ORIGINAL ARTICLE

# DNA metabarcoding suggests adaptive seasonal variation of individual trophic traits in a critically endangered fish

Kurt Villsen[1] | Emmanuel Corse[1,2,3] | Emese Meglécz[1] | Gaït Archambaud-Suard[4] | Hélène Vignes[5] | Alexander V. Ereskovsky[1,6,7] | Rémi Chappaz[4] | Vincent Dubut[1]

[1]Aix Marseille Université, CNRS, IRD, Avignon Université, IMBE, Marseille, France

[2]Centre Universitaire de Formation et de Recherche de Mayotte (CUFR), Dembeni, France

[3]MARBEC, University of Montpellier, CNRS, Ifremer, IRD, Montpellier, France

[4]INRAE, Aix Marseille Université, RECOVER, Aix-en-Provence, France

[5]CIRAD, University of Montpellier, INRAE, Montpellier SupAgro, AGAP, Montpellier, France

[6]St. Petersburg State University, St. Petersburg, Russia

[7]Koltzov Institute of Developmental Biology of Russian Academy of Sciences, Moscow, Russia

**Correspondence**
Kurt Villsen and Vincent Dubut, Aix Marseille Université, CNRS, IRD, Avignon Université, IMBE, Marseille, France.
Email: kurt.villsen@imbe.fr; v.dubut@gmail.com

**Handling Editor:** Holly Bik

## Abstract

Dietary studies are critical for understanding foraging strategies and have important applications in conservation and habitat management. We applied a robust metabarcoding protocol to characterize the diet of the critically endangered freshwater fish *Zingel asper* (the Rhone streber). We conducted modelling and simulation analyses to identify and characterize some of the drivers of individual trophic trait variation in this species. We found that population density and ontogeny had minor effects on the trophic niche of *Z. asper*. Instead, our results suggest that the majority of trophic niche variation was driven by seasonal variation in ecological opportunity. The total trophic niche width of *Z. asper* seasonally expanded to include a broader range of prey. Furthermore, null model simulations revealed that the increase of between-individual variation in autumn indicates that *Z. asper* become more opportunistic relative to summer and spring, rather than being associated with a seasonal specialization of individuals. Overall, our results suggest an adaptive variation of individual trophic traits in *Z. asper*: the species mainly consumes a few ephemeropteran taxa (*Baetis fuscatus* and *Ecdyonurus*) but seems to be capable of adapting its foraging strategy to maintain its body condition. This study illustrates how metabarcoding data obtained from faeces can be validated and combined with individual-based modelling and simulation approaches to explore inter- and intrapopulational individual trophic traits variation and to test hypotheses in the conventional analytic framework of trophic ecology.

### KEYWORDS

conservation, freshwater fish, individual trait variation, metabarcoding, trophic niche

# 1 | INTRODUCTION

Trophic studies are essential to understand ecological interactions, providing insights into the processes that structure ecological communities and regulate energy-flow through trophic networks (Garvey & Whiles, 2016; Nielsen et al., 2018). Functional differences among species are thought to be one of the main sources of ecological variability in food webs, and many diet studies have been performed to investigate interspecific trophic niche variation (Bolnick et al., 2003). However, there is now a growing body of evidence that intraspecific trait variability (ITV) is crucial for maintaining functional diversity in ecosystems, species and populations (Des Roches et al., 2018; Musseau et al., 2020; Raffard et al., 2019). Intraspecific trait diversity promotes functional complementarity among individuals through niche partitioning, facilitating more efficient use of ecological opportunities (see Table 1) over space and time (Bolnick et al., 2011), which in turn may increase individual fitness and population stability (MacColl, 2011). Intraspecific trait variability can be partitioned into three components: within-individual variation, between-individual variation and population-level variation (Albert et al., 2011). In the context of trophic ecology, these three components correspond to the average individual trophic niche width within a population (WIC; related to α-diversity), the average trophic variation between individuals (BIC; related to β-diversity) and the total trophic niche width (TNW; related to γ-diversity; Roughgarden, 1974). TNW is a function of WIC and BIC, and BIC is usually related to individual specialization (Bolnick et al., 2003; Roughgarden, 1974; see glossary in Table 1). According to this framework, many populations considered to be generalists are in fact composed of specialized individuals with relatively narrow diet breadths (Araújo et al., 2011; Bolnick et al., 2002, 2003).

Ecological opportunity, that is, the availability of ecological resources that may be exploited at a given moment (Araújo et al., 2011; Evangelista et al., 2014; Sánchez-Hernández et al., 2021; Stroud & Losos, 2016), varies across space and time (seasonality). Environments are subject to temporal changes, which in turn interact with prey phenology (Conover, 1992; Marshall & Burgess, 2015; Merritt et al., 2001). In rivers, for instance, the abundance of invertebrates is known to be influenced by flow regime dynamics (Monk et al., 2008; White et al., 2017), temperature (Arscott et al., 2003; Haidekker & Hering, 2008), substratum composition (Downes et al., 2000), and water chemistry (Cross et al., 2006; Gafner & Robinson, 2007). Moreover, aquatic insects have complex life histories with most having a short, aerial adult period and an aquatic juvenile stage. The periodicity of each species' juvenile stage can vary from a few months to a few years depending on their particular reproductive strategy (e.g., voltinism) and local environmental conditions (Clifford, 1982; Corbet et al., 2006; Kong et al., 2019). The diverse and overlapping nature of prey phenologies, and their interaction with environmental temporal changes, can lead to significant seasonal variation in community assemblages and in abundances (Erba et al., 2003; García & Añón Suárez, 2007) and consequently on the availability of prey for fishes. Empirical studies have confirmed that seasonal variation in the availability of prey is a major source of trophic niche variation (e.g., Falke et al., 2020; Hoenig et al., 2021; Shutt et al., 2020; Varpe & Fiksen, 2010). The optimal foraging theory predicts that when resource availability declines, consumers will add novel resources into their diet to maintain their fitness (Perry & Pianka, 1997). Between-individual dietary variation is expected to be a determinant component in the diversification of trophic niches when faced with low resource availability (Bolnick et al., 2011; Sjödin et al., 2018) or with seasonal change in ecological opportunities

**TABLE 1** Glossary

| Term | Explanation |
|---|---|
| Trait | Any measurable characteristic of an individual organism that is measured at either the individual or other relevant level of organization, for example, body condition, individual niche width[a] |
| Intraspecific trait variability (ITV) | The individual-based variability of a given trait within a species or population |
| Ecological opportunity | The availability of ecological resources that may be exploited at a given moment by an individual or a species, for example, prey abundance |
| Total trophic niche width (TNW) | A species- or population-level trophic trait summarizing the total realized niche of a given species or population in terms of prey species richness and abundance. Related to γ-diversity |
| Individual niche width (INW) | An individual trophic trait relating to the richness and abundance of prey species consumed by a given individual. Related to α-diversity<br>Also referred to as the within-individual component (WIC) in other studies |
| Between-individual component (BIC) | An individual trophic trait related to the average difference between an individual's trophic niche and the other trophic niches observed within a given species or population. Relating to interindividual dietary variability (β-diversity) |
| Prey turnover | A population-level trophic trait indicating the degree of interindividual dietary variation in a given population or species |
| Body condition | An individual life-history trait related to the potential fitness of a given individual as measured by the ratio between length and weight. Body condition was measured using the scaled-mass index ($\hat{M}_i$) in this study |

[a]Definition from: Dawson et al. (2021).

(Costa-Pereira et al., 2017). And yet, differential responses of individual diets to seasonal prey fluctuation has widespread implications for individual fitness (Durant et al., 2005; Hipfner, 2008) and can affect the demography of populations (Miller-Rushing et al., 2010).

Recent methodological advances in diet analysis by metabarcoding now provides a non-destructive and valuable alternative to methods based on the morphological identification of prey or stable isotopes, to study trophic niche variation (Alberdi et al., 2019; de Sousa et al., 2019). Notably, metabarcoding offers better taxonomic resolution of prey types, and has proven to be a powerful tool for characterizing complex trophic networks (Clare, 2014; Roslin & Majaneva, 2016), niche partitioning between species (Pansu et al., 2019; Soininen et al., 2015), and for guiding the conservation and management of endangered species (Brown et al., 2014; Quéméré et al., 2013). However, metabarcoding data has rarely been used to estimate individual traits for studying inter- and intrapopulation level of trophic niche variation (but see: Bison et al., 2015; Pansu et al., 2019; Pornon et al., 2019). Intraspecific trophic trait variation and the conventional analytic framework of trophic analysis were initially developed for diet data obtained by stable isotope analysis or from gut content (or faeces) analysis. Metabarcoding approaches more closely resemble classical morphological analyses of gut or faeces contents as they use the same type of samples to identify prey. Gut or faeces content analyses (being morphological or DNA-based) greatly differ from stable isotope approaches in terms of the temporal window accounted for by the dietary data (Novak & Tinker, 2015; Petta et al., 2020). While stable isotope analyses are very integrative and can recapitulate the diet of an individual over several weeks or months, metabarcoding data generally integrates one or a few days of feeding (Corse, Valladares, et al., 2015). Dietary data that integrate such short temporal windows usually contain a limited fraction of the total prey range of an individual (Aizpurua et al., 2018; Pansu et al., 2019). However, DNA-based analyses have the potential to provide much higher taxonomic resolution compared to morphological analyses, which are dependent on the visual identification of prey and stable isotope analysis which do not resolve prey at species-level information (Jakubavičiute et al., 2017; Zarzoso-Lacoste et al., 2016). Furthermore, DNA metabarcoding may also detect prey that are morphologically unidentifiable due to the level of degradation of prey species, especially in the case of soft-bodies species (Berry et al., 2015; Egeter et al., 2015).

The Rhone streber (*Zingel asper* (L.) [Actinopterygii: Perciformes: Percidae]) is a critically endangered fish (Crivelli, 2006) endemic to the Rhône River basin, that lost roughly 85% of its historical range during the 20th century and is now restricted to five disconnected populations located in eastern France and Switzerland (Georget, 2019; Olivier et al., 2022). *Zingel asper* is a benthic species with a low diel displacement range, that has naturally low population density (Cavalli et al., 2009; Danancher et al., 2007; Labonne et al., 2003) and mainly feeds on macroinvertebrates (Cavalli et al., 2003; Corse et al., 2019). The current *Z. asper* populations are located in several distinct climatic zones and in both nonregulated (e.g., Beaume and Loue rivers) and regulated rivers (Durance and Verdon rivers; Bravard & Gaydou, 2015; Olivier et al., 2022). In the Durance and Verdon rivers, the natural water flow regime is regulated by hydropower damming (Warner, 2012). This has greatly altered the temporal patterns of water flow and contributed to the homogenization of long-term river dynamics and to restructuring riverine habitats and macroinvertebrate communities (Cazaubon & Giudicelli, 1999; Warner, 2012). However, our current knowledge of the *Z. asper* diet is based on a single population in the Durance river (Cavalli et al., 2003; Corse et al., 2019). Here we used a metabarcoding approach to characterize spatial and seasonal diet variation across the remaining *Z. asper* populations. Second, we characterized variation in both individual (WIC and BIC) and population (TNW) trophic traits to study some of the potential drivers of trophic niche variation in *Z. asper*. Studying the response of individual trophic traits to ecological drivers has proven to be essential to understand ecological processes like dietary specialization (Bolnick et al., 2003), niche expansion (Sjödin et al., 2018), ontogenic niche shift (Sánchez-Hernández et al., 2019) and intraspecific competition (Svanbäck & Bolnick, 2005, 2007). We therefore modelled individual trophic traits against the effect of seasonality, body size (related to ontogeny) and population density, which can be related to intraspecific competition when prey availability is a limiting factor (e.g., Bolnick et al., 2010; Svanbäck et al., 2008; Vander Zanden et al., 2000). Lastly, changes in predator diet may subsequently affect predator body condition (e.g., Skinner et al., 2016), a life-history trait related to energy reserves (Peig & Green, 2009) that can drive fitness potential (Kotiaho, 1999; Wells et al., 2016). Using body condition as a proxy, we therefore aimed to determine whether trophic niche variation could affect the individual future fitness of *Z. asper*.

## 2 | MATERIALS AND METHODS

### 2.1 | Fish and faeces sampling

Five sites were sampled, which represent four of the five remnant *Z. asper* populations (Figure 1). Sampling was performed over one to four campaigns per site in spring, summer, and autumn in 2014 and 2015 (Table S1), in accordance with permits from the French *Directions Départementales des Territoires* (DDTs) from *Hautes-Alpes*, *Alpes-de-Haute-Provence*, *Ardèche* and *Jura*. Fishes were caught by electrofishing and then laid in a plastic, wire mesh fishpond until biometrical measures and faeces collection were performed. Fishes were weighed (precision 0.1 g) and their fork-length (i.e., from the tip of the snout to the fork of the caudal fin) measured (Lf; precision 1 mm; Table S2). The abdomen of *Z. asper* individuals was then pressed by hand to drain out faeces. Faeces were immediately placed in a 2 ml vial containing 96% ethanol and stored at −20°C. Fishes were then released within the sampling area. A total of 967 *Z. asper* individuals were caught, but only 498 provided faeces samples (maximum of one faecal sample per fish per sampling campaign). Lastly, the surface area of each sampling site was measured for each sampling campaign to estimate the population density of *Z. asper*.
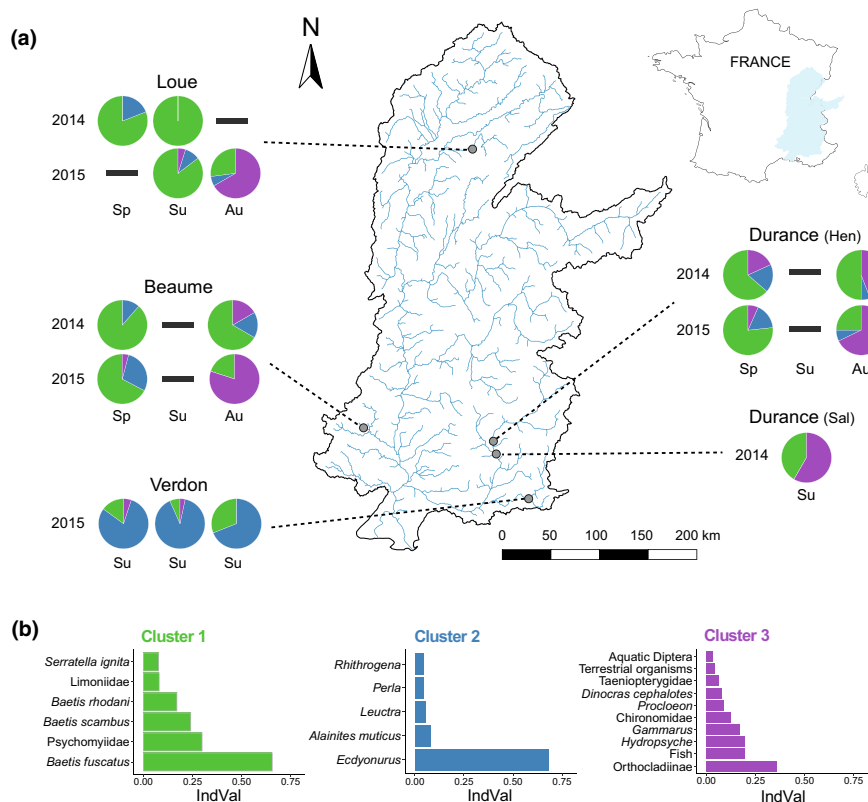
**FIGURE 1** The diets clusters (a) and their indicator species (b) for *Zingel asper* populations in the Rhône River basin. Diet clusters were determined using hierarchical agglomerative clustering based on dissimilarity measurements. The proportion of faeces assigned to clusters is summarized by pie charts. au, autumn; IndVal, indicator value for prey taxa; Su, summer; Sp, spring.

## 2.2 | Diet metabarcoding

Faecal DNA extractions were conducted in a room dedicated to the handling of degraded DNA (*Plateforme ADN Dégradé* of the LabEx CeMEB, Montpellier, France) following the method described by Corse et al. (2017) and the specific safety measures described by Monti et al. (2015). The DNA was extracted from the whole faeces for all individuals. In order to further minimize cross-sample contamination, extraction series were limited to 25 samples, including 21 *Z. asper* faeces, two "alien" faeces (i.e., from marine or non-European continental predators; see: Corse et al., 2019), one negative control for extraction and one negative control for aerosols (for more details on controls, see: Corse et al., 2017, 2019). Additionally, the analyses also included two distinct mock samples communities (described in Corse et al., 2019) as both positive controls and standards across MiSeq runs. Samples and controls were amplified by PCR in triplicates using three primer sets (MFZR, ZFZR and LFCR; Corse et al., 2019) that target ~150 bp overlapping sequences located in the 5′ end of the cytochrome *c* oxidase subunit I gene (COI). Thus, nine separate PCRs were generated per sample/control. The PCR-enrichment step included the one-locus-several-primers (OLSP) strategy developed by Corse et al. (2019), which aims to minimize false negatives in metabarcoding data by using several primer sets that target overlapping but complementary invertebrate taxa. Amplicons were then processed and sequenced on an Illumina MiSeq version 3 platform (as detailed in Corse et al., 2017).

High-throughput sequencing (HTS) data were filtered using an ASV-based procedure (Amplicon Sequence Variant; Corse et al., 2017) recently reimplemented in VTAM (Gonzáles et al., 2020). Several abundance and frequency thresholds were determined from the HTS data of negative and positive controls and from exogenous samples in order to filter out all ASVs that could not be distinguished from Low Frequency Noise (LFN, sensu De Barba et al., 2014), thereby minimizing false positives in faecal samples (i.e., experimental/molecular artefacts such as PCR/sequencing errors, tag-switching and cross-sample contaminations). Filtering thresholds were determined by sequencing run, based on variant occurrence and frequencies (filtering parameters including LFN thresholds are reported in Table S1) and were then applied separately for each run. We further ensured the reproducibility of ASVs by (i) eliminating PCR replicates considered to be too distant compared to the other replicates from the same sample (Renkonnen dissimilarity used), by (ii) retaining only ASVs that were present in at least two replicates, and by (iii) discarding chimeras and pseudogenes. Ultimately, ASVs obtained from the different primer sets that were identical in their overlapping regions (~130 bp) were combined into contigs (further details in: Corse et al., 2017, 2019).

The taxonomic assignment of ASVs/contigs was conducted both automatically using the lowest taxonomic group approach (Corse et al., 2017) and manually using BOLD systems (www.boldsystems.org; Ratnasingham & Hebert, 2007). When necessary (i.e., insufficient assignment level, conflicting results between lowest

taxonomic group approach and BOLD), we built phylogenetic trees and/or integrate biogeographic information to finalize taxonomic assignment. Previous studies have shown that this procedure (which includes three distinct assignment approaches) allows for species-level identification for up to 75% of the ASVs/contigs (Corse et al., 2017, 2019). As *Z. asper* mainly feeds on macroinvertebrates but can also feed on fishes (Cavalli et al., 2003; Corse et al., 2019; Raveret-Wattel, 1900), we considered macroinvertebrates and fishes as relevant prey and collectively referred to them as Macrometazoans (see Table S3). Hence, taxa that most probably result from passive ingestion or secondary predation such as microinvertebrates (e.g., Tardigrada, Rotifera), diatoms, algae, and plants, as well as potential parasites (e.g., Acanthocephala), were excluded from the analyses (for a similar approach, see Hardy et al., 2017).

## 2.3 | Statistical analysis

All statistical analyses and data formatting were performed in R version 3.5.2 (R Core Team, 2020). Prey abundances were estimated using the Minimal Number of Individuals (MNI; White, 1953) approach. MNI is a quantitative statistic that corresponds to the number of distinct ASVs/contigs validated in each sample for a given prey taxon (Corse et al., 2017). As substantial differences in genetic diversity can be observed between invertebrate species (e.g., Weigand et al., 2020), the MNI may overestimate or underestimate prey relative abundance if prey genetic diversity is too variable between taxa. Therefore, we tested the relationship between MNI and prey haplotype diversity using a linear regression model. For the 11 most prevalent taxa in faeces, haplotype diversity was calculated at the genus-level and standardized (at the smallest sample size; $n = 21$) using Contrib version 1.02 (Petit et al., 1998). Lastly, to evaluate a possible ontogenetic effect on the diet of *Z. asper*, we defined four size-classes: young-of-the-year (size-class 1), 63–108 mm (size-class 2), 109–151 mm (size-class 3), and 152–205 mm (size-class 4). Except for the young-of-the-year, the size classes were based on quartiles.

### 2.3.1 | Qualitative trophic variation

The diet of *Z. asper* was first examined qualitatively, both spatially (sampling sites), and temporally (seasons and years) by conducting (i) a principal component analysis based on proportions (pPCA; de Crespin de Billy et al., 2000), performed on the relative abundances (based on MNI) of prey species, and (ii) a hierarchical agglomerative clustering analysis (Ward, 1963). The cluster analysis was performed using the Ward clustering algorithm (function agnes, package cluster; Maechler et al., 2019) based on dissimilarity measurements calculated from Hill number β-diversities ($q = 1$, function pair_dis, package HILLDIV; Alberdi & Gilbert, 2019b). The optimal number of clusters was determined using the Gap-statistic (fviz_nbclust function) from the package NBCLUSTER (Charrad et al., 2014). The diet

clusters were then characterized using Dufrêne-Legendre indicator species analysis (Dufrêne & Legendre, 1997), which calculates an indicator value and significance-level for each prey taxon in the respective clusters (function indval, package LABDSV; Roberts, 2019). Indicator values are highest (IndVal = 1.00) when all individuals of a given taxon are solely found in a single cluster. Lastly, to test for differences in the proportion of diet clusters between different modalities (i.e., seasons, size-class etc.) we performed one-tailed two-proportions *z*-tests.

### 2.3.2 | Estimation of trophic traits

Quantitative dietary niche variation of *Z. asper* was examined for two individual trophic traits: the individual diet niche width (INW; related to α-diversity) and the between individual component of diet niche width (BIC; related to β-diversity). The INW is related to the within-individual component (WIC) of the trophic niche width when using diet data (Bolnick et al., 2002). Both INW and BIC were estimated using "traditional" estimators and using the Hill numbers diversity indices (Hill, 1973). The sensitivity of Hill numbers ($^q D$) to rare prey types can be adjusted with the order of diversity parameter q (Alberdi & Gilbert, 2019b). We calculated Hill numbers at $q = 1$, which includes the relative abundance of prey (in our case, MNI) in the diversity calculation. INW was estimated by the Shannon-Wiener index ($INW_S$) (Shannon, 1948) using the R package vegan (Oksanen et al., 2020), and by Hill numbers $^1D$ ($INW_D$) (see Alberdi & Gilbert, 2019a), using hilldiv (Alberdi & Gilbert, 2019b). BIC was first estimated using the individual specialization index V ($BIC_V$) (Bolnick et al., 2007) which corresponds to 1—proportional similarity index, which measures the diet overlap between an individual and its population (Bolnick et al., 2002). $BIC_V$ was estimated based on the average population diet (pop.diet = "average") using the PSicalc function of the package RInSp (Zaccarelli et al., 2013). Second, BIC was estimated using dissimilarity measurements derived from β-diversities based on the Hill number $^1D$ ($BIC_D$) (see above). $BIC_D$ is the mean dissimilarity between an individual and the other individuals of its population.

In addition to individual trophic traits, we estimated two population-level trophic traits within the Hill number framework. The total niche width (TNW; related to γ-diversity) was calculated for each sampling campaign as $^1D$ using a coverage-based rarefaction approach (Chao & Jost, 2012). In order to standardize the TNW estimates between sampling campaigns with different sample sizes, $^1D$ rarefaction and extrapolation estimates were standardized at 90% sample-coverage using the function EstimateD from the package INEXT (Hsieh et al., 2016). Second, prey turnover was calculated for each sampling campaign using Jaccard-type turnover (see: Alberdi & Gilbert, 2019a) which is calculated from Hill β-diversity values ($q = 1$; function beta_dis, package hilldiv). Jaccard-type turnover quantifies the normalized prey turnover rate (across individuals) with respect to the whole system (in our case: sampling campaign) (Chiu et al., 2014).

### 2.3.3 | A proxy for fitness potential: the body condition

We estimated the body condition of *Z. asper* using the scaled mass index ($\hat{M}_i$), which was calculated using the formula from Peig and Green (2009):

$$\text{Scaled mass index} = M_i \left[ \frac{L_0}{L_i} \right]^{b_{SMA}}$$

where $M_i$ and $L_i$ are the body mass and length, respectively of individual "i". $L_0$ represents an arbitrary length value; here, we used the mean length value of the fishes caught in this study. $b_{SMA}$ is the scaling component calculated by standardized major axis regression of body mass on length (Peig & Green, 2009). In order to be comparable between individuals of different lengths, body condition needs to account for species-specific asymmetric growth patterns, for example, if shorter individuals are relatively heavier than longer individuals or vice versa. A linear regression model was therefore performed to test for a relationship between body condition and body length.

### 2.3.4 | The effect of seasonality, population density and ontogeny on ITV

To quantify the effect of season, ontogeny and *Z. asper* population density on INW and BIC, linear mixed-effects regression models (LMM) were fitted using the LME4 package (Bates et al., 2015). All models were fitted with a Gaussian distribution and the normality of model residuals was determined visually by q-q plot. The $INW_D$ model residuals failed to meet normality, $INW_D$ was therefore log-transformed to meet model assumptions. The best performing model was selected based on conditional Akaike information criterion (cAIC), and in cases of very similar cAIC values (differences <2), the simplest model was selected (function cAIC, package cAIC4; Säfken et al., 2021). *p*-Values were fitted and calculated for all fixed effects in the best performing LMMs using the package lmerTest (Kuznetsova et al., 2017). Model performance was evaluated by conditional $R^2$ ($R^2_C$; variation explained including random effects) while fixed effects performance was evaluated by marginal $R^2$ ($R^2_M$; variation explained excluding random effects; function r.squaredGLMM, package MUMIN; Bartoń, 2019). The respective contribution of each fixed effect to the marginal $R^2$ was determined by type III ANOVA (with Kenward Roger's degrees of freedom corrections). Differences in traits between the factorial fixed effects were determined by post hoc Tukey tests on the best performing models using the emmeans function with Tukey corrections (function emmeans, package EMMEANS; Lenth, 2019).

In order to ensure that our inferences based on LMMs were robust and overcoming potential issues associated with pseudoreplication (due to repeated measurements from each sampling campaign) we included two random effects (Arnqvist, 2020): a sampling campaign random effect nested within a site random effect. Additionally, we used a Bayesian predictive approach, which has been proven to make reliable biological inferences even when samples are pseudoreplicated (Lazic et al., 2020). The Bayesian predictive approach

determines the probability that twogroup means are different by sampling from posterior predictive distributions. All models were fitted with a Gaussian family distribution except for $INW_D$, which followed a lognormal distribution. Bayesian models were performed via the package brms (function brm; Bürkner, 2017) with default priors, four chains, 30,000 iterations and a warm-up of 10,000. Model selection was performed by leave-one-out cross-validation (LOOic) via the package LOO (Vehtari et al., 2017). We assumed our results to be reliable and robust when (i) LMM model coefficients overlapped with Bayesian 95% confidence intervals and (ii) differences between factorial variables were in accordance.

### 2.3.5 | Partitionning the variance of TNW

The total niche width of a given population is described as the sum of WIC and BIC (Bolnick et al., 2003; Roughgarden, 1974). Determining whether the TNW is mainly driven by variation in BIC and/or WIC provides important insights into the biological mechanisms that drive TNW (Sjödin et al., 2018). We therefore isolated the relative contribution of INW (proxy for WIC) and BIC to variation in TNW using relative importance analysis (package RELAIMPO; Grömping, 2006). However, for this type of analysis, the dietary data used to estimate dietary traits should account for a substantial temporal window (MacColl, 2011). If the sampled diet is substantially underestimated due, for instance, to single time-point data and/or limited stomach size, BIC will tend to be overestimated (Bolnick et al., 2007). This can drive artefactual correlations between BIC and TNW. It is therefore necessary to use null models to test whether the observed BIC values are different than what would be expected by random subsampling of the population's diet (Bolnick et al., 2007). We simulated dietary data using a null model that constrained the number of prey consumed by each fish to the number in the observed diet data (as described in Bison et al., 2015). Simulations were calculated based on the average diet of each sampling campaign using the package RinSp, function Null.Hp.RInSp (Zaccarelli et al., 2013). The between-individual component ($BIC_{Dsimulated}$) was then calculated for the simulated diet data using the method described above (Section 2.3.2). In order to test whether an increase in TNW corresponded to a larger increase in BIC than would be predicted under the null model, we compared the slope of (BIC–TNW) between observed and simulated BIC values using linear regression. We added an interaction fixed effect (TNW*data type) to test whether the relationship between BIC and TNW significantly differed from the null model. Lastly, we tested for differences between the simulated and observed BIC values for each sampling campaign, separately using Welch's *t*-tests.

### 2.3.6 | The effect of trophic variation, seasonality, and sampling site on body condition

To evaluate the effect of dietary variation on body condition, LMM and BRM model selection was performed using quantitative trophic niche traits (INW and BIC) and qualitative dietary clusters

(see Section 2.3.1). Models were fitted using "traditional" estimates of INW and BIC, as well as their Hill number equivalents. We also tested for seasonal differences in body condition by fitting LMMs and BRMs, again including sampling site and year as random effects. These models were fitted with a sampling campaign random effect nested within a site random effect. Lastly, we tested for differences in body condition between sampling sites, by performing LMMs and BRMs. For this final model selection procedure, we used a season random effect nested within a year effect. LMMs and BRMs were performed as detailed above (Section 2.3.4).

## 3 | RESULTS

### 3.1 | Metabarcoding data

The raw data set was gathered from 21 distinct MiSeq runs. Once past the filtering procedure, 393 ASVs were validated for MFZR, 447 for ZFZR, and 417 for LFCR. These corresponded to 0.3% of the ASVs initially identified as COI and 70.7% of the reads identified as COI. After combining the MFZR, ZFZR, and LFCR ASVs, 391 contigs and 354 ASVs were obtained. A total of 12 samples were removed after the filtering process as they did not contain any validated ASVs or contigs. None of the negative controls had validated ASVs or contigs. All ASVs expected in mock samples were retrieved. One to two extra ASVs were also validated in most of our mock samples (contig_0238, contig_0124, MFZR_000591; Table S3; see also Corse et al., 2019). A total of 536 distinct Macrometazoan ASVs/contigs (corresponding to 197 prey taxa) were obtained from the 486 Z. asper faaeces containing validated ASVs or contigs. 72% of the prey ASVs/contigs were identified to the species level, another 15% to the genus level, 11% to the family or subfamily level and 2% to the order or class level (Table S3).

### 3.2 | Genetic diversity and MNI

Although haplotype richness varied greatly between prey genera in our data set (Hk = [3–18]), the correlation between MNI and prey haplotype richness was weak ($R^2 = .03$, $p < .001$; Figure S4). This suggests that the differences in genetic diversity between prey only marginally biased the MNI. We therefore assumed the MNI to be a reliable quantitative estimate of prey abundance in the faeces of Z. asper.

### 3.3 | Spatial and seasonal qualitative diet variation

In accordance with previous local-scale studies (Cavalli et al., 2003; Corse et al., 2019), we found that the Z. asper diet was characterized by Ephemeropteran species (Figure S1). Ephemeropteran species constituted 60% of the total prey abundance and occurred in 86% of all faecal samples. Baetidae was the most represented Ephemeropteran family (39% of the total prey abundance), with a

single species (Baetis fuscatus) representing 28% of the total prey abundance and being present in 70% of faecal samples. The pPCA analysis (Figure 2) identified three main prey taxa that characterized spatial and temporal variation in the diet of Z. asper: PC1 was primarily characterized by a gradient of increasing abundance of the Ephemeroptera genus Ecdyonurus (64.0%) while PC2 was mainly characterized by contrasting variation of B. fuscatus (55.3%) versus Orthocladiinae (Diptera) (23.6%). During spring and summer, the diet of Z. asper was primarily composed of Ephemeroptera larvae (B. fuscatus and Ecdyonurus). In autumn, Ephemeroptera consumption declined while the consumption of secondary (e.g., Chironomidae, Hydropsyche) and rare prey (e.g, Plecoptera, fish) markedly increased (Figures S1 and S2). Spatial diet variation was discernible in summer: in the Verdon River the Z. asper diet was dominated by Ecdyonurus, while at Salignac (SAL) on the Durance River, the summer diet more closely resembled the autumn diet of the other sampled locations. However, it should be noted that the Salignac population was almost exclusively composed of young-of-the-year individuals (Table S1).

Hierarchical cluster analysis revealed three distinct diet clusters (Figure 1; Figure S2). The clusters were largely characterized by a single or a few prey taxa (Figure 1): Cluster 1, by B. fuscatus (Indval = 0.66), Cluster 2 by Ecdyonurus (Indval = 0.68) and Cluster 3 by Orthocladiinae, fish and Hydropsyche (Indval = 0.36, 0.20 and 0.20, respectively) as well as several other rare prey. The clustering analysis associated with IndValue analysis indicated that the diet of Z. asper diversified in autumn when compared to spring and summer, this pattern was consistent among the different sampling sites and between the two sampling years (Figure 1). In spring and summer, Cluster 1 (B. fuscatus) was the dominant cluster (except in the Verdon River, where Cluster 2 was dominant). This was supported by two-proportions z-test comparisons, with significantly higher Cluster 1 in spring (0.74) and summer (0.57) compared to in autumn (0.32; $X^2 = 44.45$, df = 1, $p < .001$). In autumn, the diet of Z. asper diversified: prey from Cluster 1 declined, and prey from Cluster 3 (Orthocladiinae and rare prey) proportionately increased. Accordingly, the proportion of Cluster 3 diets in autumn (0.62) was significantly higher than in spring (0.06) and summer (0.06; $X^2 = 183.25$, df = 1, $p < .001$). Moreover, in accordance with the pPCA results, the clustering analysis indicated that the young-of-the-year diets differed from the adult and juvenile individuals. Most young-of-the-year diets were assigned to Cluster 3 (0.63), irrespective of season (Figure S2), while Cluster 3 accounted for only 20% of diets in the other size-classes ($X^2 = 37.08$, df = 1, $p < .001$).

### 3.4 | Individual trophic traits variation in Z. asper

Traditional and Hill number estimates of individual trophic traits were highly correlated (Figure S4). According to LMMs fixed effects alone accounted for 11% (for INW) and 29%–37% (for BIC) of the observed variance. Population density and size-class fixed effects jointly explained the variance of both INW indices (Table 2).
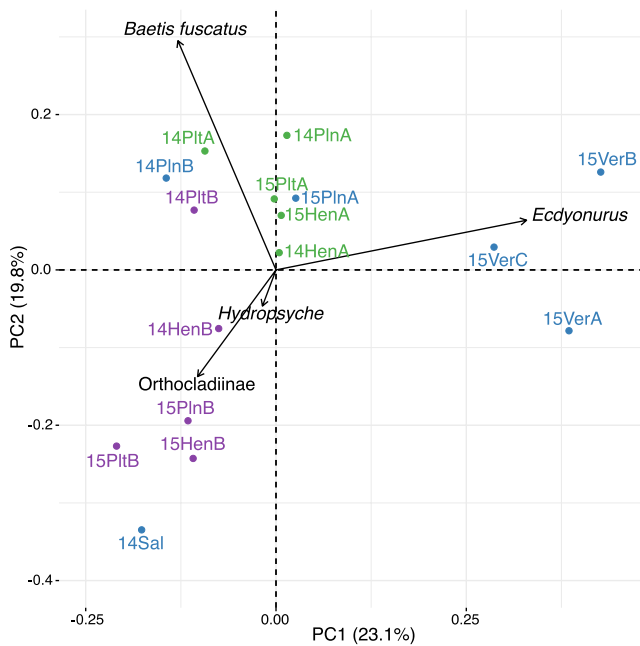
**FIGURE 2** Principal coordinate analysis based on proportions (pPCA) of *Zingel asper* prey consumption inferred from DNA metabarcoding. The proportions of prey taxa are based on the cumulative minimal number of individuals (MNIs). Only the vectors of the four most contributive prey are represented. Each point represents the centroid position of each sampling campaign at each sampling site: Hen and Sal, Durance River; Ver, Verdon River; Plt, Beaume River; Pln, Loue River. Colours correspond to seasons: Green, spring; blue, summer; purple, autumn.

Population density was negatively associated with INW, while INW increased incrementally between size-classes. Sum of square analysis revealed that population density and size-class fixed effects accounted for 4% and 7% of INW variance, respectively (Table S7), and BRM posterior predictive distribution sampling indicated a 60 and a 64% probability that INW was lower in young-of-the-year (size-class 1) individuals compared to size-classes 3 and 4, respectively. LMM and BRM parameter estimates were in accordance. However, contrary to the best LMM, the best performing BRM also included a seasonal effect: BRM estimates indicated that there was respectively a 60% and 59% probability that INW was lower in autumn compared to spring and summer.

BIC indices were predicted by a seasonal fixed effect, which was retained as the sole predictor for both LMM and BRM models (Table 2; Table S7). The seasonal effect was characterized by a significant increase in BIC in autumn compared to both summer and spring. BRM predictions indicated that there was 87% probability that BIC was higher in autumn compared to spring and summer (Figure 4; Table 2). Site and sampling campaign random effects greatly increased the predicted variance for both INW ($R^2_M = .11$ vs. $R^2_C = .20$) and BIC indices (BIC$_V$: $R^2_M = .29$ vs. $R^2_C = .56$; BIC$_D$: $R^2_M = .37$ vs. $R^2_C = .73$). The Bayesian 95% CIs of model coefficients and predictions based on Bayesian predictive inference were in accordance with LMMs for both BIC and INW (Table 2; Table S6).

## 3.5 | Populational trophic traits variation in *Z. asper*

Total niche width varied from 5.9 to 32.2 across sampling campaigns, with minimum values observed in summer, and maximum values in autumn (Table S5; Figure 4). A similar trend was observed in prey turnover, which was generally higher in autumn (from 0.47 to 0.63) compared to spring and summer (from 0.21 to 0.58; see Table S5 for full details). Linear regression models (TNW ~ INW + BIC) revealed that TNW was slightly better explained by Hill number estimates, accounting for 52% of the variation in TNW ($R^2 = .52$, $p < .001$), compared to 46% by traditional estimates ($R^2 = .46$, $p < .001$). In both cases however, between-individual variation accounted for vast majority of TNW variation (BIC$_D = 51%$; BIC$_V = 43%$). While INW had a significant positive effect on TNW according to linear models, it only accounts for a small proportion of the variance of TNW (INW$_D = 1%$; INW$_S = 3%$).

## 3.6 | Comparison between simulated and observed BIC values

Simulated and observed BIC were both positively correlated with TNW values (Figure 5) however, the slope of the BIC$_{observed}$ ~ TNW regression was significantly greater than for the BIC$_{simulated}$ values (TNW*data type interaction, $t = -2.97$, $p = .003$). However, within all sampling campaigns, BIC$_{observed}$ was never significantly higher than BIC$_{simulated}$ (Figure 5).

## 3.7 | Determinants of body condition variation

Linear regression modelling revealed that there was no significant relationship between body condition and body length in *Z. asper* ($t = 1.43$, $p = .155$), indicating that body condition was not biased by the relationship between length and weight in *Z. asper*.

We found no relationship between body condition and trophic niche variation (INW, BIC or dietary cluster) for both traditional indices and Hill number equivalents. Body condition was predicted by season and site effects (Table 3; Figure 3), which respectively accounted for 8% and 12% of variation. The Bayesian 95% CIs of model coefficients and predictions based on Bayesian predictive inference were in accordance with LMMs for body condition (Table 3; Table S6). Bayesian predictive inference indicated a probability of 68% and 61% that body condition was higher in spring compared to autumn and summer, respectively. While the highest body condition was observed at location Plt with a probability of 75%, 66% and 57% that body condition was higher than at the locations Pln, Ver and Hen, respectively.

## 4 | DISCUSSION

By combining nondestructive sampling, a high resolution metabarcoding data set and both modelling and simulation approaches, we

**TABLE 2** Effect of season, ontogeny and intraspecific competition on *Zingel asper*'s individual dietary traits

| Trait | Trait estimate | Fixed effect | LMM Estimate | SE | df | t | p-Value | BRM $R^2_M$–$R^2_C$ | BRM P | Effect summary |
|---|---|---|---|---|---|---|---|---|---|---|
| Individual trophic niche width | $INW_S$ and $INW_D$ | Intercept | 1.75 | 0.33 | 9.09 | 5.27 | <.001 | 0.11–0.020 | 0.98 to 2.76 | |
| | | Population density | −0.23 | 0.09 | 8.72 | −2.60 | .029 | | −0.48 to −0.03 | Negative |
| | | Size-class 2 | 0.13 | 0.11 | 350.96 | 1.14 | .254 | | −0.11 to 0.34 | Positive |
| | | Size-class 3 | 0.26 | 0.10 | 301.50 | 2.64 | .009 | | 0.05 to 0.45 | Positive |
| | | Size-class 4 | 0.26 | 0.10 | 366.98 | 2.64 | .009 | | 0.05 to 0.45 | Positive |
| Between-individual component | $BIC_V$ | Intercept | 0.74 | 0.05 | 5.81 | 15.10 | <.001 | 0.29–0.56 | 0.60 to 0.92 | |
| | | Summer | −0.22 | 0.06 | 9.59 | −3.56 | .006 | | −0.39 to −0.07 | Negative |
| | | Spring | −0.22 | 0.05 | 7.78 | −3.95 | .004 | | −0.34 to −0.09 | Negative |
| | $BIC_D$ | Intercept | 0.81 | 0.62 | 4.86 | 13.11 | <.001 | 0.37–0.73 | 0.63 to 1.05 | |
| | | Summer | −0.27 | 0.07 | 11.66 | −3.62 | .004 | | −0.45 to −0.10 | Negative |
| | | Spring | −0.27 | 0.06 | 7.55 | −4.79 | .002 | | −0.41 to −0.14 | Negative |

Abbreviations: df, degrees of freedom; P, Bayesian posterior distribution of parameter estimates; $R^2_C$, conditional $R^2$; $R^2_M$, marginal $R^2$; SE, standard error; t, t-value.

provided the first fine-scale characterization of dietary variation in the critically endangered *Z. asper*. Moreover, we were able to relate individual trophic trait variation to several ecological drivers, the seasonal driver being the most important and consistent across the species' range. While *Z. asper* adopts largely the same invertivorous diet across its range, like many riverine fishes (e.g., Angermeier, 1982; Sánchez-Hernández et al., 2019; Slack, 1934; Smyly, 1957; Zhao et al., 2014) *Z. asper* modifies its diet between seasons, ontogenic stages and to a lesser extent, spatial scales (in the Verdon River). Furthermore, our results suggest that individual trophic trait variation in *Z. asper* is adaptive.

## 4.1 | Diet variation and plasticity in *Z. asper*

Interestingly, similar patterns of both diet composition and seasonal diet variation were observed across populations despite the fact that *Z. asper* inhabits distinct climatic regions and both non-regulated (Loue and Beaume rivers) and regulated rivers (Durance and Verdon rivers) (Olivier et al., 2022). Especially, *Z. asper* populations share an autumnal diet shift (previously reported in the Durance population; Cavalli et al., 2003) that is associated with a decline in the consumption of its main prey (*B. fuscatus*) and an increase in the consumption of secondary prey (Figures 1, 2 and 3). Qualitative dietary diversification coincided with both a population niche (TNW) expansion and an increase in inter-individual dietary variation (BIC) in most populations (Figures 3 and 4), with between-individual dietary variation (BIC) being the main driver of TNW. According to the prevailing trophic niche theory, BIC rather than individual niche width (INW), is expected to drive trophic niche variation (Araújo et al., 2011; Bolnick et al., 2003; Sjödin et al., 2018). Most empirical studies conclude that BIC variation is driven by interindividual variation in prey preference, microhabitat specialization or individual variation in foraging behaviour (e.g., Endo & Watanabe, 2020; Lewis, 1986; Persson, 1985; Svanbäck & Bolnick, 2005). However, in our case, simulations revealed that the measured BIC in *Z. asper* did not significantly differ from the null hypothesis of random foraging (Figure 5), rejecting the hypothesis of individual specialization. In other words, individuals faced with the same prey community did not differ more than would be expected by chance, and individual diets would be expected to converge over time as more prey taxa are incorporated into their diets (see: Aizpurua et al., 2018; Novak & Tinker, 2015). At a seasonal scale, BIC variation may simply relate to temporal variability in prey encounter rates, which can promote dietary divergence only over short-time periods (Novak & Tinker, 2015). Indeed, the increase of BIC and population diet breadth (TNW) in autumn coincided with both narrower individual niche widths (INW) (Figure 3) and a higher hindgut emptiness (which may be indicative of lower short-term foraging success; Table S1). This suggests that fewer prey captures per individual in autumn contribute to an increase in short-term dietary divergence between individuals.

**TABLE 3** Effect of season and sampling site on body condition of *Zingel asper*. Scaled mass index ($\hat{M}$ i) modelled against season and individual dietary traits (INW and BIC)

| Variable type | Fixed effect | LMM | | | | | | BRM | Effect summary |
|---|---|---|---|---|---|---|---|---|---|
| | | Estimate | SE | df | t | p-Value | $R^2_M$–$R^2_C$ | P | |
| Season | Intercept | 12.85 | 0.32 | 12.45 | 39.69 | <.001 | 0.08–0.28 | 11.66 to 13.79 | |
| | Summer | 0.20 | 0.45 | 12.07 | 0.44 | .665 | | −0.80 to 1.73 | Positive |
| | Spring | 1.00 | 0.45 | 11.93 | 2.22 | .046 | | 0.00 to 2.02 | Positive |
| Sampling site | (Intercept) | 13.43 | 0.42 | 1.72 | 31.79 | .002 | 0.12–0.28 | 11.08 to 15.63 | |
| | Hen | 0.27 | 0.14 | 217.11 | 1.94 | .054 | | 0.00 to 0.56 | Positive |
| | Pln | −0.72 | 0.11 | 406.44 | −6.61 | <.001 | | −0.94 to −0.51 | Negative |
| | Plt | 0.64 | 0.14 | 200.34 | 4.66 | <.001 | | 0.37 to 0.93 | Positive |

Abbreviations: df, degrees of freedom; P, Bayesian posterior distribution of parameter estimates; $R^2_C$, conditional $R^2$; $R^2_M$, marginal $R^2$; SE, standard error; t, t-value.

However, despite temporal (between seasons) and spatial (in the Verdon River) trophic niche variation, body condition in *Z. asper* was not related to either quantitative (e.g., BIC) or qualitative (dietary clusters) dietary variation. In fish, body condition is driven by a variety of biotic and abiotic factors, and food quality, quantity, and availability have proven to be major drivers of body condition (Bojsen, 2005; Pazianoto et al., 2016; Pothoven et al., 2001). Body condition summarizes one part of the health and physiological state of individuals (Cavraro et al., 2019; Stevenson & Woods Jr, 2006) and therefore their potential fitness (Kotiaho, 1999; Wells et al., 2016). Population-level trophic niche expansion is a well-established mechanism in predators to meet energetic requirements (Ivlev, 1961; Perry & Pianka, 1997). In this context, a transient increase in BIC may be related to opportunistic foraging, allowing individuals to acquire sufficient nutrients and maintain their fitness potential when facing changing ecological opportunities.

## 4.2 | Conservation implications for *Z. asper*

Diet studies have proven to be critical for guiding the conservation and management of species and habitats (Nunn et al., 2012; Zarzoso-Lacoste et al., 2019). This study revealed the possible adaptive dietary plasticity of *Z. asper*, that is, its capacity to modify its diet while maintaining comparable body condition across its range and seasons. For instance, fishes in regulated rivers are commonly associated with distinct diets and body condition in relation to conspecifics in nonregulated streams (Alexandre et al., 2015; Corse, Pech, et al., 2015; Luz-Agostinho et al., 2009; Pusey et al., 2010). Flow regulation can alter the natural composition and seasonality of macroinvertebrates (e.g., Brittain & Saltveit, 1989; Cortes et al., 2002; Poff et al., 1997) which can affect seasonal diet variation in some fishes (e.g., Alexandre et al., 2015). In the Durance River, the modification of the natural flow regime has significantly modified the macroinvertebrate community (Cazaubon & Giudicelli, 1999), and both the diet and body condition in some fish species (*Parachondrostoma toxostoma*

and *Chondrostoma nasus*) were shown to be affected by flow regulation (Corse, Pech, et al., 2015). However, the dietary variation of *Z. asper* in the Durance River was comparable to the populations in the unregulated Beaume and Loue rivers, and neither the Durance nor the Verdon populations of *Z. asper* exhibited lower body condition relative to populations in natural flow regimes. To some extent, *Z. asper* may therefore be more resilient than some other fish species to environmental changes that would affect its prey distribution and abundance.

Nevertheless, despite some ubiquitous taxa also occurring in the diet of *Z. asper* (e.g., *Gammarus*, fishes), the diet of *Z. asper* is characterized by a few main prey (*B. fuscatus* and *Ecdyonurus*) and secondary prey (Orthocladiinae, *Hydropsyche*, *Simulium)* which are all rheophilic taxa (Tachet et al., 2020). Our results therefore suggest that high flow velocity habitats (e.g., runs and riffles) and their associated prey are essential for the long-term survival of *Z. asper*. Additionally, the diet of young-of-the-year *Z. asper* was mostly composed of smaller prey like chironomids (Orthocladiinae) (Figure 1). The availability of smaller prey may therefore be important during the first year of growth of *Z. asper* (Monnet et al., 2022) until they can transition to larger, potentially more beneficial prey (i.e., *B. fuscatus* and *Ecdyonurus*). Moreover, chironomids are similarly important for the autumnal diet shift of adults. Consequently, the availability of both its main prey and smaller chironomids as well as of their rheophilic habitats should be accounted in the conservation and management of the critically endangered *Z. asper*, especially for the selection of potential reintroduction sites or for river restoration programmes.

## 4.3 | Advice for future diet studies using metabarcoding data

The metabarcoding approach employed in this study was designed to produce a robust dietary data set. Critically, our metabarcoding approach aimed to limit both type I errors (false negatives), and type II errors (false positives). The choice of PCR primers is determinant
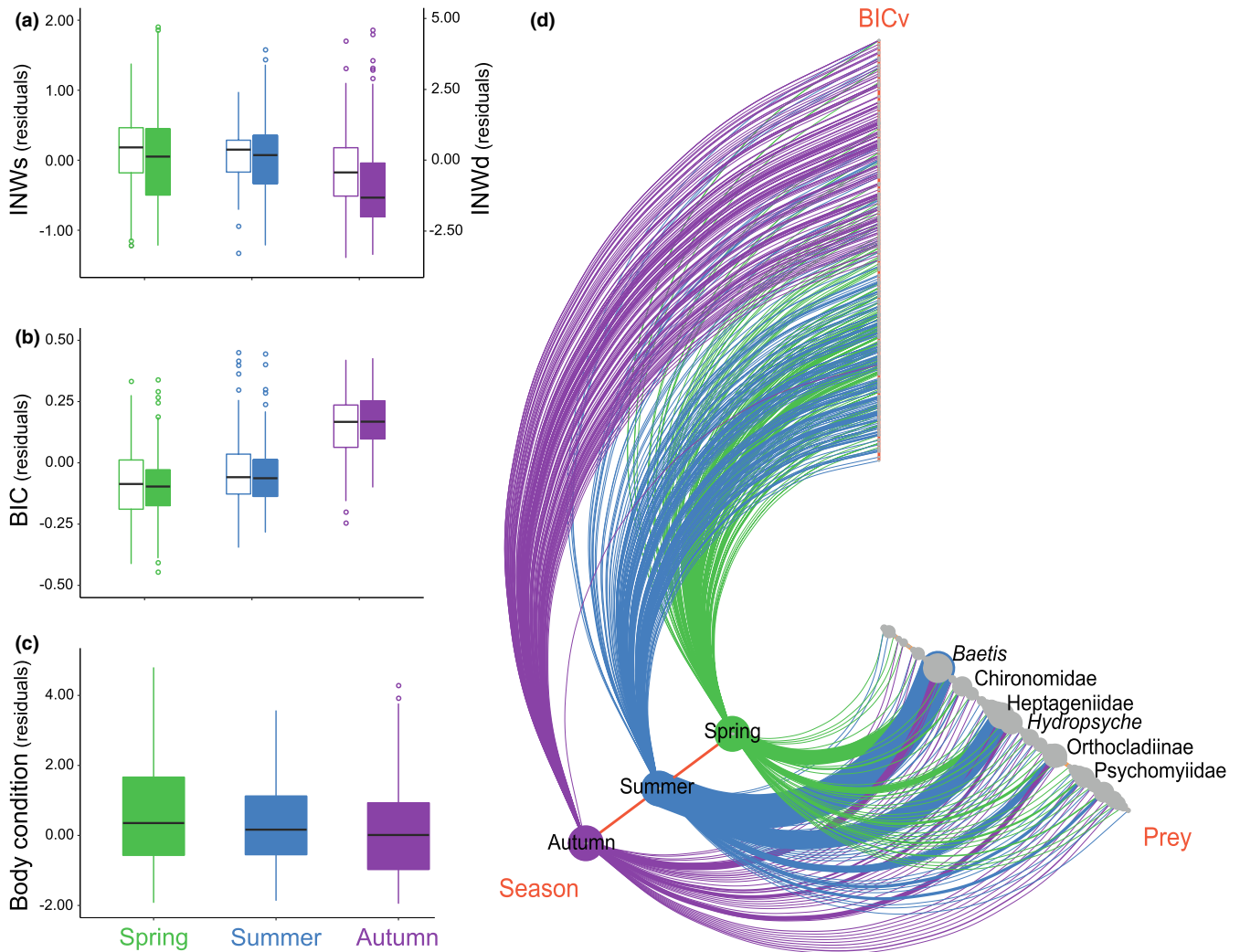
**FIGURE 3** Seasonal variation of INW (a), BIC (b) and body condition (c), and the network (hive plot) of relationships of prey, season and *Zingel asper*'s BIC. Boxplots were produced using residuals extracted from $x \sim$ year + station to account for spatio-temporal variation in INW, BIC and body condition. For INW and BIC, blank boxes correspond to Shannon (INW$_S$) and V indices (BIC$_V$), filled boxes correspond to Hill number indices (INW$_D$ and BIC$_D$). The hive plot was produced using FUNCMAP (Hanson, 2018). BIC, between individual component; INW, individual niche width.

for minimizing false negatives. Indeed, no single primer set can perfectly amplify every species, with the "best" primers covering a maximum of 80% of taxonomic diversity in environmental samples (see: Elbrecht et al., 2019; Tournayre et al., 2020). Consequently, using multiple primer sets together is now recommended to produce both more comprehensive characterizations of diet composition (Aldasoro et al., 2019; Corse et al., 2019; Esnaola et al., 2018) and more accurate estimates of both α- and β-diversity (Browett et al., 2021; Forsman et al., 2022; Hajibabaei et al., 2019).

Furthermore, the choice of metabarcoding data filtering and validation strategies is critical for obtaining robust data and accurate ecological estimates (Drake et al., 2022; Tercel & Cuff, 2022; Zinger et al., 2019). Many authors are in search of nonarbitrary and adequate metabarcoding data filtering strategies to standardize biodiversity analyses (e.g., Antich et al., 2021; Corse et al., 2017; Gonzáles et al., 2020; O'Rourke et al., 2020). Indeed, accurate and exhaustive curation of false positives (e.g., sequencing and PCR

errors, chimeras, internal and external contamination) and ensuring repeatability by using technical replicates has proven essential for producing accurate biodiversity estimates (Calderón-Sanou et al., 2020; Littleford-Colquhoun et al., 2022). Therefore, we recommend using a robust experimental design to produce relevant estimates of diet traits for characterizing individual and population trophic niche variation using metabarcoding data. From our experience, a robust metabarcoding protocol should include (i) several primer sets (three primer sets in our case) to minimize false negatives and to comprehensively cover the taxonomic diversity of prey and (ii) a filtering procedure that explicitly integrates negative controls, positive controls, and technical replicates to minimize false positives, ensure repeatability and validate dietary metabarcoding data within and between high-throughput sequencing runs. Especially when dealing with several distinct high-throughput sequencing runs, mock communities should be used as standards for the filtering parametrization. This should minimize the effect of random fluctuations
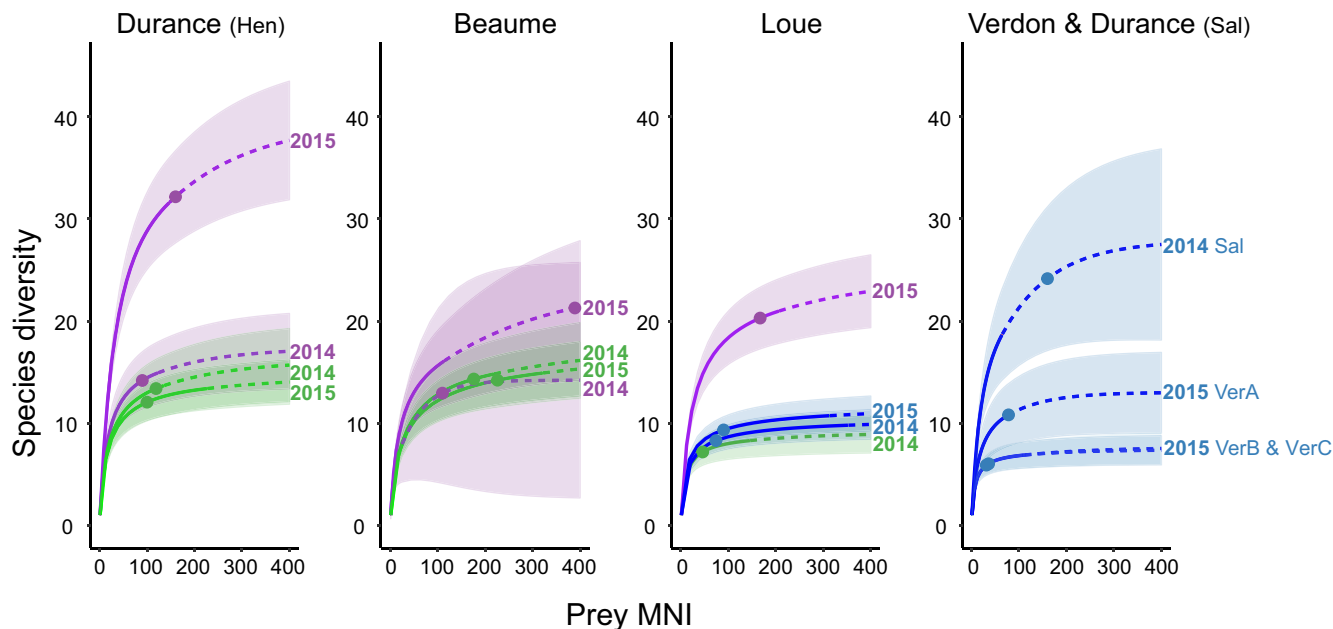
**FIGURE 4** Rarefaction curves of prey in *Zingel asper* and coverage-based estimation of total trophic niche widths (TNW). TNW corresponds to species diversity calculated using Hill numbers ($q = 1$), based on 90% diversity coverage. Colours correspond to seasons: Green, spring; blue, summer; purple, autumn. Line types indicate whether estimates are interpolated (solid) or extrapolated (dashed). Ribbons indicate the 95% confidence intervals.

and make samples comparable between runs (Bakker, 2018; Corse et al., 2019). Also, the metabarcoding protocol should preferably be developed and tested on a subset of faeces before conducting large-scale studies to ensure it is fully adapted to the predator–prey context (Alberdi et al., 2018; Corse et al., 2019).

Additionally, metabarcoding data is often criticized for poorly quantifying abundance (Lamb et al., 2019; but see: Thomas et al., 2016; Vasselon et al., 2018). Here, we employed the minimum number of individuals (MNI), a quantitative estimate widely used in archaeological sciences (Domínguez-Rodrigo, 2012; Lyman, 2018), to provide a conservative estimate of prey abundance based on the number of distinct variants detected per prey species (Corse et al., 2017). However, this estimate may be sensitive to false positives and to variation in the genetic diversity of prey taxa (the abundance of taxa that exhibit high genetic diversity may be overestimated). Future studies that adopt the MNI approach should therefore (i) implement a robust ASV-based filtering protocol to minimize false positives and (ii) verify that the MNI is not biased by the genetic diversity of prey (Figure S4).

Individual-based approaches are commonly used in ecology to understand how population-level patterns emerge from interactions between individuals and their environment (DeAngelis & Grimm, 2014; Durrett & Levin, 1994). In trophic ecology, individual-based modelling has proven to be essential to characterize the patterns and drivers of intraspecific diet variation (Bolnick et al., 2011) but remains rare for the analysis of metabarcoding data sets (but see: Bison et al., 2015; Pansu et al., 2019; Pornon et al., 2019). However, in ecology, and especially in the case of endangered species, the number of locations that can be studied are most often limited, experimental units (here, locations) will inevitably be the object of

multiple interdependent measurements, leading to pseudoreplication in the data (Hurlbert & White, 1993). In this context, linear mixed models (LMMs) should be correctly fitted with random effects and interpreted with caution (Arnqvist, 2020). Alternatively, Bayesian predictive approaches have been recently proposed to make valid inferences even when there are pseudoreplicates (Lazic et al., 2020). Here, we used the congruence between both LMMs and Bayesian approaches as a cross-validation to support the accuracy of our conclusions about the variation of individual trophic traits in *Z. asper*.

Finally, while simulations can also be used to test the impact of pseudoreplication on ecological conclusions (e.g., Zuckerberg et al., 2020), they have previously been employed to test the significance of between-individual component (BIC) as the main driver of population diet breadth (Bison et al., 2015; Bolnick et al., 2002). In this study, simulations allow us to reject the hypothesis that higher BIC in autumn reflected specialization between individuals. Rather, simulations indicated that high BIC in autumn probably arose due to an increase in stochastic diet variation in *Z. asper*, suggesting a seasonal increase of dietary opportunism when facing changing ecological opportunities.

In summary, we would advise future diet metabarcoding to combine: (i) a thorough experimental design, including temporal replicates, (ii) the production of a robust metabarcoding data set, (iii) the use of standardized estimates (derived from Hill numbers), (iv) appropriately structured individual-based LMMs and Bayesian predictive approaches to deal with potential pseudoreplication, and (v) simulations in order to test null hypotheses. In our case, this combination proved to be sufficiently robust to provide fine-scale information about the trophic ecology of *Z. asper* and offered valuable
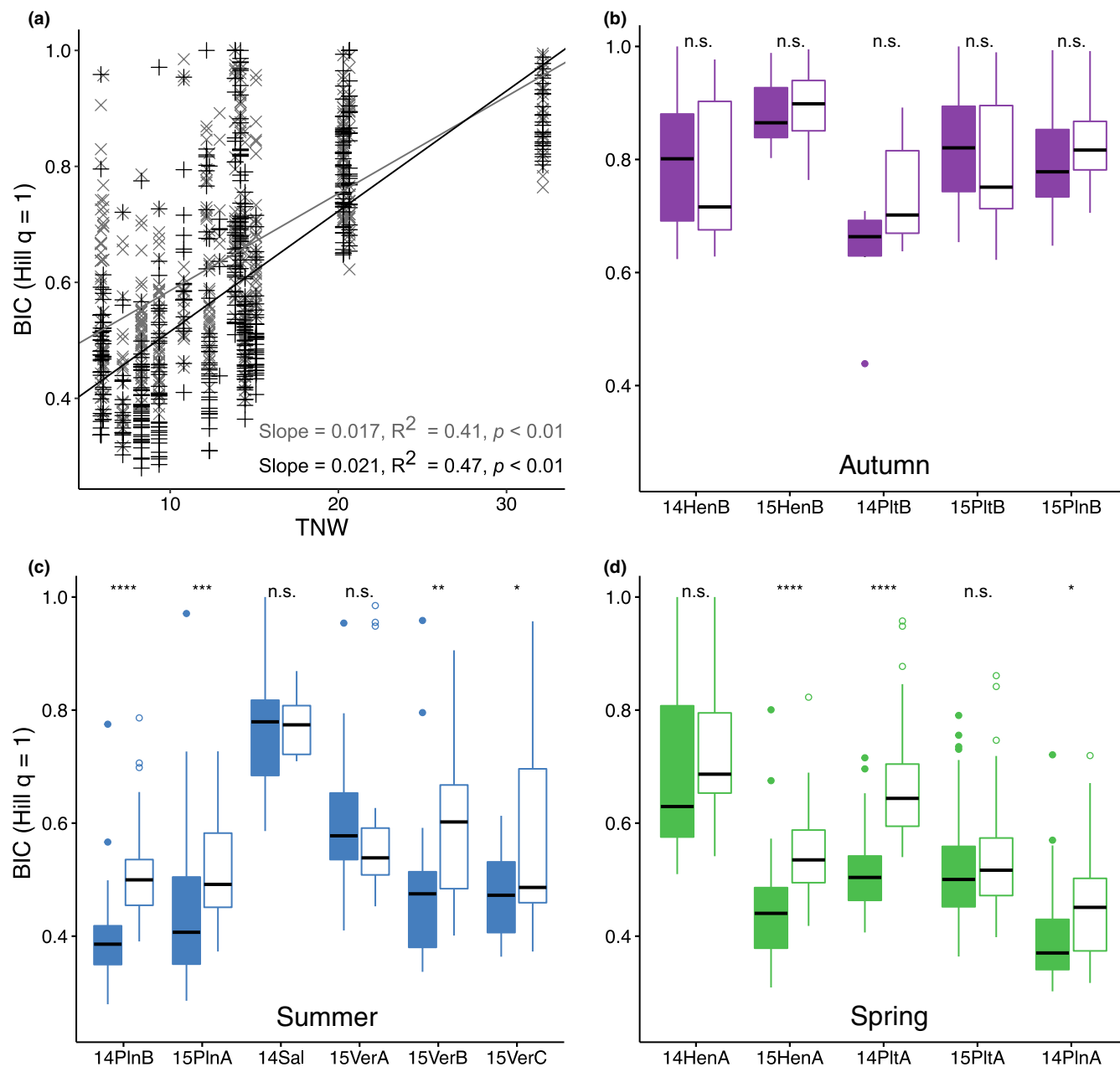
**FIGURE 5** Observed and simulated (based on a null model) $BIC_D$ values in *Zingel asper*. (a) $BIC_D \sim TNW$ relationship between simulated (grey) and observed $BIC_D$ values (black). Black and grey lines figure linear regression slopes. (b–d) within campaigns distributions of observed $BIC_D$ values (filled boxes) and simulated $BIC_D$ values (blank boxes) by season. $BIC_D$, between individual component measured from Hill number; TNW, total trophic niche width. \*\*\*$p < .001$; n.s., not significant

perspectives for its conservation. We believe that it may be fully applied to any invertivorous vertebrate to respond to trophic niche-related research questions. Our methodology would be particularly effective for studying endangered predators: the collection of faeces being nondestructive, it allows for larger sample sizes.

## 4.4 | Perspectives

To date, most studies that investigate individual trophic traits variation have focused on species with large fundamental niches and/or

multiple foraging strategies (e.g., Costa-Pereira et al., 2019; Sánchez-Hernández et al., 2021; Vrede et al., 2011), and the biotic and, to a lesser extent, abiotic drivers of individual trophic trait variation are now relatively well understood in these species (Araújo et al., 2011). Future studies should aim to determine the underlying drivers of individual trophic niche variation in species with a more constrained trophic niche, such as *Z. asper*.

Our study improved the understanding of how *Z. asper* varies its trophic niche across its range and thus provides valuable information for its conservation. Especially, though unrelated to individual specialization, transient variation in BIC appears to be linked to the

maintenance of body condition in *Z. asper*. Transient individual trophic trait variation is a foraging response often related to fluctuations in ecological opportunity (e.g., Brooke McEachern et al., 2006; Matich et al., 2011; Woo et al., 2008), in particular, BIC has been shown to increase as preferred prey declines (Pires et al., 2013; Tinker et al., 2008). In fact, seasonal dietary variation in *Z. asper* has been previously shown to be driven by the seasonal decline in the abundance of its main prey (*B. fuscatus* and *Ecdyonurus*) in the Durance River (Cavalli et al., 2003). Furthermore, seasonal variation in BIC was related with signs of resource depression in autumn (i.e., narrower INW and higher hindgut emptiness). We therefore hypothesize that the diet shift observed in the Beaume and Loue *Z. asper* populations are probably driven by a similar seasonal depletion of main prey taxa. Determining the exact biotic drivers of the adaptive trophic niche variation highlighted in this study should guide future studies. This will be achieved only with the acquisition of the biotic (e.g., prey abundance, prey diversity) and even abiotic (e.g., water temperature, substrate granulometry) data for conducting mechanistic analyses and modelling. Riverine fishes are among the most endangered species in the world (Olden et al., 2010). Combining diet metabarcoding, prey and habitat data to conduct causal analyses would yield further critical insights into the trophic ecology of endangered fish species and the potential threats facing them.

## AUTHOR CONTRIBUTIONS

Vincent Dubut and Emmanuel Corse conceived and designed the study. Emmanuel Corse, Vincent Dubut, Gaït Archambaud-Suard and Rémi Chappaz conducted the fieldwork. Emmanuel Corse, Emese Meglécz and Hélène Vignes did the molecular work and/or bioinformatics. Kurt Villsen performed statistical analyses, with contributions from Emmanuel Corse and Vincent Dubut. Kurt Villsen and Vincent Dubut wrote the original draft, and all authors contributed to further writing and editing.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

## DATA AVAILABILITY STATEMENT

Supplementary data deposited in Dryad (https://doi.org/10.5061/dryad.2ck7120) including: (i) unfiltered HTS data; (ii) the sample/tag combination correspondences; (iii) the filtered and validated sequence data, and the taxonomic assignment of prey detected in Z. asper's feces; (iv) the final diet dataset used to estimate individual trophic traits; and (iv) the dataset used for statistical analysis and modelling process.

## ORCID

*Emmanuel Corse* https://orcid.org/0000-0003-0992-726X
*Emese Meglécz* https://orcid.org/0000-0001-7547-4858
*Gaït Archambaud-Suard* https://orcid.org/0000-0001-9493-2279
*Alexander V. Ereskovsky* https://orcid.org/0000-0003-1079-7204
*Vincent Dubut* https://orcid.org/0000-0002-5619-6909

## REFERENCES

Aizpurua, O., Budinski, I., Georgiakakis, P., Gopalakrishnan, S., Ibañez, C., Mata, V., Rebelo, H., Russo, D., Szodoray-Parádi, F., Zhelyazkova, V., Zrncic, V., MTP, G., & Alberdi, A. (2018). Agriculture shapes the trophic niche of a bat preying on multiple pest arthropods across Europe: Evidence from DNA metabarcoding. *Molecular Ecology*, 27(3), 815–825. https://doi.org/10.1111/mec.14474

Alberdi, A., Aizpurua, O., Bohmann, K., Gopalakrishnan, S., Lynggaard, C., Nielsen, M., & Gilbert, M. T. P. (2019). Promises and pitfalls of using high-throughput sequencing for diet analysis. *Molecular Ecology Resources*, 19(2), 327–348. https://doi.org/10.1111/1755-0998.12960

Alberdi, A., Aizpurua, O., Gilbert, M. T. P., & Bohmann, K. (2018). Scrutinizing key steps for reliable metabarcoding of environmental samples. *Methods in Ecology and Evolution*, 9(1), 134–147. https://doi.org/10.1111/2041-210X.12849

Alberdi, A., & Gilbert, M. T. P. (2019a). A guide to the application of Hill numbers to DNA-based diversity analyses. *Molecular Ecology Resources*, 19(4), 804–817. https://doi.org/10.1111/1755-0998.13014

Alberdi, A., & Gilbert, M. T. P. (2019b). Hilldiv: An R package for the integral analysis of diversity based on Hill numbers. *BioRxiv*, 545665. https://doi.org/10.1101/545665

Albert, C. H., Grassein, F., Schurr, F. M., Vieilledent, G., & Violle, C. (2011). When and how should intraspecific variability be considered in trait-based plant ecology? *Perspectives in Plant Ecology, Evolution and Systematics*, 13(3), 217–225. https://doi.org/10.1016/j.ppees.2011.04.003

Aldasoro, M., Garin, I., Vallejo, N., Baroja, U., Arrizabalaga-Escudero, A., Goiti, U., & Aihartza, J. (2019). Gaining ecological insight on dietary allocation among horseshoe bats through molecular primer combination. *PLoS One*, *14*(7), e0220081. https://doi.org/10.1371/journal.pone.0220081

Alexandre, C. M., Sales, S., Ferreira, M. T., & Almeida, P. R. (2015). Food resources and cyprinid diet in permanent and temporary Mediterranean rivers with natural and regulated flow. *Ecology of Freshwater Fish*, *24*(4), 629–645. https://doi.org/10.1111/eff.12176

Angermeier, P. L. (1982). Resource seasonality and fish diets in an Illinois stream. *Environmental Biology of Fishes*, *7*(3), 251–264. https://doi.org/10.1007/BF00002500

Antich, A., Palacin, C., Wangensteen, O. S., & Turon, X. (2021). To denoise or to cluster, that is not the question: Optimizing pipelines for COI metabarcoding and metaphylogeography. *BMC Bioinformatics*, *22*(1), 1–24.

Araújo, M. S., Bolnick, D. I., & Layman, C. A. (2011). The ecological causes of individual specialisation. *Ecology Letters*, *14*(9), 948–958. https://doi.org/10.1111/j.1461-0248.2011.01662.x

Arnqvist, G. (2020). Mixed models offer no freedom from degrees of freedom. *Trends in Ecology & Evolution*, *35*(4), 329–335. https://doi.org/10.1016/j.tree.2019.12.004

Arscott, D. B., Tockner, K., & Ward, J. V. (2003). Spatio-temporal patterns of benthic invertebrates along the continuum of a braided alpine river. *Archiv für Hydrobiologie*, *158*(4), 431–460. https://doi.org/10.1127/0003-9136/2003/0158-0431

Bakker, M. G. (2018). A fungal mock community control for amplicon sequencing experiments. *Molecular Ecology Resources*, *18*(3), 541–556. https://doi.org/10.1111/1755-0998.12760

Bartoń, K. (2019). *MuMIn: R package for model selection and multi-model inference.* R Package Version 1.43.4. https://mumin.r-forge.r-project.org

Bates, D., Mächler, M., Bolker, B., & Walker, S. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1), 1–48. https://doi.org/10.18637/jss.v067.i01

Berry, O., Bulman, C., Bunce, M., Coghlan, M., Murray, D. C., & Ward, R. D. (2015). Comparison of morphological and DNA metabarcoding analyses of diets in exploited marine fishes. *Marine Ecology Progress Series*, *540*, 167–181. https://doi.org/10.3354/meps11524

Bison, M., Ibanez, S., Redjadj, C., Boyer, F., Coissac, E., Miquel, C., Rioux, D., Said, S., Maillard, D., Taberlet, P., Yoccoz, N. G., & Loison, A. (2015). Upscaling the niche variation hypothesis from the intra- to the inter-specific level. *Oecologia*, *179*(3), 835–842. https://doi.org/10.1007/s00442-015-3390-7

Bojsen, B. H. (2005). Diet and condition of three fish species (Characidae) of the Andean foothills in relation to deforestation. *Environmental Biology of Fishes*, *73*(1), 61–73. https://doi.org/10.1007/s10641-004-5330-y

Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., Rudolf, V. H., Schreiber, S. J., Urban, M. C., & Vasseur, D. A. (2011). Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution*, *26*(4), 183–192. https://doi.org/10.1016/j.tree.2011.01.009

Bolnick, D. I., Ingram, T., Stutz, W. E., Snowberg, L. K., Lau, O. L., & Pauli, J. S. (2010). Ecological release from interspecific competition leads to decoupled changes in population and individual niche width. *Proceedings of the Royal Society B: Biological Sciences*, *277*(1689), 1789–1797. https://doi.org/10.1098/rspb.2010.0018

Bolnick, D. I., Svanbäck, R., Araújo, M. S., & Persson, L. (2007). Comparative support for the niche variation hypothesis that more generalized populations also are more heterogeneous. *Proceedings of the National Academy of Sciences of the United States of America*, *104*(24), 10075–10079. https://doi.org/10.1073/pnas.0703743104

Bolnick, D. I., Svanbäck, R., Fordyce, J. A., Yang, L. H., Davis, J. M., Hulsey, C. D., & Forister, M. L. (2003). The ecology of individuals: Incidence and implications of individual specialization. *American Naturalist*, *161*(1), 1–28. https://doi.org/10.1086/343878

Bolnick, D. I., Yang, L. H., Fordyce, J. A., Davis, J. M., & Svanbäck, R. (2002). Measuring individual-level resource specialization. *Ecology*, *83*(10), 2936–2941. https://doi.org/10.1890/0012-9658(2002)083[2936:MILRS]2.0.CO;2

Bravard, J.-P., & Gaydou, P. (2015). Historical development and integrated management of the Rhône River floodplain, from the Alps to the Camargue Delta, France. In P. F. Hudson & H. Middelkoop (Eds.), *Geomorphic approaches to integrated floodplain management of lowland fluvial systems in North America and Europe* (pp. 289–320). Springer.

Brittain, J. E., & Saltveit, S. J. (1989). A review of the effect of river regulation on mayflies (Ephemeroptera). *Regulated Rivers: Research & Management*, *3*(1), 191–204. https://doi.org/10.1002/rrr.3450030119

Brooke McEachern, M., A. Eagles-Smith, C., M. Efferson, C., & H. Van Vuren, D. (2006). Evidence for local specialization in a generalist mammalian herbivore, *Neotoma fuscipes*. *Oikos*, *113*(3), 440–448. https://doi.org/10.1111/j.2006.0030-1299.14176.x

Browett, S. S., Curran, T. G., O'Meara, D. B., Harrington, A. P., Sales, N. G., Antwis, R. E., O'Neill, D., & McDevitt, A. D. (2021). Primer biases in the molecular assessment of diet in multiple insectivorous mammals. *Mammalian Biology*, *101*(3), 293–304. https://doi.org/10.1007/s42991-021-00115-4

Brown, D. S., Burger, R., Cole, N., Vencatasamy, D., Clare, E. L., Montazam, A., & Symondson, W. O. C. (2014). Dietary competition between the alien Asian musk shrew (*Suncus murinus*) and a re-introduced population of Telfair's skink (*Leiolopisma telfairii*). *Molecular Ecology*, *23*(15), 3695–3705. https://doi.org/10.1111/mec.12445

Bürkner, P.-C. (2017). brms: An R package for Bayesian multilevel models using Stan. *Journal of Statistical Software*, *80*(1), 1–28. https://doi.org/10.18637/jss.v080.i01

Calderón-Sanou, I., Münkemüller, T., Boyer, F., Zinger, L., & Thuiller, W. (2020). From environmental DNA sequences to ecological conclusions: How strong is the influence of methodological choices? *Journal of Biogeography*, *47*(1), 193–206. https://doi.org/10.1111/jbi.13681

Cavalli, L., Knight, C. M., Durbec, M., Chappaz, R., & Gozlan, R. E. (2009). Twenty-four hours in the life of *Zingel asper*. *Journal of Fish Biology*, *75*(3), 723–727. https://doi.org/10.1111/j.1095-8649.2009.02315.x

Cavalli, L., Pech, N., & Chappaz, R. (2003). Diet and growth of the endangered *Zingel asper* in the Durance River. *Journal of Fish Biology*, *63*(2), 460–471. https://doi.org/10.1046/j.1095-8649.2003.00168.x

Cavraro, F., Bettoso, N., Zucchetta, M., D'Aietti, A., Faresi, L., & Franzoi, P. (2019). Body condition in fish as a tool to detect the effects of anthropogenic pressures in transitional waters. *Aquatic Ecology*, *53*(1), 21–35. https://doi.org/10.1007/s10452-018-09670-4

Cazaubon, A., & Giudicelli, J. (1999). Impact of the residual flow on the physical characteristics and benthic community (algae, invertebrates) of a regulated Mediterranean river: The durance, France. *Regulated Rivers: Research & Management*, *15*(5), 441–461. https://doi.org/10.1002/(SICI)1099-1646(199909/10)15:5<441::AID-RRR558>3.0.CO;2-9

Chao, A., & Jost, L. (2012). Coverage-based rarefaction and extrapolation: Standardizing samples by completeness rather than size. *Ecology*, *93*(12), 2533–2547. https://doi.org/10.1890/11-1952.1

Charrad, M., Ghazzali, N., Boiteau, V., & Niknafs, A. (2014). NbClust: An R package for determining the relevant number of clusters in a data set. *Journal of Statistical Software*, *61*(6), 1–36. https://doi.org/10.18637/jss.v061.i0

Chiu, C. H., Jost, L., & Chao, A. (2014). Phylogenetic beta diversity, similarity, and differentiation measures based on Hill numbers. *Ecological Monographs*, *84*(1), 21–44. https://doi.org/10.1890/12-0960.1

Clare, E. L. (2014). Molecular detection of trophic interactions: Emerging trends, distinct advantages, significant considerations and conservation applications. *Evolutionary Applications*, *7*(9), 1144–1157. https://doi.org/10.1111/eva.12225

Clifford, H. (1982). Life cycles of mayflies (Ephemeroptera), with special reference to voltinism. *Quaestiones Entomologicae*, *18*(1), 15–90.

Conover, D. O. (1992). Seasonality and the scheduling of life history at different latitudes. *Journal of Fish Biology*, *41*, 161–178. https://doi.org/10.1111/j.1095-8649.1992.tb03876.x

Corbet, P. S., Suhling, F., & Soendgerath, D. (2006). Voltinism of Odonata: A review. *International Journal of Odonatology*, *9*(1), 1–44. https://doi.org/10.1080/13887890.2006.9748261

Corse, E., Meglécz, E., Archambaud, G., Ardisson, M., Martin, J.-F., Tougard, C., Chappaz, R., & Dubut, V. (2017). A from-benchtop-to-desktop workflow for validating HTS data and for taxonomic identification in diet metabarcoding studies. *Molecular Ecology Resources*, *17*(6), e146–e159. https://doi.org/10.1111/1755-0998.12703

Corse, E., Pech, N., Sinama, M., Costedoat, C., Chappaz, R., & Gilles, A. (2015). When anthropogenic river disturbance decreases hybridisation between non-native and endemic cyprinids and drives an ecomorphological displacement towards juvenile state in both species. *PLoS One*, *10*(11), e0142592. https://doi.org/10.1371/journal.pone.0142592

Corse, E., Tougard, C., Archambaud-Suard, G., Agnèse, J. F., Messu Mandeng, F. D., Bilong Bilong, C. F., Duneau, D., Zinger, L., Chappaz, R., Xu, C. C. Y., Meglécz, E., & Dubut, V. (2019). One-locus-several-primers: A strategy to improve the taxonomic and haplotypic coverage in diet metabarcoding studies. *Ecology and Evolution*, *9*(8), 4603–4620. https://doi.org/10.1002/ece3.5063

Corse, E., Valladares, S., Planas, M., Chamorro, A., & Pintado, J. (2015). Analysis of the diet of the long-snouted seahorse *Hippocampus guttulatus* by 18SrDNA amplification of prey in faeces. *Aquaculture Nutrition*, *21*(5), 528–540. https://doi.org/10.1111/anu.12189

Cortes, R. M. V., Ferreira, M. T., Oliveira, S. V., & Oliveira, D. (2002). Macroinvertebrate community structure in a regulated river segment with different flow conditions. *River Research and Applications*, *18*(4), 367–382. https://doi.org/10.1002/rra.679

Costa-Pereira, R., Araújo, M. S., Souza, F. L., & Ingram, T. (2019). Competition and resource breadth shape niche variation and overlap in multiple trophic dimensions. *Proceedings of the Royal Society B: Biological Sciences*, *286*(1902), 20190369. https://doi.org/10.1098/rspb.2019.0369

Costa-Pereira, R., Tavares, L. E. R., de Camargo, P. B., & Araújo, M. S. (2017). Seasonal population and individual niche dynamics in a tetra fish in the Pantanal wetlands. *Biotropica*, *49*(4), 531–538. https://doi.org/10.1111/btp.12434

Crivelli, A. J. (2006). *Zingel asper. The IUCN Red List of Threatened Species 2006: e.T23207A9427850*. https://doi.org/10.2305/IUCN.UK.2006.RLTS.T23207A9427850.en

Cross, W. F., Wallace, J. B., Rosemond, A. D., & Eggert, S. L. (2006). Whole-system nutrient enrichment increases secondary production in a detritus-based ecosystem. *Ecology*, *87*(6), 1556–1565. https://doi.org/10.1890/0012-9658(2006)87[1556:WNEISP]2.0.CO;2

de Crespin de Billy, V., Doledec, S., & Chessel, D. (2000). Biplot presentation of diet composition data: an alternative for fish stomach contents analysis. *Journal of Fish Biology*, *56*(4), 961–973. https://doi.org/10.1111/j.1095-8649.2000.tb00885.x

Danancher, D., Labonne, J., Gaudin, P., & Joly, P. (2007). Scale measurements as a conservation tool in endangered *Zingel asper* (Linnaeus, 1758). *Aquatic Conservation: Marine and Freshwater Ecosystems*, *17*(7), 712–723. https://doi.org/10.1002/aqc.810

Dawson, S. K., Carmona, C. P., González-Suárez, M., Jönsson, M., Chichorro, F., Mallen-Cooper, M., Melero, Y., Moor, H., Simaika, J. P., & Duthie, A. B. (2021). The traits of "trait ecologists": An analysis of the use of trait and functional trait terminology. *Ecology and Evolution*, *11*(23), 16434–16445. https://doi.org/10.1002/ece3.8321

De Barba, M., Miquel, C., Boyer, F., Mercier, C., Rioux, D., Coissac, E., & Taberlet, P. (2014). DNA metabarcoding multiplexing and validation of data accuracy for diet assessment: Application to omnivorous diet. *Molecular Ecology Resources*, *14*(2), 306–323. https://doi.org/10.1111/1755-0998.12188

de Sousa, L. L., Silva, S. M., & Xavier, R. (2019). DNA metabarcoding in diet studies: Unveiling ecological aspects in aquatic and terrestrial ecosystems. *Environmental DNA*, *1*(3), 199–214. https://doi.org/10.1002/edn3.27

DeAngelis, D. L., & Grimm, V. (2014). Individual-based models in ecology after four decades. *F1000Prime Reports*, *6*, 39. https://doi.org/10.12703/P6-39

Des Roches, S., Post, D. M., Turley, N. E., Bailey, J. K., Hendry, A. P., Kinnison, M. T., Schweitzer, J. A., & Palkovacs, E. P. (2018). The ecological importance of intraspecific variation. *Nature Ecology and Evolution*, *2*(1), 57–64. https://doi.org/10.1038/s41559-017-0402-5

Domínguez-Rodrigo, M. (2012). Critical review of the MNI (minimum number of individuals) as a zooarchaeological unit of quantification. *Archaeological and Anthropological Sciences*, *4*(1), 47–59. https://doi.org/10.1007/s12520-011-0082-z

Downes, B. J., Lake, P. S., Schreiber, E. S. G., & Glaister, A. (2000). Habitat structure, resources and diversity: The separate effects of surface roughness and macroalgae on stream invertebrates. *Oecologia*, *123*(4), 569–581. https://doi.org/10.1007/PL00008862

Drake, L. E., Cuff, J. P., Young, R. E., Marchbank, A., Chadwick, E. A., & Symondson, W. O. (2022). An assessment of minimum sequence copy thresholds for identifying and reducing the prevalence of artefacts in dietary metabarcoding data. *Methods in Ecology and Evolution*, *13*(3), 694–710. https://doi.org/10.1111/2041-210X.13780

Dufrêne, M., & Legendre, P. (1997). Species assemblages and indicator species: The need for a flexible asymmetrical approach. *Ecological Monographs*, *67*(3), 345–366. https://doi.org/10.2307/2963459

Durant, J. M., Hjermann, D., Anker-Nilssen, T., Beaugrand, G., Mysterud, A., Pettorelli, N., & Stenseth, N. C. (2005). Timing and abundance as key mechanisms affecting trophic interactions in variable environments. *Ecology Letters*, *8*(9), 952–958. https://doi.org/10.1111/j.1461-0248.2005.00798.x

Durrett, R., & Levin, S. (1994). The importance of being discrete (and spatial). *Theoretical Population Biology*, *46*(3), 363–394. https://doi.org/10.1006/tpbi.1994.1032

Egeter, B., Bishop, P. J., & Robertson, B. C. (2015). Detecting frogs as prey in the diets of introduced mammals: A comparison between morphological and DNA-based diet analyses. *Molecular Ecology Resources*, *15*(2), 306–316. https://doi.org/10.1111/1755-0998.12309

Elbrecht, V., Braukmann, T. W. A., Ivanova, N. V., Prosser, S. W. J., Hajibabaei, M., Wright, M., Zakharov, E. V., Hebert, P. D. N., & Steinke, D. (2019). Validation of COI metabarcoding primers for terrestrial arthropods. *PeerJ*, *7*, e7745. https://doi.org/10.7717/peerj.7745

Endo, C., & Watanabe, K. (2020). Morphological variation associated with trophic niche expansion within a lake population of a benthic fish. *PLoS One*, *15*(4), e0232114. https://doi.org/10.1371/journal.pone.0232114

Erba, S., Melissano, L., & Buffagni, A. (2003). Life cycle of Baetidae (Ephemeroptera) in a north Italian prealpine stream. In E. Gaino (Ed.), *Research update on Ephemeroptera & Plecoptera* (pp. 177–176). University of Perugia.

Esnaola, A., Arrizabalaga-Escudero, A., González-Esteban, J., Elosegi, A., & Aihartza, J. (2018). Determining diet from faeces: Selection

of metabarcoding primers for the insectivore Pyrenean desman (*Galemys pyrenaicus*). *PLoS One*, *13*(12), e0208986. https://doi.org/10.1371/journal.pone.0208986

Evangelista, C., Boiche, A., Lecerf, A., & Cucherousset, J. (2014). Ecological opportunities and intraspecific competition alter trophic niche specialization in an opportunistic stream predator. *Journal of Animal Ecology*, *83*(5), 1025–1034. https://doi.org/10.1111/1365-2656.12208

Falke, L. P., Henderson, J. S., Novak, M., & Preston, D. L. (2020). Temporal shifts in intraspecific and interspecific diet variation among 3 stream predators. *Freshwater Science*, *39*(1), 115–125. https://doi.org/10.1086/707599

Forsman, A. M., Hoenig, B. D., Gaspar, S. A., Fischer, J. D., Siegrist, J., & Fraser, K. (2022). Evaluating the impacts of metabarcoding primer selection on DNA characterization of diet in an aerial insectivore, the purple Martin. *Ornithology*, *139*(1), ukab075. https://doi.org/10.1093/ornithology/ukab075

Gafner, K., & Robinson, C. T. (2007). Nutrient enrichment influences the responses of stream macroinvertebrates to disturbance. *Journal of the North American Benthological Society*, *26*(1), 92–102. https://doi.org/10.1899/0887-3593(2007)26[92:NEITRO]2.0.CO;2

García, P. E., & Añón Suárez, D. A. (2007). Community structure and phenology of chironomids (Insecta: Chironomidae) in a Patagonian Andean stream. *Limnologica*, *37*(1), 109–117. https://doi.org/10.1016/j.limno.2006.09.005

Garvey, J. E., & Whiles, M. (2016). *Trophic ecology*. CRC Press.

Georget, M. (2019). Retours sur deux programmes Life et un plan national d'action en faveur de l'apron du Rhône. *Sciences Eaux & Territoires*, *(Cahier spécial IV)*, 2–5. https://doi.org/10.14758/SET-REVUE.2019.cs4.01

Gonzáles, A., Dubut, V., Corse, E., Mekdad, R., Dechatre, T., & Meglécz, E. (2020). VTAM: A robust pipeline for validating metabarcoding data using internal controls. *bioRxiv*, 2020.11.06.371187. https://doi.org/10.1101/2020.11.06.371187

Grömping, U. (2006). Relative importance for linear regression in R: The package relaimpo. *Journal of Statistical Software*, *17*(1), 1–27.

Haidekker, A., & Hering, D. (2008). Relationship between benthic insects (Ephemeroptera, Plecoptera, coleoptera, Trichoptera) and temperature in small and medium-sized streams in Germany: A multivariate study. *Aquatic Ecology*, *42*(3), 463–481. https://doi.org/10.1007/s10452-007-9097-z

Hajibabaei, M., Porter, T. M., Wright, M., & Rudar, J. (2019). COI metabarcoding primer choice affects richness and recovery of indicator taxa in freshwater systems. *PLoS One*, *14*(9), e0220953. https://doi.org/10.1371/journal.pone.0220953

Hanson, B. A. (2018). *FuncMap: Hive plots of R package function calls.* R package version 1.0.10. https://cran.r-project.org/web/packages/FuncMap/

Hardy, N., Berry, T., Kelaher, B., Goldsworthy, S., Bunce, M., Coleman, M., Gillanders, B., Connell, S., Blewitt, M., & Figueira, W. (2017). Assessing the trophic ecology of top predators across a recolonisation frontier using DNA metabarcoding of diets. *Marine Ecology Progress Series*, *573*, 237–254. https://doi.org/10.3354/meps12165

Hill, M. O. (1973). Diversity and evenness: A unifying notation and its consequences. *Ecology*, *54*(2), 427–432. https://doi.org/10.2307/1934352

Hipfner, J. M. (2008). Matches and mismatches: Ocean climate, prey phenology and breeding success in a zooplanktivorous seabird. *Marine Ecology Progress Series*, *368*, 295–304. https://doi.org/10.3354/meps07603

Hoenig, B. D., Trevelline, B. K., Nuttle, T., & Porter, B. A. (2021). Dietary DNA metabarcoding reveals seasonal trophic changes among three syntopic freshwater trout species. *Freshwater Biology*, *66*(3), 509–523. https://doi.org/10.1111/fwb.13656

Hsieh, T. C., Ma, K. H., & Chao, A. (2016). iNEXT: An R package for rarefaction and extrapolation of species diversity (Hill numbers). *Methods in Ecology and Evolution*, *7*(12), 1451–1456. https://doi.org/10.1111/2041-210X.12613

Hurlbert, S. H., & White, M. D. (1993). Experiments with freshwater invertebrate zooplanktivores: Quality of statistical analyses. *Bulletin of Marine Science*, *53*(1), 128–153.

Ivlev, V. S. (1961). *Experimental ecology of the feeding of fishes*. Yale University Press.

Jakubavičiute, E., Bergström, U., Eklöf, J. S., Haenel, Q., & Bourlat, S. J. (2017). DNA metabarcoding reveals diverse diet of the three-spined stickleback in a coastal ecosystem. *PLoS One*, *12*(10), e0186929. https://doi.org/10.1371/journal.pone.0186929

Kong, J. D., Hoffmann, A. A., & Kearney, M. R. (2019). Linking thermal adaptation and life-history theory explains latitudinal patterns of voltinism. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *374*(1778), 20180547. https://doi.org/10.1098/rstb.2018.0547

Kotiaho, J. S. (1999). Estimating fitness: Comparison of body condition indices revisited. *Oikos*, *87*(2), 399–400. https://doi.org/10.2307/3546755

Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest package: Tests in linear mixed effects models. *Journal of Statistical Software*, *82*(13), 1–26. https://doi.org/10.18637/jss.v082.i13

Labonne, J., Allouche, S., & Gaudin, P. (2003). Use of a generalised linear model to test habitat preferences: The example of *Zingel asper*, an endemic endangered percid of the river Rhône. *Freshwater Biology*, *48*(4), 687–697. https://doi.org/10.1046/j.1365-2427.2003.01040.x

Lamb, P. D., Hunter, E., Pinnegar, J. K., Creer, S., Davies, R. G., & Taylor, M. I. (2019). How quantitative is metabarcoding: A meta-analytical approach. *Molecular Ecology*, *28*(2), 420–430. https://doi.org/10.1111/mec.14920

Lazic, S. E., Mellor, J. R., Ashby, M. C., & Munafo, M. R. (2020). A Bayesian predictive approach for dealing with pseudoreplication. *Scientific Reports*, *10*(1), 1–10. https://doi.org/10.1038/s41598-020-59384-7

Lenth, R. (2019). *emmeans: Estimated marginal means, aka least-squares means.* R Package version 1.3.5. https://cran.r-project.org/web/packages/emmeans/

Lewis, A. C. (1986). Memory constraints and flower choice in *Pieris rapae*. *Science*, *232*(4752), 868–871. https://doi.org/10.1126/science.232.4752.863

Littleford-Colquhoun, B. L., Freeman, P. T., Sackett, V. I., Tulloss, C. V., McGarvey, L. M., Geremia, C., & Kartzinel, T. R. (2022). The precautionary principle and dietary DNA metabarcoding: Commonly used abundance thresholds change ecological interpretation. *Molecular Ecology*, *31*(6), 1615–1626. https://doi.org/10.1111/mec.16352

Luz-Agostinho, K. D. G., Agostinho, A. A., Gomes, L. C., & Fugi, R. (2009). Effects of flooding regime on the feeding activity and body condition of piscivorous fish in the upper Paraná River floodplain. *Brazilian Journal of Biology*, *69*, 481–490.

Lyman, R. L. (2018). The history of MNI in north American zooarchaeology. In C. Giovas & M. LeFebvre (Eds.), *Zooarchaeology in practice* (pp. 13–33). Springer.

MacColl, A. D. C. (2011). The ecological causes of evolution. *Trends in Ecology and Evolution*, *26*(10), 514–522. https://doi.org/10.1016/j.tree.2011.06.009

Maechler, M., Rousseeuw, P., Struyf, A., Hubert, M., Hornik, K. (2019). cluster: Cluster analysis basics and extensions. R package version 2.1.0. https://cran.r-project.org/web/packages/cluster/

Marshall, D. J., & Burgess, S. C. (2015). Deconstructing environmental predictability: Seasonality, environmental colour and the biogeography of marine life histories. *Ecology Letters*, *18*(2), 174–181. https://doi.org/10.1111/ele.12402

Matich, P., Heithaus, M. R., & Layman, C. A. (2011). Contrasting patterns of individual specialization and trophic coupling in two marine apex predators. *Journal of Animal Ecology*, 80(1), 294–305. https://doi.org/10.1111/j.1365-2656.2010.01753.x

Merritt, J. F., Lima, M., & Bozinovic, F. (2001). Seasonal regulation in fluctuating small mammal populations: Feedback structure and climate. *Oikos*, 94(3), 505–514. https://doi.org/10.1034/j.1600-0706.2001.940312.x

Miller-Rushing, A. J., Høye, T. T., Inouye, D. W., & Post, E. (2010). The effects of phenological mismatches on demography. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 365(1555), 3177–3186. https://doi.org/10.1098/rstb.2010.0148

Monk, W. A., Wood, P. J., Hannah, D. M., & Wilson, D. A. (2008). Macroinvertebrate community response to inter-annual and regional river flow regime dynamics. *River Research and Applications*, 24(7), 988–1001. https://doi.org/10.1002/rra.1120

Monnet, G., Corse, E., Archambaud-Suard, G., Grenier, R., Chappaz, R., & Dubut, V. (2022). Growth variation in the endangered fish *Zingel asper*: Contribution of substrate quality, hydraulics, prey abundance and water temperature. *Aquatic Conservation: Marine and Freshwater Ecosystems*, 32(7), 1156–1170. https://doi.org/10.1002/aqc.3818

Monti, F., Duriez, O., Arnal, V., Dominici, J. M., Sforzi, A., Fusani, L., Grémillet, D., & Montgelard, C. (2015). Being cosmopolitan: Evolutionary history and phylogeography of a specialized raptor, the osprey *Pandion haliaetus*. *BMC Evolutionary Biology*, 15(1), 255. https://doi.org/10.1186/s12862-015-0535-6

Musseau, C., Vincenzi, S., Santoul, F., Boulêtreau, S., Jesenšek, D., & Crivelli, A. J. (2020). Within-individual trophic variability drives short-term intraspecific trait variation in natural populations. *Journal of Animal Ecology*, 89(3), 921–932. https://doi.org/10.1111/1365-2656.13149

Nielsen, J. M., Clare, E. L., Hayden, B., Brett, M. T., & Kratina, P. (2018). Diet tracing in ecology: Method comparison and selection. *Methods in Ecology and Evolution*, 9(2), 278–291. https://doi.org/10.1111/2041-210X.12869

Novak, M., & Tinker, M. T. (2015). Timescales alter the inferred strength and temporal consistency of intraspecific diet specialization. *Oecologia*, 178(1), 61–74. https://doi.org/10.1007/s00442-014-3213-2

Nunn, A. D., Tewson, L. H., & Cowx, I. G. (2012). The foraging ecology of larval and juvenile fishes. *Reviews in Fish Biology and Fisheries*, 22(2), 377–408. https://doi.org/10.1007/s11160-011-9240-8

Oksanen, J., Blanchet, F. G., Kindt, R., Legendre, P., Minchin, P. R., O'Hara, R. B., Simpson, G. L., Solymos, P., Stevens, M. H. H., & Wagner, H. (2020). *Vegan: Community ecology package*. R package version 2.5-7. https://cran.r-project.org/web/packages/vegan/

Olden, J. D., Kennard, M. J., Leprieur, F., Tedesco, P. A., Winemiller, K. O., & García-Berthou, E. (2010). Conservation biogeography of freshwater fishes: Recent progress and future challenges. *Diversity and Distributions*, 16(3), 496–513. https://doi.org/10.1111/j.1472-4642.2010.00655.x

Olivier, J.-M., Carrel, G., Lamouroux, N., Dole-Olivier, M.-J., Malard, F., Bravard, J.-P., Piégay, H., Castella, E., & Barthélemy, C. (2022). The Rhône River basin. In K. Tockner, C. Zarfl, & C. T. Robinson (Eds.), *Rivers of Europe* (2d ed., pp. 391–451). Elsevier.

O'Rourke, D. R., Bokulich, N. A., Jusino, M. A., MacManes, M. D., & Foster, J. T. (2020). A total crapshoot? Evaluating bioinformatic decisions in animal diet metabarcoding analyses. *Ecology and Evolution*, 10(18), 9721–9739. https://doi.org/10.1002/ece3.6594

Pansu, J., Guyton, J. A., Potter, A. B., Atkins, J. L., Daskin, J. H., Wursten, B., Kartzinel, T. R., & Pringle, R. M. (2019). Trophic ecology of large herbivores in a reassembling African ecosystem. *Journal of Ecology*, 107(3), 1355–1376. https://doi.org/10.1111/1365-2745.13113

Pazianoto, L. H. R., Cionek, V. M., Fogaça, F. N. O., & Benedito, E. (2016). Feeding and body condition of an invasive fish species under different environmental conditions. *Neotropical Ichthyology*, 14(1), e150164. https://doi.org/10.1590/1982-0224-20150164

Peig, J., & Green, A. J. (2009). New perspectives for estimating body condition from mass/length data: The scaled mass index as an alternative method. *Oikos*, 118(12), 1883–1891. https://doi.org/10.1111/j.1600-0706.2009.17643.x

Perry, G., & Pianka, E. R. (1997). Animal foraging: Past, present and future. *Trends in Ecology & Evolution*, 12(9), 360–364. https://doi.org/10.1016/S0169-5347(97)01097-5

Persson, L. (1985). Optimal foraging: The difficulty of exploiting different feeding strategies simultaneously. *Oecologia*, 67(3), 338–341. https://doi.org/10.1007/BF00384938

Petit, R. J., El Mousadik, A., & Pons, O. (1998). Identifying populations for conservation on the basis of genetic markers. *Conservation Biology*, 12(4), 844–855. https://doi.org/10.1111/j.1523-1739.1998.96489.x

Petta, J. C., Shipley, O. N., Wintner, S. P., Cliff, G., Dicken, M. L., & Hussey, N. E. (2020). Are you really what you eat? Stomach content analysis and stable isotope ratios do not uniformly estimate dietary niche characteristics in three marine predators. *Oecologia*, 192(4), 1111–1126. https://doi.org/10.1007/s00442-020-04628-6

Pires, M. M., Martins, E. G., Araújo, M. S., & Dos Reis, S. F. (2013). Between-individual variation drives the seasonal dynamics in the trophic niche of a neotropical marsupial. *Austral Ecology*, 38(6), 664–671. https://doi.org/10.1111/aec.12011

Poff, N. L. R., Allan, J. D., Bain, M. B., Karr, J. R., Prestegaard, K. L., Richter, B. D., Sparks, R. E., & Stromberg, J. C. (1997). The natural flow regime: A paradigm for river conservation and restoration. *Bioscience*, 47(11), 769–784. https://doi.org/10.2307/1313099

Pornon, A., Baksay, S., Escaravage, N., Burrus, M., & Andalo, C. (2019). Pollinator specialization increases with a decrease in a mass-flowering plant in networks inferred from DNA metabarcoding. *Ecology and Evolution*, 9(24), 13650–13662. https://doi.org/10.1002/ece3.5531

Pothoven, S. A., Nalepa, T. F., Schneeberger, P. J., & Brandt, S. B. (2001). Changes in diet and body condition of lake whitefish in southern Lake Michigan associated with changes in benthos. *North American Journal of Fisheries Management*, 21(4), 876–883. https://doi.org/10.1577/1548-8675(2001)021<0876:CIDABC>2.0.CO;2

Pusey, B. J., Arthington, A. H., Stewart-Koster, B., Kennard, M. J., & Read, M. G. (2010). Widespread omnivory and low temporal and spatial variation in the diet of fishes in a hydrologically variable northern Australian river. *Journal of Fish Biology*, 77(3), 731–753. https://doi.org/10.1111/j.1095-8649.2010.02712.x

Quéméré, E., Hibert, F., Miquel, C., Lhuillier, E., Rasolondraibe, E., Champeau, J., Rabarivola, C., Nusbaumer, L., Chatelain, C., Gautier, L., Ranirison, P., Crouau-Roy, B., Taberlet, P., & Chikhi, L. (2013). A DNA metabarcoding study of a primate dietary diversity and plasticity across its entire fragmented range. *PLoS One*, 8(3), e58971. https://doi.org/10.1371/journal.pone.0058971

R Core Team. (2020). *R: A language and environment for statistical computing*. Vienna, Austria: R Foundation for Statistical Computing. http://www.r-project.org

Raffard, A., Santoul, F., Cucherousset, J., & Blanchet, S. (2019). The community and ecosystem consequences of intraspecific diversity: A meta-analysis. *Biological Reviews*, 94(2), 648–661. https://doi.org/10.1111/brv.12472

Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The barcode of life data system (www.barcodinglife.org). *Molecular Ecology Notes*, 7(3), 355–364. https://doi.org/10.1111/j.1471-8286.2007.01678.x

Raveret-Wattel, C. (1900). *Atlas de poche des poissons d'eau douce de la France de la Suisse romande et de la Belgique*. Paul Klincksieck.

Roberts, D. W. (2019). *Labdsv: Ordination and multivariate analysis for ecology*. R package version 2.0-1. https://cran.r-project.org/web/packages/labdsv/

Roslin, T., & Majaneva, S. (2016). The use of DNA barcodes in food web construction-terrestrial and aquatic ecologists unite! *Genome*, *59*(9), 603–628. https://doi.org/10.1139/gen-2015-0229

Roughgarden, J. (1974). Niche width: Biogeographic patterns among *Anolis* lizard populations. *The American Naturalist*, *108*(962), 429–442. https://doi.org/10.1086/282924

Säfken, B., Rügamer, D., Kneib, T., & Greven, S. (2021). Conditional model selection in mixed-effects models with cAIC4. *Journal of Statistical Software*, *99*(8), 1–30. https://doi.org/10.18637/jss.v099.i08

Sánchez-Hernández, J., Finstad, A. G., Arnekleiv, J. V., Kjærstad, G., & Amundsen, P. A. (2021). Beyond ecological opportunity: Prey diversity rather than abundance shapes predator niche variation. *Freshwater Biology*, *66*(1), 44–61. https://doi.org/10.1111/fwb.13606

Sánchez-Hernández, J., Nunn, A. D., Adams, C. E., & Amundsen, P. A. (2019). Causes and consequences of ontogenetic dietary shifts: A global synthesis using fish models. *Biological Reviews*, *94*(2), 539–554. https://doi.org/10.1111/brv.12468

Shannon, C. (1948). A mathematical theory of communication. *Bell Systems Technological Journal*, *27*(3), 379–423.

Shutt, J. D., Nicholls, J. A., Trivedi, U. H., Burgess, M. D., Stone, G. N., Hadfield, J. D., & Phillimore, A. B. (2020). Gradients in richness and turnover of a forest passerine's diet prior to breeding: A mixed model approach applied to faecal metabarcoding data. *Molecular Ecology*, *29*(6), 1199–1213. https://doi.org/10.1111/mec.15394

Sjödin, H., Ripa, J., & Lundberg, P. (2018). Principles of niche expansion. *Proceedings of the Royal Society B: Biological Sciences*, *285*(1893), 20182603. https://doi.org/10.1098/rspb.2018.2603

Skinner, H. M., Durso, A. M., Neuman-Lee, L. A., Durham, S. L., Mueller, S. D., & French, S. S. (2016). Effects of diet restriction and diet complexity on life history strategies in side-blotched lizards (*Uta stansburiana*). *Journal of Experimental Zoology Part A: Ecological Genetics and Physiology*, *325*(9), 626–637. https://doi.org/10.1002/jez.2056

Slack, H. D. (1934). The winter food of brown trout (*Salmo trutta* L.). *Journal of Animal Ecology*, *3*(1), 105–108. https://doi.org/10.2307/1286

Smyly, W. J. P. (1957). The life-history of the bullhead or Miller's thumb (*Cottus gobio* L.). *Proceedings of the Zoological Society of London*, *128*(3), 431–454. https://doi.org/10.1111/j.1096-3642.1957.tb00336.x

Soininen, E. M., Gauthier, G., Bilodeau, F., Berteaux, D., Gielly, L., Taberlet, P., Gussarova, G., Bellemain, E., Hassel, K., Stenøien, H. K., Epp, L., Schrøder-Nielsen, A., Brochmann, C., & Yoccoz, N. G. (2015). Highly overlapping winter diet in two sympatric lemming species revealed by DNA metabarcoding. *PLoS One*, *10*(1), e0115335. https://doi.org/10.1371/journal.pone.0115335

Stevenson, R. D., & Woods, W. A., Jr. (2006). Condition indices for conservation: New uses for evolving tools. *Integrative and Comparative Biology*, *46*(6), 1169–1190. https://doi.org/10.1093/icb/icl052

Stroud, J. T., & Losos, J. B. (2016). Ecological opportunity and adaptive radiation. *Annual Review of Ecology, Evolution, and Systematics*, *47*, 507–532. https://doi.org/10.1146/annurev-ecolsys-121415-032254

Svanbäck, R., & Bolnick, D. I. (2005). Intraspecific competition affects the strength of individual specialization: An optimal diet theory method. *Evolutionary Ecology Research*, *7*(7), 993–1012.

Svanbäck, R., & Bolnick, D. I. (2007). Intraspecific competition drives increased resource use diversity within a natural population. *Proceedings of the Royal Society B: Biological Sciences*, *274*(1611), 839–844. https://doi.org/10.1098/rspb.2006.0198

Svanbäck, R., Eklöv, P., Fransson, R., & Holmgren, K. (2008). Intraspecific competition drives multiple species resource polymorphism in fish communities. *Oikos*, *117*(1), 114–124. https://doi.org/10.1111/j.2007.0030-1299.16267.x

Tachet, H., Richoux, P., Bournaud, M., & Usseglio-Polatera, P. (2020). *Invertébrés d'eau douce: Systématique, biologie, écologie*. CNRS éditions.

Tercel, M. P. T. G., & Cuff, J. P. (2022). The complex epistemological challenge of data curation in dietary metabarcoding: Comment on "the precautionary principle and dietary DNA metabarcoding: Commonly used abundance thresholds change ecological interpretation" by Littleford-Colquhoun et al.(2022). *Molecular Ecology*, in press. https://doi.org/10.1111/mec.16576

Thomas, A. C., Deagle, B. E., Eveson, J. P., Harsch, C. H., & Trites, A. W. (2016). Quantitative DNA metabarcoding: Improved estimates of species proportional biomass using correction factors derived from control material. *Molecular Ecology Resources*, *16*(3), 714–726. https://doi.org/10.1111/1755-0998.12490

Tinker, M. T., Bentall, G., & Estes, J. A. (2008). Food limitation leads to behavioral diversification and dietary specialization in sea otters. *Proceedings of the National Academy of Sciences of the United States of America*, *105*(2), 560–565. https://doi.org/10.1073/pnas.0709263105

Tournayre, O., Leuchtmann, M., Filippi-Codaccioni, O., Trillat, M., Piry, S., Pontier, D., Charbonnel, N., & Galan, M. (2020). In silico and empirical evaluation of twelve metabarcoding primer sets for insectivorous diet analyses. *Ecology and Evolution*, *10*(13), 6310–6332. https://doi.org/10.1002/ece3.6362

Vander Zanden, M. J., Shuter, B. J., Lester, N. P., & Rasmussen, J. B. (2000). Within- and among-population variation in the trophic position of a pelagic predator, lake trout (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences*, *57*(4), 725–731. https://doi.org/10.1139/f00-011

Varpe, Ø., & Fiksen, Ø. (2010). Seasonal plankton-fish interactions: Light regime, prey phenology, and herring foraging. *Ecology*, *91*(2), 311–318. https://doi.org/10.1890/08-1817.1

Vasselon, V., Bouchez, A., Rimet, F., Jacquet, S., Trobajo, R., Corniquel, M., Tapolczai, K., & Domaizon, I. (2018). Avoiding quantification bias in metabarcoding: Application of a cell bio-volume correction factor in diatom molecular biomonitoring. *Methods in Ecology and Evolution*, *9*(4), 1060–1069. https://doi.org/10.1111/2041-210X.12960

Vehtari, A., Gelman, A., & Gabry, J. (2017). Practical Bayesian model evaluation using leave-one-out cross-validation and WAIC. *Statistics and Computing*, *27*(5), 1413–1432. https://doi.org/10.1007/s11222-016-9696-4

Vrede, T., Drakare, S., Eklöv, P., Hein, A., Liess, A., Olsson, J., Persson, J., Quevedo, M., Stabo, H. R., & Svanbäck, R. (2011). Ecological stoichiometry of Eurasian perch—Intraspecific variation due to size, habitat and diet. *Oikos*, *120*(6), 886–896. https://doi.org/10.1111/j.1600-0706.2010.18939.x

Ward, J. H. (1963). Hierarchical grouping to optimize an objective function. *Journal of the American Statistical Association*, *58*(301), 236–244. https://doi.org/10.1080/01621459.1963.10500845

Warner, R. F. (2012). Environmental impacts of hydroelectric power and other anthropogenic developments on the hydromorphology and ecology of the durance channel and the Etang de Berre, Southeast France. *Journal of Environmental Management*, *104*, 35–50. https://doi.org/10.1016/j.jenvman.2012.03.011

Weigand, A. M., Michler-Kozma, D., Kuemmerlen, M., & Jourdan, J. (2020). Substantial differences in genetic diversity and spatial structuring among (cryptic) amphipod species in a mountainous river basin. *Freshwater Biology*, *65*(9), 1641–1656. https://doi.org/10.1111/fwb.13529

Wells, K., O'Hara, R. B., Cooke, B. D., Mutze, G. J., Prowse, T. A. A., & Fordham, D. A. (2016). Environmental effects and individual body condition drive seasonal fecundity of rabbits: Identifying acute and lagged processes. *Oecologia*, *181*(3), 853–864. https://doi.org/10.1007/s00442-016-3617-2