.doi.org/10.1890/0012-9658(2003)084[1298:IVITOT]2.0.CO;2)

- Quevedo, M., Svanbäck, R., Eklöv, P., 2009. Intrapopulation niche partitioning in a generalist predator limits food web connectivity. *Ecology* 90, 2263–2274, <http://dx.doi.org/10.1890/07-1580.1>
- Rawcliffe, R., Sayer, C.D., Woodward, G.U.Y., Grey, J., Davidson, T.A., Iwan Jones, J., 2010. Back to the future: using palaeolimnology to infer long-term changes in shallow lake food webs. *Freshwater Biol.* 55, 600–613, <http://dx.doi.org/10.1111/j.1365-2427.2009.02280.x>
- Sagouis, A., Cucherousset, J., Villéger, S., Santoul, F., Bouletreau, S., 2015. Non-native species modify the isotopic structure of freshwater fish communities across the globe. *Ecography*, <http://dx.doi.org/10.1111/ecog.01348> (in press).
- Schmidt, S.N., Olden, J.D., Solomon, C.T., Zanden, M.J.V., 2007. Quantitative approaches to the analysis of stable isotope food web data. *Ecology* 88, 2793–2802, <http://dx.doi.org/10.1890/07-0121.1>
- Solomon, C.T., Carpenter, S.R., Clayton, M.K., Cole, J.J., Coloso, J.J., Pace, M.L., Vander Zanden, M.J., Weidel, B.C., 2011. Terrestrial, benthic, and pelagic resource use in lakes: results from a three-isotope Bayesian mixing model. *Ecology* 92, 1115–1125, <http://dx.doi.org/10.1890/i0012-9658-92-5-1115>
- Soto, D.X., Wassenaar, L.I., Hobson, K.A., 2013. Stable hydrogen and oxygen isotopes in aquatic food webs are tracers of diet and provenance. *Funct. Ecol.* 27, 535–543, <http://dx.doi.org/10.1111/1365-2435.12054>
- Swanson, H.K., Lysy, M., Power, M., Stasko, A.D., Johnson, J.D., Reist, J.D., 2015. A new probabilistic method for quantifying n -dimensional ecological niches and niche overlap. *Ecology* 96, 318–324, (<http://dx.doi.org/10.1890/14-0235.1>) (In press).
- Syväranta, J., Lensu, A., Marjomäki, T.J., Oksanen, S., Jones, R.I., 2013. An empirical evaluation of the utility of convex hull and standard ellipse areas for assessing population niche widths from stable isotope data. *PLoS ONE* 8 (2), e56094, <http://dx.doi.org/10.1371/journal.pone.0056094>
- Trebilco, R., Baum, J.K., Salomon, A.K., Dulvy, N.K., 2013. *Ecosystem ecology: size-based constraints on the pyramids of life*. *Trends Ecol. Evol.* 28, 423–431, <http://dx.doi.org/10.1016/j.tree.2013.03.008>
- Vander Zanden, H.B., Bjorndal, K.A., Reich, K.J., Bolten, A.B., 2010. Individual specialists in a generalist population: results from a long-term stable isotope series. *Biol. Lett.* 6, 711–714, <http://dx.doi.org/10.1098/rsbl.2010.0124>
- Vander Zanden, M.J., Cabana, G., Rasmussen, J.B., 1997. Comparing trophic position of freshwater fish calculated using stable nitrogen isotope ratios ($\delta^{15}\text{N}$) and literature dietary data. *Can. J. Fish. Aquat. Sci.* 54, 1142–1158.
- Vander Zanden, M.J., Casselman, J.M., Rasmussen, J.B., 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. *Nature* 401, 464–467.
- Villéger, S., Grenouillet, G., Brosse, S., 2013. Decomposing functional β -diversity reveals that low functional β -diversity is driven by low functional turnover in European fish assemblages. *Glob. Ecol. Biogeogr.* 22, 671–681, <http://dx.doi.org/10.1111/geb.12021>
- Villéger, S., Mason, N.W.H., Mouillot, D., 2008. New multidimensional functional diversity indices for a multifaceted framework in functional ecology. *Ecology* 89, 2290–2301, <http://dx.doi.org/10.1890/07-1206.1>
- Villéger, S., Novack-Gottshall, P.M., Mouillot, D., 2011. The multidimensionality of the niche reveals functional diversity changes in benthic marine biotas across geological time. *Ecol. Lett.* 14, 561–568, <http://dx.doi.org/10.1111/j.1461-0248.2011.01618.x>
- Zhao, T., Villéger, S., Lek, S., Cucherousset, J., 2014. High intraspecific variability in the functional niche of a predator is associated with ontogenetic shift and individual specialization. *Ecol. Evol.* 4, 4649–4657, <http://dx.doi.org/10.1002/ece3.12>