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

Kurt Villsen¹, Emmanuel Corse^{1,2,3}, Emese Megléc¹, Gaït Archambaud-Suard⁴, Hélène Vignes⁵, Alexander V. Ereskovsky^{1,6,7}, Rémi Chappaz⁴, Vincent Dubut¹

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

²Centre Universitaire de Mayotte, Dembeni, Mayotte, France

³MARBEC, Univ Montpellier, CNRS, Ifremer, IRD, Montpellier, France

⁴INRAE, Aix Marseille Université, RECOVER, Aix-en-Provence, France.

⁵CIRAD, Univ Montpellier, INRAE, Montpellier SupAgro, AGAP, Montpellier, France

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

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

Correspondence: Kurt Villsen, kurt.villsen@imbe.fr; or Vincent Dubut, vincent.dubut@gmail.com

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* and conducted modelling and simulation analyses to characterize and identify some of the drivers of individual trophic trait variation in this species. We found that intra-specific competition 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 (in our case, the seasonal variation in the availability of preferred prey types). Overall, our results are in line with the optimal foraging theory and suggest that *Z. asper* is specialized on a few ephemeropteran prey species (*Baetis fuscatus* and *Ecdyonurus*) but adapts its foraging by becoming more opportunistic as its favoured prey seasonally decline. Despite the now widespread usage of metabarcoding, very few studies have attempted to study inter- and intra-population individual trophic traits variation with metabarcoding data. This study illustrates how metabarcoding data obtained from feces can be combined with modelling and simulation approaches to test hypotheses in the conventional analytic framework of trophic analysis.

Keywords

Trophic niche, individual trait variation; metabarcoding; conservation; freshwater fish

1-Introduction

Trophic studies are central to our understanding of 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 inter-specific trophic niche variation (Bolnick et al., 2003). However, there is now a growing body of evidence that intra-specific trait variability (ITV) is crucial for maintaining functional diversity in ecosystems, species and populations (Des Roches et al., 2018; Raffard et al., 2019). Intra-specific trait diversity promotes functional complementarity among individuals through niche partitioning, facilitating more efficient use of ecological opportunities over space and time (Bolnick et al., 2011), which in turn may increase individual fitness and population stability (MacColl, 2011). Intra-specific 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), respectively, TNW being the sum of WIC and BIC ($TNW = WIC + BIC$) and BIC being related to individual specialization (IS) (Bolnick et al., 2003; Roughgarden, 1974). According to this framework, many *generalist* populations are in fact composed of specialized individuals with relatively narrow diet breadths (Araújo et al., 2011; Bolnick et al., 2002, 2003).

For predators, ecological opportunity, i.e. the availability of ecological resources that may be exploited at a given moment (Stroud & Losos, 2016; Wellborn & Langerhans, 2015), 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; J. C. 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). In parallel, aquatic insects have a complex life history with most having a short, aerial adult period and an aquatic juvenile stage. The periodicity of the juvenile stage can vary from a few months to a few years depending on each species' 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 et al., 2007) and consequently on the availability of prey for fishes.

Empirical studies have confirmed that seasonality is a major source of trophic niche variation (e.g. Falke et al., 2020; Varpe & Fiksen, 2010; Shutt et al., 2020) that has implications for individual fitness (Durant et al., 2005; Hipfner, 2008) and affects the demography of populations (Miller-Rushing et al., 2010). In response to low resource availability, trophic niche expansion is predicted by the Optimal Foraging Theory (OFT; see Perry & Pianka 1997). This niche expansion is achieved by the introduction of novel resources into the population's niche when preferred resources are less abundant. Trophic ITV has been shown to be a determinant component of this process (Bolnick et al., 2011; Sjödin et al., 2018), notably in the case of seasonal change in ecological opportunities (Costa-Pereira et al., 2017). The relationship between trophic niche variation (especially ITV) and ecological opportunity is therefore central for the understanding of biodiversity evolution (Bolnick et al., 2003, 2011; Brodersen et al., 2018), and for conservation and management issues (Agosta, 2002; Johnson et al., 2009; Titulaer et al., 2017).

For studying trophic niche variation, 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 (Alberdi et al., 2019; 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 et al., 2016), the 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). Here we used a metabarcoding approach to characterize intra-specific trophic niche variation in the fish *Zingel asper* (L.) [Actinopterygii: Perciformes: Percidae]. *Zingel asper* is a critically endangered species (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 metapopulations in eastern France and Switzerland (Georget, 2019). *Zingel asper* is a benthic species with a low diel displacement range, that has naturally low population density (Cavalli et al., 2003, 2009; Danancher et al., 2007; Labonne et al., 2003) and mainly feeds on macro-invertebrates (Cavalli et al., 2003; Corse et al., 2019).

Dietary data based on the metabarcoding of *Z. asper*'s feces were used to investigate qualitative and quantitative trophic variation in this species. The Rhône river basin covers an area of ~98 500 km² and contains several climatic zones: northern areas receive the highest precipitation, while southern areas have a Mediterranean climate with hot and dry summer periods (Olivier et al.,

2009). Therefore, we investigated the trophic variation in five populations of *Z. asper* that live in representative environmental conditions over the remnant species range. We characterized variation in *Z. asper*'s trophic niche for both individual (WIC and BIC) and population (TNW) trophic traits. In addition to seasonal variation, we tested the effect of two well-known determinants of ITV: body size (a functional trait related to ontogeny) and intra-specific competition (e.g. Bolnick et al., 2010; Svanbäck et al., 2008; Vander Zanden et al., 2000; Zhao et al., 2014). Lastly, changes in predator diet may subsequently affect predator body condition (e.g. Skinner et al., 2016), a life-history trait related to the size of energy reserves of individuals (Peig & Green, 2009) that can drive the future fitness of individuals (Kotiaho, 1999; Wells et al., 2016). We therefore aimed to determine whether trophic niche variation could affect the individual future fitness of *Z. asper* (using body condition as a proxy).

2-Materials and methods

2.1-Fish and feces sampling

Our sampling scheme included five sites which represent four of the five remnant *Z. asper* populations (see Figure 1). Sampling was performed over several campaigns in spring, summer and autumn in 2014 and 2015 (see 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 feces collection were performed. Fishes were weighted (precision 0.1g) and their fork-length measured (Lf; precision 1mm). The abdomen of *Z. asper* individuals was then pressed by hand in order to drain out feces. Feces were immediately placed in a 2ml vial containing 96% ethanol and stored at -20°C. After biometric measures and feces collection, fishes were released within the sampling area. A total of 967 *Z. asper* individuals were caught, and 498 feces samples were collected. Additionally, the surface of the prospected area was measured each sampling season in order to estimate the density of *Z. asper* (which was used as a proxy for intraspecific competition).

2.2-Diet metabarcoding

Fecal 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 feces for all individuals. In order to further minimize cross-sample contamination, extraction series were limited to 25 samples, including 21 *Z. asper* feces, two 'alien' feces (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, our 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 ~150bp 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. Our 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 (see also: Esnaola et al., 2018; Hajibabaei et al., 2019). Amplicons were then processed and sequenced on an Illumina MiSeq v3 platform (as detailed in Corse et al. 2017). High-throughput sequencing (HTS) data were filtered using the ASV-centered (Amplicon Sequence Variant) procedure developed by Corse et al. (2017). 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 fecal samples (i.e. experimental/molecular artefacts such as PCR/sequencing errors, tag switching and cross-sample contaminations). 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 (Renkonen 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 (~130bp) 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 (LTG) 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 LTG and BOLD), we built phylogenetic trees and/or integrate biogeographic information to finalize taxonomic assignment. In the case of the prey of *Z. asper*, this procedure (which includes three distinct assignment approaches) allowed for species-level identification for up to 75% of the ASVs/contigs (Corse et al., 2017). As the *Z. asper* mainly feeds on macroinvertebrates but can also feed on fishes (Cavalli et al., 2003; Corse et al., 2019; Raveret-Wattel & Bessin, 1900), we considered macroinvertebrates and fishes as relevant prey and collectively referred to them as Macrometazoans. All other taxa (listed in Corse et al. 2019) were excluded from the analyses.

2.3-Statistical analyses

All statistical analyses and data formatting were performed in R v 3.5.2 (R Core Development Team, 2018), using the RStudio interface (RStudio, 2010). Prey abundances were estimated using the Minimal Number of Individuals (MNI; White, 1953) approach. MNI is a semi-quantitative statistic that corresponds to the number of distinct ASVs/contigs validated in each sample for a given prey item (see Corse et al. 2017). To evaluate a possible ontogenetic effect on the diet of

Z. asper, we defined four size-classes: Young of the Year (YOY; Size-class 1), 63-108 mm (Size-class 2), 109-151 mm (Size-class 3), and 152-205 mm (Size-class 4). With the exception of the YOY, the size classes were based on quartiles.

2.3.1-Qualitative trophic variation

Zingel asper's diet was first examined qualitatively, both spatially (sampling sites), and temporally (seasons and year) by conducting (i) a Principal Component Analysis based on proportions (pPCA), 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., 2012) based on dissimilarity measurements calculated from Hill number β -diversities ($q = 1$, function *pair_dis*, package *hilldiv*, Alberdi and Gilbert 2019a). The optimal number of clusters was determined by the Gap-statistic calculated using the *fviz_nbclust* function from the package *NbCluster* (Charrad et al., 2014). The diet clusters were then characterized by Dufrene-Legendre indicator species analysis (Dufrene & Legendre, 1997), which calculates an indicator value for each prey item in the respective clusters (function *indval*, package *labdsv*, Roberts & Roberts, 2016).

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 (qD) to rare prey types can be adjusted with the order of diversity parameter q (Alberdi & Gilbert, 2019a). 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) (Weaver, 1949) using the R package *vegan* (Oksanen et al., 2008), 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 $1 - P_{Si}$, where P_{Si} (proportional similarity index) 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). Secondly, 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 conducted at 90% sample-coverage using the function *EstimateD* from the package *iNEXT* (Hsieh et al., 2016). Secondly, prey turnover was calculated for each sampling campaign as an estimate of the overall BIC. Prey turnover was calculated using Jaccard-type turnover (see: Alberdi and 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 & Green (2009):

$$\hat{M}_i = M_i \left[\frac{L_0}{L_i} \right]^{b_{SMA}}$$

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 (SMA) regression of M on L (Peig & Green, 2009). A one-way ANOVA was performed to confirm that no significant differences in $\hat{M}_i$ existed between individuals from different size-classes.

2.3.4-The effect of seasonality, intra-specific competition and ontogeny on ITV

To quantify the effect of season, ontogeny and intra-specific competition on INW and BIC, we fitted linear mixed-effects regression models (LMM) using the *lme4* package (Bates et al., 2019).

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. LMMs were fitted using season, size-class and *Z. asper* density as fixed effects and using sampling site and year as random effects. 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., 2018). *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*; Barton, 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 et al., 2018).

In addition to LMMs, Bayesian regression models (BRMs) were fitted to quantify the effect of season ontogeny and intra-specific competition on INW and BIC . BRMs provide more conservative inferences on biological systems, particularly in cases of pseudo-replication (Lazic et al., 2020). BRMs therefore served to cross-validate the results obtained by LMM. All models were fitted with a Gaussian family distribution with the exception of INW_d , which followed a lognormal distribution. BRMs were performed via the package *brms* (function *brm*) with default priors, 3 chains, 20000 iterations and a warm-up of 15000. Model selection was performed by Leave-One-Out cross-validation (LOOic) via the package *loo* (Vehtari et al., 2017). Differences in the response variables between factorial variables (e.g. season) was determined by calculating the probability of selecting a random value from the posterior predictive distribution (P) for a given grouping (e.g. autumn) and it being higher or lower than another value selected from the posterior predictive distribution of another grouping (e.g. spring).

2.3.5-Partitioning the variance of TNW

The total niche width of a given population is described as the sum of WIC and BIC ($TNW \sim WIC + BIC$; Bolnick et al., 2003; Roughgarden, 1972). 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*; Groemping & Matthias, 2018). 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 items consumed by each individual 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 ($BIC_{\text{simulated}}$) 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 season using Welch's t-tests.

2.3.6 The effect of trophic variation and seasonality on fitness potential

To evaluate the effect of dietary variation on fitness potential, LMMs and BRMs were performed following the formula $\hat{M}_i \sim \text{INW} + \text{BIC}$, with sampling site and year as random effects. LMMs and BRMs were fitted using 'traditional' estimates of INW and BIC, as well as their Hill numbers equivalents. A qualitative estimate of BIC was also included in the model selection process: the dietary cluster assigned to each individual using the hierarchical agglomerative clustering procedure described above. Lastly, to test for seasonal differences in $\hat{M}_i$, we fitted LMMs and BRMs, again including sampling site and year as random effects. LMMs and BRMs were performed as detailed above (section 2.3.4), including the model selection process (i.e. selection by cAIC and LOOic for LMMs and BRMs, respectively)

3-Results

3.1-Metabarcoding data

The raw dataset was gathered from 21 distinct MiSeq runs. After pre-processing, the dataset consisted of 61 million (M) reads that corresponded to the three PCR replicates of 510 eDNA samples, 160 negative controls, and 42 mock community samples. Filtering thresholds were determined by run, based on variant occurrence and frequencies (filtering parameters including LFN thresholds are reported in Supporting Information Table S1) and were then applied separately for each run. After filtering, 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 (43 M validated reads). After combining the MFZR, ZFZR, and LFCR ASVs, 391 contigs and 356 ASVs were obtained. At the end of the filtering process, 12 of the initial eDNA samples did not contain any validated ASV or contigs. None of the negative controls had validated ASVs or contigs. All ASVs expected in mock samples were retrieved. As previously reported (Corse et al. 2019), one or two extra ASVs were also validated in most of our mock samples (contig_0238, contig_0124, MFZR_000591; Supporting Information Table S2). By recovering all of the expected taxa from the mock communities in all of the different runs, we assumed that we had minimized random fluctuations, making our samples comparable between runs (see Bakker 2018). A total of 745 distinct Macrometazoan ASVs/contigs (corresponding to 141 prey items) were obtained from the 498 *Z. asper* feces. About 70% of the prey items were identified to the species level, another ~20% to the genus level, ~8% to the family level and ~2% to the order level (Supporting Information Table S2).

3.2-Spatial and seasonal qualitative diet variation

The most important prey group for *Z. asper* were Ephemeropteran species (Figure S1): 60% of the total prey abundance (estimated using the MNI), and occurrence in 86% of all fecal 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 fecal samples. The pPCA analysis (Figure S2) identified three main prey types that characterized spatial and temporal variation in the diet of *Z. asper*. PC1 was primarily characterized by the Ephemeroptera genus *Ecdyonurus* (64.0%) while PC2 was mainly characterized by *B. fuscatus* (55.3%) and 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 types (e.g. Plecoptera, fish) markedly increased (Figures S1 & S2). Spatial diet variation was discernible in summer: in the Verdon River the *Z. asper* diet was dominated by *Ecdyonurus*, while at site SAL on the Durance River, the summer diet more closely resembled the autumn diet of the other sampled locations. However, the SAL sampling campaign was almost exclusively composed of feces from Young Of the Year (YOY) individuals (see Table S1).

Hierarchical cluster analysis revealed three distinct diet clusters (Figure 1; Figure S3). The clusters were largely characterized by a single or a few prey-types (see 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 indicated that the diet of *Z. asper* diversified in autumn when compared to spring and summer, this pattern being 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). In autumn, the diet of *Z. asper* diversified: prey from Cluster 1 declined, and prey from Cluster 3 (Orthocladiinae and rare prey) proportionately increased. Moreover, in accordance with the pPCA results, the clustering analysis indicated that the YOY differ from the 1yr + individuals in their diet. Most YOY diets were assigned to Cluster 3, irrespective of season (see Figure S3), while Cluster 3 accounted for only ~ 5% to 10% of diets in the other size-classes.

3.3-Individual trophic trait variation in *Zingel asper*

Variation in individual traits (Individual Niche Width; INW and Between-Individual dietary Component; BIC) was examined using traditional indices (INW_S and BIC_V) and their Hill numbers equivalents (INW_D and BIC_D). In both cases, the corresponding traditional and Hill number estimates were highly correlated: for BIC_V vs. BIC_D, $R^2 = 0.91$ ($p < 0.001$); and INW_S vs. log(INW_D), $R^2 = 1$, ($p = 0$; Figure S4).

According to LMMs, roughly 16% (for INW) and 40% (for BIC) of the observed variance was explained by fixed effects alone. Both INW indices were jointly explained by density (intra-specific competition) and size-class fixed effects (Table 2). Density was negatively associated with INW, while INW increased incrementally between size-classes. According to Sum of Square analysis, density and size-class fixed effects explained roughly half of the total variance explained by the

best LMM (55% and 45%, respectively) (see Table S3). Post-hoc Tukey tests on model *emmeans* revealed that the differences between size-classes were only significant between YOY (size-class 1) and size-classes 3 and 4 ($p = 0.006$ and 0.007 , respectively). In accordance with the LMM results, BRM posterior predictive distribution sampling indicated a 60% and a 64% probability that INW was lower in YOY individuals compared to size-classes 3 and 4, respectively. For the most part, 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 modelling results revealed that both indices were explained by a seasonal fixed effect, which was retained as the sole predictor for both LMM and BRM models. The seasonal effect was characterized by a significant increase in BIC in autumn compared to both summer and spring (Tukey $p < 0.001$) (Figure 4; Table 2). This autumnal BIC increase was further supported by BRM predictions. For the BIC_v model, the probability that BIC was higher in autumn than in summer or spring was 90% and 86%, respectively; compared to 93% and 90% for the BIC_D model.

Site and year random effects greatly increased the variance explained for INW indices ($R^2_M = 0.16$ vs. $R^2_C = 0.27$). Including sampling site as a random effect also greatly increased the explained variance for both BIC indices (BIC_v: $R^2_M = 0.37$ vs. $R^2_C = 0.57$; BIC_D: $R^2_M = 0.41$ vs. $R^2_C = 0.73$) however, the year random effect failed to improve model fit, indicating negligible annual variation in BIC.

3.4-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 (see Table S2 and Figure 3). 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 S2 for full details). Linear regression models (TNW ~ INW + BIC) revealed that TNW was slightly best explained by Hill number estimates, accounting for 52% of the variation in TNW ($R^2 = 0.52$, $p < 0.001$), compared to 46% by traditional estimates ($R^2 = 0.46$, $p < 0.001$). 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 explained a minimal proportion of the variance of TNW (INW_d = 1%; INW_s = 3%).

3.5-Comparison between simulated and observed BIC values

Simulated and observed BIC were both strongly correlated with TNW values (Figure 4) however, the slope of the $BIC_{\text{observed}} \sim \text{TNW}$ regression was significantly greater than for the simulated BIC values (TNW*data type interaction $t = -2.97$, $p = 0.003$). T-tests revealed that simulated BIC values were significantly higher than the observed BIC values in spring (Welch's t test: $t = -4.67$, $p < 0.001$) and summer (Welch's t test: $t = -5.56$, $p < 0.001$; Figure 4). However, in autumn observed and simulated BIC did not significantly differ (Welch's t test: $t = -0.63$, $p = 0.528$).

3.6-Determinants of body condition variation

In order to be comparable between individuals of different lengths, body condition ($\hat{M}_i$) need to account for species-specific asymmetric growth patterns e.g. if shorter individuals are relatively heavier than longer individuals or vice versa. A one-way ANOVA revealed that there were no significant differences in $\hat{M}_i$ between size-classes in *Z. asper* ($p > 0.05$), indicating that $\hat{M}_i$ was not biased by the relationship between length and weight in *Z. asper*.

For both Hill numbers and traditional trophic indices, the best performing LMMs and BRMs included a significant negative effect of BIC on $\hat{M}_i$ (BIC_D : $t = 3.01$, $p = 0.003$; BIC_V : $t = -2.75$, $p = 0.006$) (Table 2). In both cases, neither INW nor dietary cluster was included in the final $\hat{M}_i$ models. According to emmeans performed on the best $\hat{M}_i$ LMM was significantly lower in autumn (spring-autumn: $p = 0.005$; summer-autumn: $p = 0.018$) (Table 2; Figure 2). The BRM also supported a decline in $\hat{M}_i$ in autumn compared to spring ($P = 0.60$) and summer ($P = 0.62$). Overall, individual trophic traits and seasonal variation explained a small proportion of the observed variance in $\hat{M}_i$ (R^2_M ranged from 0.01 to 0.03), and higher R^2_C values (0.26-0.29) indicated marked spatial and annual variation in $\hat{M}_i$.

4-Discussion

We implemented a robust metabarcoding protocol to characterize individual and population trophic niche variation in *Z. asper*. This protocol used multiple primer pairs to target the COI region to maximize the taxonomic coverage of prey and used an ASV-based filtering procedure that explicitly integrates negative and positive controls to validate dietary metabarcoding data within and among sequencing runs. 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 Ephemeroptera species, closely resembling the diet of its sister species *Romanichthys valsanicola* (Găldean et al., 1997). Additionally, we found qualitative spatial dietary variation of *Z. asper* to be relatively sparse, with *Baetis fuscatus* (Baetidae) and the genus *Ecdyonurus* (Heptageniidae) constituting the two main prey items regardless of the sampling location. The only notable spatial variation was observed in the Verdon River, where the diet of *Z. asper* was characterized by high consumption of *Ecdyonurus* compared to other populations.

4.1-Evidence for an ontogenetic trophic niche shift

Cluster analysis revealed a clear distinction between the diet of Young Of the Year (YOY) individuals and 1yr + individuals (size-classes 2-4) (Figure S3). YOY diets more closely resembled the autumn diet of adults (Cluster 3), with higher consumption of Chironomidae. While qualitative dietary variation between size-classes 2 to 4 was minimal, we detected a general positive association between size-class and INW, with the lowest INW observed in YOY individuals. Such dietary niche expansion associated with ontogeny is common in many fish species, owing to size-related limitations in prey consumption, differential nutritional requirements, differences in habitat use or to avoid competition with adult individuals (for a review see: Sánchez-Hernández et al., 2019). Interestingly, adult individuals appear to undergo a seasonal “back-shift” in autumn. Adult individuals both had lower INW and a higher proportion of Cluster 3 diets in autumn (Figure S3), closely resembling the YOY diet.

4.2-Seasonal individual trophic trait variation as adaptive foraging?

We found evidence of a seasonal diet shift in autumn that was shared by several *Z. asper* populations in distinct climatic areas of the Rhône river basin. This autumnal shift was associated with a decline in the consumption of *Z. asper*'s main prey type (*B. fuscatus*) and an increase in

the consumption of secondary prey (Figure 1). This qualitative dietary diversification coincided with both a TNW expansion and an increase in inter-individual dietary variation (BIC) in most populations (Figures 2 & 3). A seasonal dietary shift was previously reported in the Durance River and was associated with a decline in the abundance of *Z. asper*'s main prey (Baetidae) (Cavalli et al., 2003). Though in our study we did not conduct invertebrate inventories to evaluate prey abundance, it is very likely that the diet shift observed in the Beaume River and Loue River was also driven by the seasonal depletion of *Z. asper*'s main prey species (i.e. *B. fuscatus* and *Ecdyonurus*). Indeed, Trophic Niche Variation (TNV) is driven, among other factors, by variation in ecological opportunity (i.e. the abundance and diversity of prey species) (Varpe & Fiksen, 2010; Shutt et al., 2020; Araújo et al., 2011), and the Optimal Foraging Theory predicts that when the availability of preferred prey species declines, previously disregarded or less preferred prey species will be incorporated into the diet, thus leading to trophic niche expansion at the population level (Perry & Pianka, 1997).

In terms of ITV, we found that the autumnal TNW expansion was associated with a marked increase in inter-individual dietary variation (BIC) (Figure 2b). Transient ITV 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). BIC is often related to the degree of individual specialization in a given population (Bolnick et al., 2003; Roughgarden, 1974; Thoday, 1974), which can arise due to inter-individual variation in prey preference, microhabitat specialization or individual variation in foraging behavior (Lewis, 1986; Persson, 1985; Svanbäck & Bolnick, 2005). However, as BIC is inherently correlated with TNW it is important to compare the observed BIC against simulated values (based on a null model) to determine whether the observed individual specialization is significant (Bison et al., 2015; Bolnick et al., 2003). While we found that the observed BIC~TNW relationship in *Z. asper* differed significantly from the null (random foraging) model, we found that the observed and simulated BIC values did not significantly differ in autumn (Figure 4). This suggests that the observed autumnal BIC increase arose due to random foraging from a larger spectrum of prey (i.e. high TNW in autumn; Table S4). According to these results, *Z. asper* individuals appear to adapt their foraging behavior towards opportunistic foraging as opposed to specialization when faced with the autumnal depletion of their preferred prey.

Intra-specific competition is also described as one of the primary drivers of trophic Individual Trait Variation (ITV). Intra-specific competition is expected to reduce resource availability thus leading to an increase in INW as individuals expand their diet to include less favorable prey species

(MacColl, 2011). However, we found that *Z. asper* density was negatively associated with INW and had no observable effect on BIC. Nevertheless, these unexpected results should be interpreted cautiously due to the co-linearity between season and density (the highest *Z. asper* densities were found in autumn; Table S1) which may have influenced the inclusion of density in the best performing BRM and LMM models. Moreover, model performance (as per R^2 and AIC) did not greatly differ between models that included both density and season and models that included them as solo predictors, indicating that the variance of INW that is explained by density and season overlap to a degree that it is difficult to disentangle their effects. Given that *Z. asper* is naturally found at low density (e.g. Labonne, Allouche, and Gaudin 2003), we assume that the role of intra-specific competition in driving trophic ITV of *Z. asper* is most likely weak.

Lastly, using the scaled mass index ($\hat{M}_i$) as a proxy for the body condition, we aimed to determine whether the observed autumnal trophic niche expansion constituted a period of trophic stress for *Z. asper*. Body condition incorporates long-term ecological information that ultimately determine an individual's potential fitness, and/or energy reserves (Kotiaho, 1999). Although we found body condition to be lower in autumn than in summer or spring (possibly indicating that the autumnal dietary shift constitutes a minor trophic stress), we found that BIC and seasonal effects only accounted for a small proportion of the observed variation in body condition ($R^2_M = 0.01-0.03$). This minor effect of individual dietary traits on the body condition suggests that *Z. asper* optimize their foraging strategy to obtain sufficient nutrients when faced with fluctuations in ecological opportunity so that the seasonal change in individual trophic traits appear to only marginally affect the fitness of *Z. asper*.

Overall, our results associated with the autumnal diet shift in *Z. asper* populations are in line with the Optimal Foraging Theory which predicts that when 'specialist' predators are faced with the depletion of their preferred prey, they will adopt a more generalist feeding behavior to meet energy requirements (Holt & Kimbrell, 2007; Singer & Bernays, 2003). Our results suggest that *Z. asper* is specialized on a few ephemeropteran prey species (*B. fuscatus* and *Ecdyonurus*) and adapts its foraging by becoming more opportunistic as preferred prey seasonally decline, to maximize energy gain and maintain fitness.

4.3-Estimating individual dietary traits with metabarcoding data

The use of metabarcoding data is now widespread in dietary studies. However, metabarcoding data has rarely been used to estimate individual traits for studying inter- and intra-population level

of trophic niche variation (but see Bison et al., 2015; Soininen et al., 2015). Trophic ITV and the conventional analytic framework of trophic analysis were initially developed for diet data obtained by stable isotope analysis or from stomach (or gut, or feces) content analysis. Metabarcoding approaches most closely resemble classical morphological analyses of stomach, gut or feces content as they use the same type of samples to identify prey. Compared to morphological analyses, metabarcoding is known to be poorly quantitative and will provide inaccurate estimations of prey biomass in most cases (Lamb et al., 2019; but see Thomas et al., 2016; Vasselon et al., 2018). However, DNA-based analyses provide much higher taxonomic resolution compared to morphological analyses, which are dependent on the visual identification of prey (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; Sutela & Huusko, 2000). The estimation of individual dietary traits is known to be sensitive to the number of prey items considered, which is directly related to taxonomic resolution. Consequently, estimates of dietary traits like INW or BIC are expected to be higher when using metabarcoding data. In trophic studies, this may be particularly meaningful when the taxonomic units have functional differences that could affect the predator's hierarchization of prey (MacColl, 2011).

The most fundamental difference between stable isotope approaches and stomach, or gut or feces content analyses (being morphological or DNA-based) is related to the temporal window accounted for by the dietary data (Novak & Tinker, 2015; Petta et al., 2020). Compared to stable isotope analyses which are very integrative and can recapitulate the diet of an individual over several weeks or months, the metabarcoding approach employed in this study provided a snapshot of the diet of a given individual, likely accounting for one or a few days of feeding (e.g. Corse et al., 2015). Dietary data that integrate such short temporal windows usually contain a limited fraction of the total prey range of a predator (Aizpurua et al., 2018). Consequently, short-term dietary data tend to underestimate the realized INW as more diverse prey can be incorporated over a longer time-period and may also result in the overestimation of BIC which declines over time as individual diets converge (Novak *et al.* 2015). However, stable isotope analyses also have limitations. For instance, stable isotope analyses are highly dependent on a thorough characterization of isotopic spectrum of prey (which may not be known a priori), and the isotopic diversity of predators does not necessarily relate to taxonomic or functional diversity of prey (Young et al., 2014). Furthermore, physiological variation, growth rate, or stress-related factors can drive significant inter-individual variation in isotopic composition irrespective of the diet

consumed (Barnes et al., 2007; Gorokhova, 2018; Karlson et al., 2018) that can hinder trophic studies based on stable isotopes.

Nevertheless, although prudence is needed for their interpretation and comparison, stable isotope-based and morphological- or metabarcoding-based analyses provide valuable, albeit different insights into different dimensions of the trophic niche, including the foraging behavior of organisms (Alberdi et al., 2019; Hette-Tronquart, 2019; Petta et al., 2020). In this study, we used metabarcoding data to describe the mechanisms behind the observed autumnal TNW expansion and BIC increase. The interpretation of our results was assisted by comparing the observed dietary traits to simulated values based on a null model (random foraging model). Simulations revealed that the higher BIC values observed in autumn resulted from a shift toward random foraging as opposed to individual specialization as such increases in BIC are classically interpreted (Bolnick et al., 2003; Tinker et al., 2008). The use of simulations therefore appears to be a valuable method to test alternative foraging hypotheses when using metabarcoding data.

4.4-Estimating individual dietary traits with Hill numbers

In this study, we used both traditional dietary estimators of INW and BIC (Shannon-Wiener index and V) and their Hill numbers-derived equivalents to characterize the trophic ITV of *Z. asper*. Hill numbers have been proposed as a unifying framework for ecological diversity indices, providing methods for calculating taxonomic, phylogenetic and functional diversity indices (Chao et al., 2014). We found that traditional dietary estimators of INW and BIC were highly correlated with their Hill numbers equivalents (Figure S4) and that the best performing LMMs or BRMs were identical in structure regardless of the type of indices used, suggesting that Hill numbers are highly comparable to traditional diversity indices. The only notable difference was that INW and BIC estimated from Hill numbers were often better explained by ecological variables compared to their traditional counterparts. Our empirical results are therefore in line with the recent calls (Alberdi & Gilbert, 2019a; Ohlmann et al., 2019) for the use of Hill number diversity indices to facilitate comparisons between taxonomic (but also phylogenetic and functional) diversity indices in trophic ecology, especially when using metabarcoding-based data (Alberdi & Gilbert, 2019a).

5-Conclusion and perspectives

Diet studies have proven to be critical for guiding the conservation and management of species and habitats (e.g. Nunn et al., 2012; Zarzoso-Lacoste et al., 2019). In particular, an understanding of seasonal trophic niche variation is critical in the current context of climate change (Damien & Tougeron, 2019). Using a high-resolution metabarcoding data and both modelling and simulation approaches to characterize dietary variation in *Z. asper* we were able to relate trophic ITV to several ecological drivers, the seasonal driver being the most important and consistent across the species' range. Our results provide important insights into how a species can modulate individual and population-level dietary traits to adapt to variation in ecological opportunity (seasonality) and optimize its fitness. However, the high site- and annual-specific variability in ITV and body condition emphasize the need to investigate the causal relationships between the prey community (including its phenological variation), the habitat and both dietary and life-history ITV (e.g. body condition, annual growth) of *Z. asper*. This will improve our understanding of how this critically endangered species adapts to variation in ecological opportunity across space and time, thus providing valuable tools for the management and the conservation of this species.

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Author contributions

VD and EC conceived and designed the study. EC, VD, GAS and RC conducted the fieldwork.
EC, EM and HV did the molecular work and/or bioinformatics. KV performed statistical analyses,
with contributions from EC and VD. KV and VD wrote the original draft, and all authors contributed
to further writing and editing.

Data accessibility

Supplementary data were deposited in Dryad (<https://doi.org/10.5061/dryad.2ck7120>), including
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Bibliography

- Agosta, S. J. (2002). Habitat use, diet and roost selection by the big brown bat (*Eptesicus fuscus*) in North America: A case for conserving an abundant species. *Mammal Review*, 32(3), 179–198. <https://doi.org/10.1046/j.1365-2907.2002.00103.x>
- Aizpurua, O., Budinski, I., Georgiakakis, P., Gopalakrishnan, S., Ibañez, C., Mata, V., Rebelo, H., Russo, D., Szodoray-Parádi, F., Zhelyazkova, V., Zrncic, V., Gilbert, M. T. P., & 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., & 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>
- 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>
- Arscott, D. B., Tockner, K., & Ward, J. V. (2003). Spatio-temporal patterns of benthic invertebrates along the continuum of a braided Alpine river. *Archiv Fur Hydrobiologie*, 158(4), 431–460. <https://doi.org/10.1127/0003-9136/2003/0158-0431>
- Barnes, C., Sweeting, C. J., Jennings, S., Barry, J. T., & Polunin, N. V. C. (2007). Effect of temperature and ration size on carbon and nitrogen stable isotope trophic fractionation.

Functional Ecology, 21(2), 356–362. <https://doi.org/10.1111/j.1365-2435.2006.01224.x>

Barton, K., & Barton, M. K. (2019). Package ‘MuMIn.’ *R Package Version*, 1(6).

Bates, D., Maechler, M., Bolker, B., Walker, S., Christensen, R. H. B., Singmann, H., Dai, B., Grothendieck, G., Green, P., & Bolker, M. Ben. (2015). Package ‘lme4.’ *Convergence*, 12(1), 2.

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>

Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M., Rudolf, V. H. W., 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.

- 693 [https://doi.org/10.1890/0012-9658\(2002\)083\[2936:MILRS\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2002)083[2936:MILRS]2.0.CO;2)
- 694 Brodersen, J., Post, D. M., & Seehausen, O. (2018). Upward Adaptive Radiation Cascades:
695 Predator Diversification Induced by Prey Diversification. *Trends in Ecology and Evolution*,
696 33(1), 59–70. <https://doi.org/10.1016/j.tree.2017.09.016>
- 697 Brooke McEachern, M., A. Eagles-Smith, C., M. Efferson, C., & H. Van Vuren, D. (2006).
698 Evidence for local specialization in a generalist mammalian herbivore, *Neotoma fuscipes*.
699 *Oikos*, 113(3), 440–448. <https://doi.org/10.1111/j.2006.0030-1299.14176.x>
- 700 Brown, D. S., Burger, R., Cole, N., Vencatasamy, D., Clare, E. L., Montazam, A., & Symondson,
701 W. O. C. (2014). Dietary competition between the alien Asian Musk Shrew (*Suncus*
702 *murinus*) and a re-introduced population of Telfair's Skink (*Leiopisma telfairii*). *Molecular*
703 *Ecology*, 23(15), 3695–3705. <https://doi.org/10.1111/mec.12445>
- 704 Cavalli, L., Knight, C. M., Durbec, M., Chappaz, R., & Gozlan, R. E. (2009). Twenty-four hours in
705 the life of *Zingel asper*. *Journal of Fish Biology*, 75(3), 723–727.
706 <https://doi.org/10.1111/j.1095-8649.2009.02315.x>
- 707 Cavalli, L., Pech, N., & Chappaz, R. (2003). Diet and growth of the endangered *Zingel asper* in
708 the Durance River. *Journal of Fish Biology*, 63(2), 460–471. <https://doi.org/10.1046/j.1095-8649.2003.00168.x>
- 710 Chao, A., Chiu, C. H., & Jost, L. (2014). Unifying species diversity, phylogenetic diversity,
711 functional diversity, and related similarity and differentiation measures through hill numbers.
712 *Annual Review of Ecology, Evolution, and Systematics*, 45, 297–324.
713 <https://doi.org/10.1146/annurev-ecolsys-120213-091540>
- 714 Chao, A., & Jost, L. (2012). Coverage-based rarefaction and extrapolation: Standardizing
715 samples by completeness rather than size. *Ecology*, 93(12), 2533–2547.
716 <https://doi.org/10.1890/11-1952.1>
- 717 Charrad, M., Ghazzali, N., Boiteau, V., Niknafs, A., & Charrad, M. M. (2014). Package 'nbclust.'
718 *Journal of Statistical Software*, 61, 1–36.
- 719 Chiu, C. H., Jost, L., & Chao, A. (2014). Phylogenetic beta diversity, similarity, and differentiation
720 measures based on Hill numbers. *Ecological Monographs*, 84(1), 21–44.
721 <https://doi.org/10.1890/12-0960.1>
- 722 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. http://www.ephemeroptera-galactica.com/pubs/pub_c/pubcliffordh1982p15.pdf
- 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., Valladares, S., Planas, M., Chamorro, A., & Pintado, J. (2015). Analysis of the diet of the long-snouted seahorse *Hippocampus guttulatus* by 18S rDNA amplification of prey in faeces. *Aquaculture Nutrition*, 21(5), 528–540. <https://doi.org/10.1111/anu.12189>
- Corse, Emmanuel, Megléc, 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, Emmanuel, Tougard, C., Archambaud-Suard, G., Agnès, J. F., Messu Mandeng, F. D., Bilong Bilong, C. F., Duneau, D., Zinger, L., Chappaz, R., Xu, C. C. Y., Megléc, 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>
- 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. (2016). *Zingel asper*. *The IUCN Red List of Threatened Species 2006: e.T23207A9427850*. <https://dx.doi.org/10.2305/IUCN.UK.2006.RLTS.T23207A9427850.en>. Downloaded on 06 November 2020.
- 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](https://doi.org/10.1890/0012-9658(2006)87[1556:WNEISP]2.0.CO;2)

Damien, M., & Tougeron, K. (2019). Prey–predator phenological mismatch under climate change. *Current Opinion in Insect Science*, 35, 60–68. <https://doi.org/10.1016/j.cois.2019.07.002>

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>

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>

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>

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>

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>

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>

Erba, S., Melissano, L., & Buffagni, A. (2003). Life cycle of Baetidae (Ephemeroptera) in a North Italian prealpine stream. *Research Update on Ephemeroptera and Plecoptera*, 177–186.

- Esnaola, A., Arrizabalaga-Escudero, A., González-Esteban, J., Elozegi, 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>
- 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>
- 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](https://doi.org/10.1899/0887-3593(2007)26[92:NEITRO]2.0.CO;2)
- Găldean, N., Nalbant, T. T., & Dragomirescu, L. (1997). The Food and Feeding Habits of the
Sculpin- Perch or Romanian Darter *Romanichthys Valsanicola* (Pisces: Perciformes:
Percidae). *Travaux du Museum National d'Histoire Naturelle "Grigore Antipa"*, XXXVII,
287–295.
- 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. In *Trophic Ecology*. CRC Press.
<https://doi.org/10.1201/9781315367804>
- Georget, M. (2019). Retours sur deux programmes Life et un plan national d'action en faveur de
l'apron du Rhône. *Sciences Eaux & Territoires, Numéro spé(IV)*, 2–5.
<https://doi.org/10.3917/set.hs06.0002>
- Gorokhova, E. (2018). Individual growth as a non-dietary determinant of the isotopic niche
metrics. *Methods in Ecology and Evolution*, 9(2), 269–277. <https://doi.org/10.1111/2041-210X.12887>
- Groemping, U., & Matthias, L. (2018). *Package 'relaimpo.'*
- 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., Robinson, C. V., Baird, D. J., Shokralla, S., & Wright, M. T. G. (2019). Watered-down biodiversity? A comparison of metabarcoding results from DNA extracted from matched water and bulk tissue biomonitoring samples. *PLoS ONE*, 14(12), e0225409. <https://doi.org/10.1371/journal.pone.0225409>
- Hette-Tronquart, N. (2019). Isotopic niche is not equal to trophic niche. *Ecology Letters*, 22(11), 1987–1989. <https://doi.org/10.1111/ele.13218>
- 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>
- Holt, R. D., & Kimbrell, T. (2007). Foraging and population dynamics. *Foraging : Behavior and Ecology*, 365–396.
- 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>
- 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>
- Johnson, C. K., Tinker, M. T., Estes, J. A., Conrad, P. A., Staedler, M., Miller, M. A., Jessup, D. A., & Mazet, J. A. K. (2009). Prey choice and habitat use drive sea otter pathogen exposure in a resource-limited coastal system. *Proceedings of the National Academy of Sciences of the United States of America*, 106(7), 2242–2247. <https://doi.org/10.1073/pnas.0806449106>
- Karlson, A. M. L., Reutgard, M., Garbaras, A., & Gorokhova, E. (2018). Isotopic niche reflects stress-induced variability in physiological status. *Royal Society Open Science*, 5(2), 171398. <https://doi.org/10.1098/rsos.171398>
- 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>

843 Kotiaho, J. S. (1999). Estimating Fitness: Comparison of Body Condition Indices Revisited.
844 *Oikos*, 87(2), 399. <https://doi.org/10.2307/3546755>

845 Kuznetsova, A., Brockhoff, P. B., & Christensen, R. H. B. (2017). lmerTest Package: Tests in
846 Linear Mixed Effects Models. *Journal of Statistical Software*, 82(13), 1–26.
847 <https://doi.org/10.18637/jss.v082.i13>

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

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

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

858 Lenth, R. (2019). Emmeans: estimated marginal means. *Aka Least-Squares Means.*, 1(1).
859 <https://cran.r-project.org/package=emmeans>

860 Lewis, A. C. (1986). Memory constraints and flower choice in *peris rapae*. *Science*, 232(4752),
861 868–871. <https://doi.org/10.1126/science.232.4752.863>

862 MacColl, A. D. C. (2011). The ecological causes of evolution. *Trends in Ecology and Evolution*,
863 26(10), 514–522. <https://doi.org/10.1016/j.tree.2011.06.009>

864 Maechler, M., Struyf, A., Hubert, M., Hornik, K., Studer, M., & Roudier, P. (2015). Package
865 ‘cluster’: Cluster Analysis Basics and Extensions. *R Topics Documented*, 1(2), 79.
866 <https://cran.r-project.org/web/packages/cluster/cluster.pdf>

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

870 Matich, P., Heithaus, M. R., & Layman, C. A. (2011). Contrasting patterns of individual
871 specialization and trophic coupling in two marine apex predators. *Journal of Animal*
872 *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>
- 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>
- 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>
- Ohlmann, M., Miele, V., Dray, S., Chalmandrier, L., O'Connor, L., & Thuiller, W. (2019). Diversity indices for ecological networks: a unifying framework using Hill numbers. *Ecology Letters*, 22(4), 737–747. <https://doi.org/10.1111/ele.13221>
- Oksanen, J., Kindt, R., Legendre, P., O'Hara, B., Simpson, G. L., Solymos, P. M., Stevens, M. H. H., & Wagner, H. (2008). The vegan package. *Community Ecology Package*, 10(631–637), 190. <https://brcr.bio.umass.edu/biometry/images/8/85/Vegan.pdf>
- Olivier, J.-M., Carrel, G., Lamouroux, N., Dole-Olivier, M.-J., Malard, F., Bravard, J.-P., & Amoros, C. (2009). The Rhône river basin. *Rivers of Europe*, 2, 47–295.

- 903 Pansu, J., Guyton, J. A., Potter, A. B., Atkins, J. L., Daskin, J. H., Wursten, B., Kartzinel, T. R., &
904 Pringle, R. M. (2019). Trophic ecology of large herbivores in a reassembling African
905 ecosystem. *Journal of Ecology*, 107(3), 1355–1376. [https://doi.org/10.1111/1365-](https://doi.org/10.1111/1365-2745.13113)
906 2745.13113
- 907 Peig, J., & Green, A. J. (2009). New perspectives for estimating body condition from mass/length
908 data: The scaled mass index as an alternative method. *Oikos*, 118(12), 1883–1891.
909 <https://doi.org/10.1111/j.1600-0706.2009.17643.x>
- 910 Perry, G., & Pianka, E. R. (1997). Animal foraging: past, present and future. *Trends in Ecology &*
911 *Evolution*, 12(9), 360–364.
- 912 Persson, L. (1985). Optimal foraging: the difficulty of exploiting different feeding strategies
913 simultaneously. *Oecologia*, 67(3), 338–341. <https://doi.org/10.1007/BF00384938>
- 914 Petta, J. C., Shipley, O. N., Wintner, S. P., Cliff, G., Dicken, M. L., & Hussey, N. E. (2020). Are
915 you really what you eat? Stomach content analysis and stable isotope ratios do not
916 uniformly estimate dietary niche characteristics in three marine predators. *Oecologia*,
917 192(4), 1111–1126. <https://doi.org/10.1007/s00442-020-04628-6>
- 918 Pires, M. M., Martins, E. G., Araújo, M. S., & Dos Reis, S. F. (2013). Between-individual variation
919 drives the seasonal dynamics in the trophic niche of a Neotropical marsupial. *Austral*
920 *Ecology*, 38(6), 664–671. <https://doi.org/10.1111/aec.12011>
- 921 Quéméré, E., Hibert, F., Miquel, C., Lhuillier, E., Rasolondraibe, E., Champeau, J., Rabarivola,
922 C., Nusbaumer, L., Chatelain, C., Gautier, L., Ranirison, P., Crouau-Roy, B., Taberlet, P., &
923 Chikhi, L. (2013). A DNA Metabarcoding Study of a Primate Dietary Diversity and Plasticity
924 across Its Entire Fragmented Range. *PLoS ONE*, 8(3), e58971.
925 <https://doi.org/10.1371/journal.pone.0058971>
- 926 Raffard, A., Santoul, F., Cucherousset, J., & Blanchet, S. (2019). The community and ecosystem
927 consequences of intraspecific diversity: a meta-analysis. *Biological Reviews*, 94(2), 648–
928 661. <https://doi.org/10.1111/brv.12472>
- 929 Ratnasingham, S., & Hebert, P. D. N. (2007). BOLD: The Barcode of Life Data System:
930 Barcoding. *Molecular Ecology Notes*, 7(3), 355–364. [https://doi.org/10.1111/j.1471-](https://doi.org/10.1111/j.1471-8286.2007.01678.x)
931 8286.2007.01678.x
- 932 Raveret-Wattel, C. (1900). Atlas de poche des poissons d'eau douce de la France de la Suisse

romande et de la Belgique avec leur description, moeurs et organisation. In *Bibliothèque de poche du naturaliste* (Vol. 11), 8. Paul Klincksieck.

Roberts, D. W., & Roberts, M. D. W. (2016). Package 'labdsv.' *Ordination and Multivariate*.

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>

RStudio. (2010). RStudio: Integrated development environment for R (RStudio 0.98.1091). *The R User Conference, UseR! 2011 August 16-18 ...*, 75(8), 538. <http://www.rstudio.org/>

Säfken, B., Rügamer, D., Kneib, T., & Greven, S. (2018). Conditional model selection in mixed-effects models with cAIC4. *ArXiv*. <http://arxiv.org/abs/1803.05664>

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>

Schoener, T. W. (1971). Theory of Feeding Strategies. *Annual Review of Ecology and Systematics*, 2(1), 369–404. <https://doi.org/10.1146/annurev.es.02.110171.002101>

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>

Singer, M. S., & Bernays, E. A. (2003). Understanding omnivory needs a behavioral perspective. *Ecology*, 84(10), 2532–2537. <https://doi.org/10.1890/02-0397>

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>

Soininen, E. M., Gauthier, G., Bilodeau, F., Berteaux, D., Gielly, L., Taberlet, P., Gussarova, G., Bellemain, E., Hassel, K., Stenøien, H. K., Epp, L., Schröder-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>

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>

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>

Sutela, T., & Huusko, A. (2000). Varying Resistance of Zooplankton Prey to Digestion: Implications for Quantifying Larval Fish Diets. *Transactions of the American Fisheries Society*, 129(2), 545–551. [https://doi.org/10.1577/1548-8659\(2000\)129<0545:vrozpt>2.0.co;2](https://doi.org/10.1577/1548-8659(2000)129<0545:vrozpt>2.0.co;2)

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., 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>

Team R Development Core. (2018). A Language and Environment for Statistical Computing. In *R Foundation for Statistical Computing* (Vol. 2, p. <https://www.R-project.org>). Vienna, Austria. <http://www.r-project.org>

Thoday, J. M. (1974). Evolution of Niche Width. *The American Naturalist*, 108(959), 142–143. <https://doi.org/10.1086/282892>

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.

992 <https://doi.org/10.1111/1755-0998.12490>

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

996 Titulaer, M., Melgoza-Castillo, A., Panjabi, A. O., Sanchez-Flores, A., Martínez-Guerrero, J. H.,
997 Macías-Duarte, A., & Fernandez, J. A. (2017). Molecular analysis of stomach contents
998 reveals important grass seeds in the winter diet of Baird's and Grasshopper sparrows, two
999 declining grassland bird species. *PLoS ONE*, 12(12), e0189695.
1000 <https://doi.org/10.1371/journal.pone.0189695>

1001 Vander Zanden, M. J., Shuter, B. J., Lester, N. P., & Rasmussen, J. B. (2000). Within- and
1002 among-population variation in the trophic position of a pelagic predator, lake trout
1003 (*Salvelinus namaycush*). *Canadian Journal of Fisheries and Aquatic Sciences*, 57(4), 725–
1004 731. <https://doi.org/10.1139/f00-011>

1005 Varpe, Ø., & Flksen, Ø. (2010). Seasonal plankton-fish interactions: Light regime, prey
1006 phenology, and herring foraging. *Ecology*, 91(2), 311–318. <https://doi.org/10.1890/08-1817.1>

1008 Vasselon, V., Bouchez, A., Rimet, F., Jacquet, S., Trobajo, R., Corniquel, M., Tapolczai, K., &
1009 Domaizon, I. (2018). Avoiding quantification bias in metabarcoding: Application of a cell
1010 biovolume correction factor in diatom molecular biomonitoring. *Methods in Ecology and*
1011 *Evolution*, 9(4), 1060–1069. <https://doi.org/10.1111/2041-210X.12960>

1012 Vehtari, A., Gelman, A., & Gabry, J. (2017). Practical Bayesian model evaluation using leave-
1013 one-out cross-validation and WAIC. *Statistics and Computing*, 27(5), 1413–1432.
1014 <https://doi.org/10.1007/s11222-016-9696-4>

1015 Ward, J. H. (1963). Hierarchical Grouping to Optimize an Objective Function. *Journal of the*
1016 *American Statistical Association*, 58(301), 236–244.
1017 <https://doi.org/10.1080/01621459.1963.10500845>

1018 Weaver, W. (1949). Recent contributions to the mathematical theory of communication. In *The*
1019 *mathematical theory of communication*. University of illinois Press.

1020 Wellborn, G. A., & Langerhans, R. B. (2015). Ecological opportunity and the adaptive
1021 diversification of lineages. *Ecology and Evolution*, 5(1), 176–195.

- 1022 <https://doi.org/10.1002/ece3.1347>
- 1023 Wells, K., O'Hara, R. B., Cooke, B. D., Mutze, G. J., Prowse, T. A. A., & Fordham, D. A. (2016).
1024 Environmental effects and individual body condition drive seasonal fecundity of rabbits:
1025 identifying acute and lagged processes. *Oecologia*, 181(3), 853–864.
1026 <https://doi.org/10.1007/s00442-016-3617-2>
- 1027 White, J. C., Hannah, D. M., House, A., Beatson, S. J. V., Martin, A., & Wood, P. J. (2017).
1028 Macroinvertebrate responses to flow and stream temperature variability across regulated
1029 and non-regulated rivers. *Ecohydrology*, 10(1), e1773. <https://doi.org/10.1002/eco.1773>
- 1030 White, T. E. (1953). A Method of Calculating the Dietary Percentage of Various Food Animals
1031 Utilized by Aboriginal Peoples. *American Antiquity*, 18(4), 396–398.
1032 <https://doi.org/10.2307/277116>
- 1033 Woo, K. J., Elliott, K. H., Davidson, M., Gaston, A. J., & Davoren, G. K. (2008). Individual
1034 specialization in diet by a generalist marine predator reflects specialization in foraging
1035 behaviour. *Journal of Animal Ecology*, 77(6), 1082–1091. [https://doi.org/10.1111/j.1365-](https://doi.org/10.1111/j.1365-2656.2008.01429.x)
1036 [2656.2008.01429.x](https://doi.org/10.1111/j.1365-2656.2008.01429.x)
- 1037 Young, J. W., Olson, R. J., Ménard, F., Kuhnert, P. M., Duffy, L. M., Allain, V., Logan, J. M.,
1038 Lorrain, A., Somes, C. J., Graham, B., Goñi, N., Pethybridge, H., Simier, M., Potier, M.,
1039 Romanov, E., Pagendam, D., Hannides, C., & Choy, C. A. (2014). Setting the stage for a
1040 global-scale trophic analysis of marine top predators: a multi-workshop review. *Reviews in*
1041 *Fish Biology and Fisheries*, 25(1), 261–272. <https://doi.org/10.1007/s11160-014-9368-4>
- 1042 Zaccarelli, N., Bolnick, D. I., & Mancinelli, G. (2013). RInSp: An r package for the analysis of
1043 individual specialization in resource use. *Methods in Ecology and Evolution*, 4(11), 1018–
1044 1023. <https://doi.org/10.1111/2041-210X.12079>
- 1045 Zarzoso-Lacoste, D., Bonnaud, E., Corse, E., Dubut, V., Lorvelec, O., Meringo, H. De, Santelli,
1046 C., Meunier, J. Y., Ghestemme, T., Gouni, A., & Vidal, E. (2019). A preliminary field trial to
1047 compare control techniques for invasive *Berberis aquifolium* in Belgian coastal dunes.
1048 *NeoBiota*, 53, 61–82. <https://doi.org/10.3897/neobiota.53.35086>
- 1049 Zarzoso-Lacoste, D., Bonnaud, E., Corse, E., Gilles, A., Meglecz, E., Costedoat, C., Gouni, A., &
1050 Vidal, E. (2016). Improving morphological diet studies with molecular ecology: An
1051 application for invasive mammal predation on island birds. *Biological Conservation*, 193,
1052 134–142. <https://doi.org/10.1016/j.biocon.2015.11.018>

1053 Zhao, T., Villéger, S., Lek, S., & Cucherousset, J. (2014). High intraspecific variability in the
 1054 functional niche of a predator is associated with ontogenetic shift and individual
 1055 specialization. *Ecology and Evolution*, 4(24), 4649–4657. <https://doi.org/10.1002/ece3.1260>

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TABLES and FIGURES

Table 1. Glossary

Term	Explanation
Trait	Any measurable feature of an organism e.g. body condition, diet.
Intra-specific Trait Variability (ITV)	The individual-based variability of a given trait within a species or population
Total trophic Niche Width (TNW)	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. 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 inter-individual dietary variability (β -diversity).
Prey turnover	A population-level trophic trait indicating the degree of inter-individual dietary variation in a given population or species.
Body Condition	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.

1063 **Table 2 Effect of season, ontogeny and intra-specific competition on *Zingel asper*'s individual dietary traits.** SE, standard
1064 error; df, degrees of freedom; *t*, *t*-value; *p*, *p*-value; R^2_M , marginal R^2 ; R^2_C , conditional R^2 ; P, Bayesian posterior distribution of
1065 parameter estimates.

Trait	Trait estimate	Fixed effect	LMM						BRM	
			Estimate	SE	df	<i>t</i>	<i>p</i>	$R^2_M - R^2_C$	P	R^2
Individual Trophic Niche Width	INW _s & INW _D	Intercept	2.14	0.26	29	8.20	< 0.001	0.16 – 0.27	[-0.16 – 3.27]	0.16
		Density	-0.30	0.07	86	-4.46	< 0.001		[-0.33 – 0.09]	
		Size-class 1	-0.20	0.07	460	-3.01	0.003		[-0.29 – -0.02]	
		Size-class 2	-0.03	0.06	459	-0.48	0.632		[-0.14 – 0.08]	
		Size-class 3	0.10	0.04	457	2.46	0.014		[-0.03 – 0.15]	
		Autumn	-	-	-	-	-		[-0.28 – -0.03]	
		Summer	-	-	-	-	-		[-0.02 – 0.21]	
Between Individual Component	BIC _V	Intercept	0.62	0.04	3	14.86	< 0.001	0.37 – 0.57	[0.47 – 0.79]	0.49
		Autumn	0.18	0.01	488	17.96	< 0.001		[0.16 – 0.20]	
		Summer	-0.11	0.01	383	-8.14	< 0.001		[- 0.12 – 0.01]	
	BIC _D	Intercept	0.66	0.06	4	11.37	< 0.001	0.41 – 0.73	[0.44 – 0.87]	0.63
		Autumn	0.21	0.01	492	24.06	< 0.001		[0.19 – 0.23]	
		Summer	-0.13	0.01	480	-10.29	< 0.001		[-0.16 – 0.11]	

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Table 3. Effect of season and individual diet traits on body condition of *Zingel asper*. Scaled Mass Index ($\hat{M}_i$) modelled against season and individual dietary traits (INW and BIC). SE, standard error; df, degrees of freedom; t , t -value; p , p -value; R^2_M , marginal R^2 ; R^2_C , conditional R^2 ; P, Bayesian posterior distribution of parameter estimates.

Variable type	Fixed effect	LMM						BRM	
		Estimate	SE	df	t	p	$R^2_M - R^2_C$	P	R^2 (Bayes)
Seasons	Intercept	19.06	0.63	3	30.11	< 0.001	0.03-0.29	[12.19 – 25.64]	0.23
	Autumn	-0.54	0.14	440	-3.72	< 0.001		[-0.82 – -0.25]	
	Summer	0.34	0.20	289	1.72	0.087		[-0.04 – 0.76]	
Traditional indices	Intercept	19.94	0.72	4	27.80	< 0.001	0.01-0.28	[12.93 – 26.66]	0.22
	BIC _V	-1.41	0.51	463	-2.75	0.006		[-2.42 – -0.40]	
Hill numbers	Intercept	20.01	0.71	5	28.03	< 0.001	0.02-0.28	[13.14 – 26.82]	0.22
	BIC _D	-1.51	0.50	463	-3.01	0.003		[-2.45 – -0.52]	

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Figure 1. Diets clusters of *Zingel asper* populations (A) and the indicator species value (Indval) histograms for each diet cluster (B).

Figure 2. The seasonal variation of INW (A), BIC (B) and $\hat{M}_i$ (C), and the network (Hive plot) of relationships of prey, season and *Zingel asper's* BIC. For INW and BIC boxplots, blank boxes correspond to Shannon and V indices, respectively while shaded plots correspond to hill number indices.

Figure 3. Coverage-based rarefaction curves of Total Niche Width (TNW) by population. TNW corresponds to species diversity calculated using Hill numbers ($q = 1$), based on 90% diversity coverage. Colors correspond to season (spring = green, summer = blue, autumn = purple).

Figure 4. Comparison of observed and simulated BICd values (based on a null model). Left, a scatterplot illustrating the BICd ~ TNW relationship between simulated (grey) and observed BICd values (black). Linear regression slopes are indicated by solid black and grey lines. Right, boxplots comparing simulated BICd values (light grey) to observed BICd values (dark grey) by season. ***, $p < 0.001$; n.s., not significant.