Direct and indirect effects of habitat and spatial heterogeneity on individual trophic traits in a critically endangered fish species

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Abstract

Within-species Individual Trait Variation (ITV) is now recognised as an important source of variability in ecological communities. Individual variation in trophic niche traits (i.e. individual niche width and between-individual variation) can greatly modify top-down and/or competitive interactions. Trophic traits vary according to variation in ecological opportunity, which represents the range of exploitable resources. While the role of prey availability in driving trophic traits is well-established, abiotic drivers (e.g. habitat structure) are rarely accounted for, or are solely considered via their effect on the prey community. We aimed to disentangle prey-mediated from direct habitat effects on trophic ITV in a critically endangered riverine fish, the Rhone streber (Zingel asper). We quantified individual trophic traits using high-resolution diet data obtained from faeces metabarcoding, and quantified prey availability and habitat structure using a fine-scale sampling protocol. Trophic traits were driven by distinct mechanisms: the individual niche width was driven by prey availability, while between-individual variation was largely driven by habitat conditions. Habitat conditions acted both directly on trophic traits and indirectly via their effect on the prey community. By simultaneously accounting for biotic and abiotic drivers of trophic ITV, we obtained a more complete understanding of how prey availability and habitat structure jointly determine ecological opportunity. Furthermore, by accounting for fine-scale variation in prey community and habitat conditions, the importance of spatial heterogeneity factors was highlighted. This study demonstrates how robust metabarcoding data, combined with detailed prey community and habitat information can be used to reveal the mechanistic pathways that drive trophic traits.

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Running title:

Abiotic and biotic drivers of trophic ITV

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Abstract

Within-species Individual Trait Variation (ITV) is now recognised as an important source of variability in ecological communities. Individual variation in trophic niche traits (i.e. individual niche width and between-individual variation) can greatly modify top-down and/or competitive interactions. Trophic traits vary according to variation in ecological opportunity, which represents the range of exploitable resources. While the role of prey availability in driving trophic traits is well-established, abiotic drivers (e.g. habitat structure) are rarely accounted for, or are solely considered via their effect on the prey community. We aimed to disentangle prey-mediated from direct habitat effects on trophic ITV in a critically endangered riverine fish, the Rhone streber (*Zingel asper*). We quantified individual trophic traits using high-resolution diet data obtained from faeces metabarcoding, and quantified prey availability and habitat structure using a fine-scale sampling protocol. Trophic traits were driven by distinct mechanisms: the individual niche width was driven by prey availability, while between-individual variation was largely driven by habitat conditions. Habitat conditions acted both directly on trophic traits and indirectly via their effect on the prey community. By simultaneously accounting for biotic and abiotic drivers of trophic ITV, we obtained a more complete understanding of how prey availability and habitat structure jointly determine ecological opportunity. Furthermore, by accounting for fine-scale variation in prey community and habitat conditions, the importance of spatial heterogeneity factors was highlighted. This study demonstrates how robust metabarcoding data, combined with detailed

prey community and habitat information can be used to reveal the mechanistic pathways that drive trophic traits.

Keywords: diet metabarcoding; individual niche variation; ecological opportunity; structural equation modelling; conservation.

Introduction

Understanding the ecological drivers of trophic niche variation is a central aim of trophic ecology, providing insights into predator-prey interactions, food-web structure and biodiversity evolution (Bolnick et al. 2011; Brodersen et al. 2018; Schreiber et al. 2011). Assuming that individuals are ecological equivalent, the trophic niche was long thought to vary primarily at the population- or species-level (MacArthur & Levins, 1967), but individual trait variation (ITV) is now recognised as an important source of trophic niche variation (Bolnick et al. 2003). Intraspecific trait diversity promotes functional complementarity, facilitating more efficient use of ecological opportunities over space and time (Bolnick et al. 2011), which in turn may increase population stability and populations' resilience to disturbance (MacColl, 2011). Ecological opportunity is the accessible niche space, that is, the availability of ecological resources that may be exploited at a given moment by an individual, a population or a species (Araújo et al. 2011; Evangelista et al. 2014; Sjödin et al. 2018). Greater ecological opportunity is expected to promote trophic niche diversification, as previously unoccupied resources may begin to be exploited (Nosil & Reimchen, 2005; Parent & Crespi, 2009; Simpson, 1953). For example, a more diverse and abundant prey community may allow individuals to exploit a wider range of resources (i.e. larger individual niche widths) while also facilitating resource partitioning among individuals, leading to greater between individual variation (Araújo et al. 2011; Roughgarden, 1972; Van Valen, 1965). Both prey abundance and diversity constitute an important dimension of ecological opportunity (e.g. Balme et al. 2020; Bolnick & Ballare, 2020; Sánchez-Hernández et al. 2021; Wray et al. 2021). Moreover, the importance of preferred prey abundance in driving trophic traits has also been demonstrated (e.g. Tinker et al. 2008), as when preferred prey are abundant, individual diets may converge resulting in low between-individual variation. However, in addition to the prey community composition and diversity, other ecological drivers may similarly constitute ecological opportunity and may therefore influence trophic niche variation in turn (Araújo et al. 2011). In particular, the influence of environmental drivers on trophic niche variation were recently highlighted (de Camargo et al. 2019; Lunghi et al. 2020; Musseau et al. 2015), however they remain largely overlooked in studies of trophic ecology. Furthermore, despite the prominent role of the spatial distribution of resources (reviewed in Walker et al. 2023) in the Optimal Foraging Theory (OFT; MacArthur & Pianka, 1966), few studies have evaluated the influence of spatial heterogeneity on individual diet variation (Araújo et al. 2011; but see Darimont et al. 2009; Lunghi et al. 2020; Walker et al. 2023).

Trophic ITV can be quantified by measuring two individual trophic traits: between-individual trophic variation (BIC; related to β-diversity, often related to individual specialization) and individual niche width (INW; related to α-diversity). These trophic traits are expected to vary according to ecological opportunity (Araújo et al. 2011; Bolnick et al. 2003). Overall, the biotic processes that drive trophic traits (e.g. prey availability, competition) are now generally well understood (Araújo et al. 2011), and have been revealed in a number of species (Bolnick & Ballare, 2020; Costa-Pereira et al. 2019; Sánchez-Hernández et al. 2021). On the contrary, how abiotic drivers (i.e. related to physical habitat conditions) drive trophic ITV remains unclear. Moreover, when abiotic conditions are accounted for in trophic studies, their effects are generally interpreted as being the result of hypothetical prey-mediated processes, with larger or more structurally diverse habitats presenting more diverse prey communities (MacArthur, 1965) and thus indirectly drive trophic ITV (Araújo et al. 2011; Layman et al. 2007). In this context, habitat conditions and structure are expected to drive variation in prey availability. For example, favourable habitat conditions (e.g. related to climate, elevation, vegetation, river flow regime) may indirectly promote more stable and/or diverse foraging options for predators (Dermond et al. 2018; Lunghi et al. 2020). However, habitat conditions are also known to modify predator foraging behaviour and could thus directly contribute to driving trophic niche variation, independent of prey availability. For example, habitat structural complexity was found to drive greater between-individual diet variation in a neotropical marsupial, likely due to reduced overlap in individual home ranges compared to

less complex habitats (de Camargo et al. 2019). Habitat conditions may also influence predator foraging strategies, for example water-turbidity may affect aquatic predators' selectivity when foraging, with predators adopting an opportunistic strategy when turbidity is high (Bartels et al. 2012). Thus, as abiotic conditions may simultaneously drive prey availability for predators and foraging behaviour, it is important to disentangle indirect (i.e. resource-mediated) and direct effects to understand how abiotic factors mechanistically drive trophic niche variation. To this end, it is necessary to account for both prey and habitat variables that may actively interact and that may also co-vary through different modalities (Grace & Keeley, 2006).

The use of metabarcoding data is now widespread in dietary studies (Alberdi et al. 2019; Cuff et al. 2022). However, metabarcoding data has rarely been used to estimate individual traits for studying inter- and intra-population level of trophic niche variation (Bison et al. 2015; Soininen et al. 2015; Villsen et al. 2022a; Villsen et al. 2022b). Trophic ITV and the conventional analytic framework of trophic analysis were initially developed for diet data obtained by stable isotope analyses or from stomach (or gut, or faeces) content analyses. Metabarcoding approaches most closely resemble classical morphological analyses of stomach, gut or faeces contents as they use the same type of samples to identify prey. Compared to morphological analyses, metabarcoding is known to be poorly quantitative and provide rough 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 usually 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). The most fundamental difference between stable isotope approaches and stomach, or gut or faeces 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, metabarcoding approaches generally provide a snapshot of the diet of a given individual, likely accounting for one or a few days of feeding (Corse et al. 2015). Trophic traits calculated from short-term metabarcoding data are therefore ideal for studying the fine-scale drivers of trophic ITV, as traits can be directly related to snapshot estimates of ecological opportunity (e.g. prey availability or habitat structure).

The Rhone streber (*Zingel asper* Linnaeus, 1758) is a critically endangered riverine fish, endemic to the Rhone River basin (France and Switzerland). Its current spatial distribution is limited to five disconnected populations, which represents less than 15% of its historical range (Olivier et al. 2022). The decline of *Z. asper* is suspected to be associated with habitat loss and fragmentation caused by human activities (*e.g.* dams, river regulation, channelization) (Mari et al. 2002). *Zingel asper* is a species with relatively sparse population densities and a limited diel displacement range (50-200m; Danancher et al. 2004; Labonne & Gaudin, 2005). It inhabits small to medium sized streams and its diet mostly consists of macroinvertebrates, but it can also occasionally consume small fishes (Cavalli et al. 2003; Villsen et al. 2022a). A recent study used faeces metabarcoding to describe spatiotemporal trophic ITV in *Z. asper* (Villsen et al. 2022a). Authors found marked seasonal diet variation, characterised by high consumption of *Baetis* and Heptageniidae mayflies in spring and summer. However, in autumn *Z. asper* shifted to consuming a broad range of secondary prey (e.g. Chironomidae,*Hydropsyche* , *Gammarus*). Coinciding with this autumnal niche expansion, *Z. asper* exhibited higher between-individual niche variation and narrower individual niche widths. Such seasonal trophic ITV is generally associated with seasonal variation in ecological opportunity (Costa-Pereira et al. 2017; Gerardo Herrera et al. 2008), for example seasonal variation in prey assemblages is a known driver of seasonal trophic niche variation (Hoenig et al. 2021; Shutt et al. 2020; Varpe & Fiksen, 2010). Riverine systems present ephemeral prey and habitat conditions for benthic predators that can vary considerably between seasons. For example, seasonal variation in macroinvertebrate assemblages is promoted by species-specific phenology (e.g. voltinism and seasonal emergence events) (Clifford, 1982; Kong et al. 2019). Aquatic insect abundances also vary according to local habitat conditions that may also vary between seasons such as substrate composition (Williams & Mundie, 1978), fine-sediment load (Kaller & Hartman, 2004), vegetation (Downes et al. 2000) or river flow dynamics (Monk et al. 2008). Trophic ITV in riverine predators like *Z.*

asper is likely determined jointly by prey availability and habitat conditions that actively interact across fine spatial and seasonal scales.

The aims of this study were (i) to determine and characterise how ecological opportunity (both prey and habitat dimensions) drive trophic ITV in *Z. asper* and (ii) to test the hypothesis that seasonal variation in ecological opportunity drives seasonal trophic niche variation in *Z. asper*. We quantified biotic conditions that were considered likely to influence the trophic niche of *Z. asper* , including prey richness and diversity as well as preferred and secondary prey abundance. Similarly, we quantified abiotic conditions that are known to affect benthic fish and macroinvertebrate assemblages (i.e. the main prey of *Z. asper*) such as substrate size, clogging and water velocity. To account for within-river variation in prey and habitat conditions and thus capture the distribution of ecology opportunities, we adopted an extensive sampling protocol aiming to characterise representative habitat types (i.e. runs, riffles and pools) available to *Z. asper* . We then related short-term trophic traits obtained via faeces metabarcoding to fine-scale estimates of prey and habitat conditions using causal analyses. In doing so, we expected to shed light on how habitat and prey conditions interact to jointly determine the ecological opportunities of a predator and thus its trophic niche. Indeed, understanding how *Z. asper* varies its trophic niche in relation to ecological opportunity promises to yield important insights into favourable foraging conditions with applications for its conservation and management (e.g. Agosta, 2002; Johnson et al. 2009; Titulaer et al. 2017).

Materials and Methods

2.1 Fish and faeces sampling.

Zingel asper faeces sampling was performed at 9 sampling sites across the Rhone River basin (Figure 1). At these sites, a total of 23 sampling campaigns were performed between 2014 and 2015 in diverse seasonal conditions, i.e. autumn, summer and spring (Table 1). Fishes were caught by electro-fishing in accordance with permits from the French Directions Départementales des Territoires (DDTs) from Hautes-Alpes, Alpes de Haute Provence, Ardèche and Jura. Once captured, fishes were laid in a plastic, wire mech fishpond until faeces collection. The abdomen of *Z. asper* individuals was gently pressed by hand to drain out faeces. Faeces were immediately placed in a 2ml vial containing 96% ethanol and stored at -20°C. Fishes were then released within the sampling area. A total of 1932 *Z. asper* individuals were caught and 726 faeces samples were collected (maximum of one faecal sample per fish per sampling campaign). The faeces data from young-of-the-year individuals were discarded from subsequent analyses due to their distinct diet compared to juveniles and adults (Villsen et al. 2022a). After removing young-of-the-year samples, the final diet dataset included 696 faeces samples, including those obtained from Villsen et al. 2022a and Villsen et al. 2022b (Table 1).

2.2 Diet metabarcoding protocol

Faecal DNA extractions and diet metabarcoding were performed as detailed in (Corse et al. 2017, 2019). We then used a robust experimental design to produce relevant estimates of diet traits for characterizing individual and population trophic niche variation using metabarcoding data. This robust metabarcoding protocol included (i) three distinct primer sets that target an overlapping region of the 5' end of the Cytochrome *c* oxidase subunit I gene (COI) (see: Corse et al. 2019) to minimize false negatives and to comprehensively cover the taxonomic diversity of prey and (ii) the filtering procedure described by Corse et al. (2017) and recently reimplemented in VTAM (González et al. 2023), which integrates negative controls, positive controls, and technical replicates (i.e. PCR triplicates) to minimize false positives, ensure repeatability and validate dietary metabarcoding data within and between high-throughput sequencing runs. VTAM notably explicitly uses the sequencing outputs of negative and positive controls (two distinct mock community samples), and of exogenous samples to set filtering thresholds for discarding false positives in faecal samples (i.e. experimental/molecular artefacts such as PCR/sequencing errors, tag switching and cross-sample contaminations). The sequencing output of technical (PCR) replicates was used to further ensure the reproducibility of ASVs, and chimeras and pseudogenes were also discarded. Lastly, ASVs that were identical in their overlapping regions (~130bp) for all three primer sets were combined into contigs (further details in Corse et al. 2017, 2019).

Prey abundances in faeces were estimated using the Minimal Number of Individuals (MNI; White, 1953) approach. The MNI provides a conservative estimate of prey abundance based on the number of distinct ASVs/contigs detected in each faeces sample for a given prey taxon (Corse et al. 2017). Admittedly, 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). However, a previous study demonstrated that the differences in genetic diversity between the prey of *Z. asper* are only marginally biased the MNI (Villsen et al. 2022a). We therefore assume the MNI to be a reliable quantitative estimate of prey abundance in the faeces of *Z. asper* .

2.3 Individual trophic traits

Individual niche widths (INW; related to α-diversity) and the between-individual Component of the trophic niche (BIC; related to β-diversity) trophic traits were calculated for each dietary sample. Individual niche widths (*INW*) were calculated using the Shannon diversity index (Shannon, 1948). Variation among individuals was based on the proportional similarity index using the *PSicalc* function (package RInSp, Zaccarelli, Bolnick, & Mancinelli, 2013) which measures the diet overlap between an individual and its population (Bolnick et al. 2002). We calculated a *BIC* estimator (see Bolnick, Yang, Fordyce, Davis, & Svanbäck, 2002), which corresponds to the inverse of the proportional similarity index to estimate the overall prevalence of individual specialization (Bolnick et al. 2007; Quevedo et al. 2009). Hence, *BIC* approaches 1 when an individual's diet differs greatly from that of the population as a whole, while *BIC* approaches 0 when its diet is similar to the population's diet.

2.4 Prey community characterisation

To obtain a fine-scale estimation of the ecological opportunities of *Z. asper* in each sampling campaign we performed extensive prey community and habitat sampling. For each sampling campaign, from 45 to 90 prey community and habitat sampling points (1708 sampling points in all) were characterized. Prey community sampling was performed 1-2 days before or after fish sampling. The sampling effort was distributed among the representative habitats (i.e. riffles, runs, glides and rare pools) in the fishing area. Samples were collected using a Surber sampler by perpendicular transects between riverbanks, from downstream to upstream. One to five Surber samples (0.05 m^2) were collected per transect in all accessible habitats (i.e. <80 cm depth; Villsen et al. 2022b). Macroinvertebrates were immediately stored in 96% ethanol for subsequent identification in the laboratory. Macroinvertebrates were assigned to genus using morphological criteria (Tachet et al. 2010), when this was not feasible using morphology however (e.g. due to the development stage of larvae), taxa were aggregated at higher taxonomic levels (i.e. family or subfamily). For comparison purposes, the resolution of taxonomic identification of macroinvertebrates was further harmonised between morphological identification obtained for Surber samples and the identification obtained via metabarcoding (most often more resolutive) for the faeces of *Z. asper* . The final macroinvertebrate inventory comprised 82 taxa (Table S1).

Using this dataset we summarized the prey community for each sampling campaign (i.e. site and date), separately. α-diversity was estimated as mean prey richness, mean prey diversity (Shannon index). The spatial heterogeneity of the prey community was estimated as the mean Bray-Curtis dissimilarity between samples. We also calculated the mean density of four key prey taxa (*Baetis,* Heptageniidae, Chironomiidae (excluding Orthocladiinae) and Orthocladiinae according to previous diet and growth studies on *Z. asper* (Monnet et al. 2022; Villsen et al. 2022). Prey size is known to affect predator-prey interactions via its effect on the visible detectability and nutritional quality of prey (e.g. Worischka et al. 2015). Furthermore, the abundance of various size cohorts of the two main prey taxa of *Z. asper* (*Baetis* and Heptageniidae) are known to greatly vary due to phenology (i.e. voltinism), river flow and seasonal dynamics (e.g. Erba et al. 2003; Haidekker & Hering, 2008). In order to account for the variation in abundance of different size cohorts, we separated*Baetis* and Heptageniidae into two size-classes: small (body length < 5 mm), and large (body length [?] 5 mm) (see: Table S1). Prey community abundance data were *ln+1* transformed for subsequent analyses. Lastly, we quantified the spatial heterogeneity (by sampling campaign) of each the above-described estimators as the coefficient of variation (*CV*):

$$CV = \frac{\sigma}{m} * 100,$$

where σ is standard deviation and μ is the sampling campaign mean. In all, the prey community was described using 22 variables: 11 mean estimates and their corresponding coefficients of variation (Supplementary information 1).

2.5. Habitat characterisation

In addition to macroinvertebrate sampling, we measured habitat conditions that both drive the distribution of macroinvertebrates at the microhabitat scale and may shape the foraging habitat of fishes. For each sampling point (see above, Section 2.4) we measured: substrate size-class richness, maximum substrate size-class, current velocity, water depth, substrate clogging and vegetal development. Substrate size-classes were evaluated using the semi-quantitative method of Malavoi & Souchon (1989), the scale ranged from 0 (silt: $0.0039 - 0.0625mm$ diameter) to 10 (bedrock: $> 1024mm$ diameter) see Table S2 for more details. The current velocity was measured at 3 cm from the river bottom. Substrate clogging was evaluated visually using a semi-quantitative scale ranging from 1 to 5 (see Table S3) designed to describe the level of substrate embeddedness and the clogging of the interstitial space by silt and algae (Archambaud et al. 2005). Vegetal development was characterised using a semi-quantitative method based on a theoretical climax condition of vegetation mass. Depending on the dominant vegetation type (e.g. *Cladophora sp.* algae thalli or *Myriophyllum sp.*) a maximum development mass was approximated corresponding to 100% development. The vegetation development of samples was then estimated based on this climax condition (0 - 100%) and separated into classes (class 1; $0 - 25\%$, class 2; $25 - 50\%$, class 3; $50 - 75\%$, class 4; $75 - 90\%$, class 5; $90 - 100\%$).

For each sampling campaign, habitat conditions were summarized as the mean value of each variable and their corresponding coefficient of variation (CV; see formula above). The structure of the habitat was described by 12 estimators (Supplementary information 2).

2.5. Statistical analyses

All data formatting and statistical analyses were performed using R v4.2.0 (Core Development Team, 2023).

2.5.1 Prey preferences of Zingel asper

Predators are expected to modify their foraging strategy in relation to fluctuations in preferred prey availability (e.g. Tinker et al. 2008). We therefore characterised prey selection to determine the preferred prey of *Z. asper* . Prey selection can be estimated by comparing observed prey consumption to the composition of prey taxa in the environment. We performed tests of selection for all taxa that occurred in at least 5% of *Z. asper* diet samples pooled across all sampling campaigns. Tests were performed for each sampling campaign separately using the *econullnetr* package (*generate_null_net* , sims = 1000) which calculates null model estimates of prey consumption based on observed individual diet breadth and prey availability (Vaughan et al. 2018). Three outcomes are possible for electivity tests according to the relation between observed consumption and null expectations: (i) observed > null 95CI%; positive electivity, (ii) observed = null 95CI%; neutral electivity and (iii) observed < null 95CI%; negative electivity.

2.5.2. Spatiotemporal variation of ecological opportunities and habitat structure

In order to summarize the underlying spatiotemporal variability in prey and habitat conditions among sampling campaigns we performed a Principal Components Analysis (function PCA, package FactoMineR; Lê et al. 2008). All variables were scaled to a zero mean and 1SD distribution. Results were presented in two separate biplots for prey and habitat conditions (function fvis_pca_biplot, package Factoextra; Kassambara & Mundt, 2017).

2.5.3. Identifying and characterising resource and habitat effects on individual trophic traits

We first aimed to identify key resource and habitat variables and to characterise their effects on individual trophic traits (INW and BIC). Variable selection for prey and habitat estimators was performed using a random forest based ranking procedure from the randomForest package (Liaw & Wiener, 2002). The

randomForest function was run (ntree = 500) for each of the four variable sets (BIC ~ habitat; BIC ~ prey; INW ~ habitat; INW ~ prey). The number of random variables used in each tree (mtry) was set as 5. Variables were ranked in function of their importance values (Increase of Mean Squared Error %IncMSE) for each variable set, with the 5 best performing variables being selected in each case. In order to avoid variable inflation in subsequent casual analyses, we screened variable sets using a *variance inflation factor approach. Variables with high variance inflation factor scores (VIF > 4) were* discarded sequentially starting from the highest scores *(function vif, package car;* Fox et al. 2013) and were replaced with the next best ranked variable according to %IncMSE. *Variable inflation was calculated based on linear mixed regression models (y ~ x1 + x2 + x3 + x4 + x5) including site and year as random effects (function lmer; package lme4;* Bates et al. 2015). *The variable selection procedure and resulting variable sets are detailed in Supplementary information 1 and 2.*

2.5.4. Causal analysis: The interaction between habitat, prey and ITV

The distribution of benthic macroinvertebrates and thus prey availability for aquatic invertivores, is in part driven by habitat conditions (Haidekker & Hering, 2008; Leitner et al. 2015; Monk et al. 2008). Thus, habitat effects on trophic niche variation may be theoretically decomposed into indirect (prey-mediated) and direct effects. To characterise habitat and prey effects on trophic traits, we adopted a casual analysis approach using d-sep tests (function*pSEM;* package *PiecewiseSEM; Lefcheck, 2016; Shipley, 2009)* . D-sep tests involve constructing a global casual model from several smaller models, thus limiting the risk of over-parametrization. We composed casual models from linear mixed models (*lmer* function, *lme4* package; Bates et al. 2015) that included site and year as random effects to account for spatiotemporal effects. The normality of response variables was evaluated visually using density plots. We found that INW had a skewed distribution and was thus log transformed to meet the assumptions of linear regression models (Bolker et al. 2009). Habitat and prey variables were selected via randomForest (detailed in 2.5.3). We considered that (i) habitat and prey variables could directly influence trophic traits (INW & BIC), (ii) habitat variables could indirectly influence trophic traits via a prey-mediated effect, (iii) trophic traits could not influence either habitat or prey variables (top-down control by *Z. asper* is unlikely due to its low population density (Villsen et al. 2022*a*). *The framework used for casual analysis is illustrated in Figure 2.* Goodness-of-fit was evaluated using Fisher's C and associated p-values, with non-significant values indicating good model fit ($p > 0.05$). Variable effects on trophic traits were assessed as the standardised regression estimate for each path and associated p-value. The direct effect of habitat variables was estimated as the standardised regression estimate (i.e. BIC/INW ~ H_i). The indirect effect was calculated as the standardised effect of habitat variable i on prey variable$j$ multiplied by its standardised effect on trophic traits (i.e. effect H_i ~ P_j * effect P_j ~ BIC/INW). Lastly, to evaluate model performance we obtained marginal (fixed effects) and conditional (fixed and random effects) R^2 values for each response variable (trophic traits & prey variables).

2.5.5. Drivers of seasonal BIC variation in *Zingel asper*

In order to understand the mechanisms driving seasonal BIC variation in*Z. asper* (Villsen et al. 2022a) we quantified seasonal variation for the prey and habitat variables identified in casual models (see section 2.5.4). To test the seasonal effect in key variables, we constructed linear mixed models (lmer function, lme4 package; Bates et al. 2015) with a seasonal fixed effect and, site and year random effects. We then tested the significance of seasonal differences via post-hoc Tukey tests (function emmeans, package emmeans; Lenth, 2019).

Results

3.1. Metabarcoding data

The raw data set was gathered from 28 distinct MiSeq runs. Once past the filtering procedure, 2724 ASVs were validated. After combining the ASVs of three COI overlapping markers, 709 contigs and 1025 ASVs were obtained. A total of 13 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 640 distinct Macrometazoan ASVs/ contigs (corresponding to 226 prey taxa) were obtained from the 742 *Z. asper* faeces containing validated ASVs or contigs. 69% of the prey ASVs/contigs were identified to the species level, another 17% to the genus level, 12% to the family or subfamily level and 2% to the order or class level (Table S4).

3.2. Prey preferences of Z. asper

Zingel asper consistently selected its main prey *Baetis* (18/23 campaigns) and Heptageniidae (16/23 campaigns) across its entire range (Figure 3). The rare cases in which *Baetis* and Heptageniidae were neutrally or slightly negatively selected mostly occurred in autumn (e.g. 14HenB & 15HenB). Other prey were also positively selected, but preferences were either river-dependent (e.g. Psychomiidae in the Loue River) or date-dependent (e.g. Gammaridae and *Hydropsyche*). *Zingel asper* also frequently consumed Orthocladiinae and other chironomids, but mostly less than would be expected based on their high availability in the environment.

3.3. Spatio-temporal variation in ecological opportunity and diet

Principal components analysis revealed marked spatial structure in prey availability and habitat conditions (Figure 4). Variation in prey availability among rivers was mostly explained by the horizontal axis which was characterised by Heptageniidae (small and total) abundance, spatial heterogeneity and to a lesser extent total α-diversity and chironomid abundance (Figure 4a). The vertical axis was characterised by spatial heterogeneity (i.e. cv-Richness and Bray-Curtis) and mostly described variation within rivers. The Durance exhibited the most diverse range of prey community conditions and was more comparable to the geographically close Buëch and Verdon Rivers. The Verdon and Loue Rivers exhibited the most distinct prey communities, characterised by depauperate and rich prey communities in terms of α-diversity indices, respectively. The spatial variation of the prey community was mirrored in the habitat conditions (Figure 4b). The Durance River exhibited the greatest range of habitat conditions and were more closely related to conditions observed in the Buëch and Verdon Rivers. Differences among rivers were equally explained by both axes, with the Durance being associated with fast-flowing habitats, high substrate clogging and size-class diversity. The Beaume and the Loue Rivers were characterised by deeper and more spatially heterogeneous habitats in terms of substrate size diversity and river flow. Finally, the Verdon River exhibited the narrowest range of habitat spatial variation. Spatio-temporal variation in diet composition was largely characterised by preferred prey (*Baetis* and Heptageniidae) and secondary prey (Orthocladiinae and fish). Diet composition also tended to follow a geographical pattern, with more similar diets between the Durance, Buëch and Verdon basins compared to the more distant Beaume and Loue basins.

3.4. Causal analysis

Using piecewise SEMs, we investigated the interaction between habitat and prey variables, decoupling direct and resource-mediated habitat effects on trophic traits (BIC and INW). Model performance was good for both BIC and INW models, but particularly for the BIC model, explaining 55% (R^2 marginal = 0.55, R^2 conditional = 0.86) of the variation compared to 11% for INW (R^2m = 0.11, R^2c = 0.28). The BIC model was characterised by habitat effects that were exclusively positive, and both positive and negative prey effects (Figure 5a). The magnitude of the direct habitat effect on BIC was comparable to the direct prey effect, but the strongest effect was the indirect (prey-mediated) habitat effect (Figure 5b) which was almost two-fold stronger than either the direct habitat or prey effects. This indirect habitat effect was largely associated with substrate clogging, which was a key driver of the prey community. Substrate clogging was associated with higher spatial heterogeneity in the prey community (i.e. prey richness, Bray-Curtis dissimilarity and large Baetis abundance), lower preferred prey abundance (i.e. large Heptageniidae) and lower prey richness. In contrast, vegetal development had largely the opposite effect on the prey community, reducing spatial heterogeneity and promoting higher preferred prey abundance and overall richness. Sediment clogging was thus doubly associated with high BIC, first by its direct positive effect and second by its indirect effect via the prey community. Direct habitat effects on BIC were all positive, including sediment clogging, substrate size-

class, vegetal development and spatial heterogeneity in sediment clogging. Prey community effects ranged from strongly positive (i.e. prey richness and spatial heterogeneity in prey richness) to negative (i.e. large Heptageniidae abundance) and slightly negative (i.e. spatial heterogeneity of large Baetis). See Table S5 for full details.

In contrast, the INW model was characterised by prey variables and the prey-mediated effect of habitat variables (Figure 5c, d). Individual Niche Width was solely explained by a negative relationship with the spatial heterogeneity of Heptageniidae. The prey-mediated habitat effect was mainly characterised by vegetal development and the spatial heterogeneity of water velocity. Like in the BIC model, vegetal development was associated with higher prey richness and secondary prey abundance (i.e. Orthocladiinae) and lower spatial heterogeneity in the prey community (i.e. prey richness, Orthocladiiane). While the spatial heterogeneity of water velocity tended to have the inverse effect, promoting spatially heterogeneous prey communities and lower overall richness and abundance. See Table S6 for full details.

3.5. Seasonal variation in ecological opportunity

In accordance with our initial hypothesis, prey variables associated with trophic traits appeared to follow a clear seasonal trajectory from spring to autumn conditions (Figure 6). This seasonal trajectory was most notable for prey richness and large preferred prey abundance (Heptageniidae) which declined progressively from spring to autumn. Heterogeneity in the prey community generally increased in autumn compared to spring and summer (i.e. prey richness and Heptageniidae), but spatial heterogeneity in the abundance of large preferred *Baetis* individuals was comparable between summer and autumn, despite being notably lower in spring. Most habitat variables also exhibited some degree of a seasonal trajectory (Figure 6), except for substrate-size class. The most marked seasonal variation was observed for substrate clogging which greatly increased in autumn and in vegetal development which declined from spring to summer.

Discussion

This aim of this study was to understand how prey and habitat factors drive trophic Individual Trait Variation (ITV) in the benthic invertivorous fish, *Zingel asper* . Thanks to our combined use of high-resolution diet data obtained via faeces metabarcoding and fine-scale prey community and habitat sampling protocol, we were able to obtain mechanistic details of the prey- and habitat-related processes that drive individual trophic traits. On one hand, individual niche width was only explained by prey-related processes (including an indirect habitat effect). While on the other hand, between-individual trophic variation (BIC) was largely explained by habitat effects which were either directly associated with higher BIC or indirectly by favouring prey conditions that were associated with higher BIC. Surprisingly, we demonstrated that the direct habitat effect was comparable in strength to the direct prey community effect on BIC. This is a significant departure from the currently accepted notion that trophic ITV is mainly driven by biotic (Araújo et al. 2011) or biotic-mediated processes (Layman et al. 2007), and rarely by direct abiotic effects (but see Bartels et al. 2012). Our results shed light on the abiotic dimension of ecological opportunity and how it interacts with prey conditions to determine trophic ITV.

4.1. Is habitat an overlooked dimension in trophic ecology studies?

In this study we demonstrated that the importance of direct habitat-related processes in driving BIC was non-negligible and actually comparable to prey community-related processes. An implication of this result is that habitat may promote trophic ITV independently of its effect on prey availability. In this sense, the resources that are available to any given individual (i.e. ecological opportunity) is not uniquely dependent on the presence and abundance of prey but also of physical conditions that likely influence predator foraging. Indeed, the *optimal foraging theory* posits that the net energetic benefits of a given prey is not only dependent on prey traits (e.g. abundance, size, nutritional content) but also environmental constraints that influence foraging efficiency (e.g. visibility, prey refuges) (Perry & Pianka, 1997; Townsend & Winfield, 1985). Thus, trophic ITV may arise in cases wherein beneficial habitat conditions promote selective foraging or when unfavourable conditions promote opportunistic foraging. For example, the direct habitat effect on BIC in

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Z. asper was largely related to fine-sediment deposition (i.e. clogging) and the size of the largest substrate (Figure 4a). As visual predators tend to exhibit variation in foraging success among different substrate-types (i.e. related to complexity, coloration, size) (Angermeier, 1985; Beekey et al. 2004; Nguyen & Crocker, 2006), it may hold that substrate conditions affect foraging success in *Z. asper* and thus promote either selective or opportunistic foraging strategies. As a benthic invertivore, substrate conditions may therefore directly influence BIC in *Z. asper* by promoting different degrees of foraging selectivity.

Within-population differences in habitat-use between *Z. asper* individuals may also contribute to explaining the direct effect of habitat on BIC. Overlap in habitat-use between predators and prey has been highlighted as a key factor that determines prey predation-risk and thus diet in riverine predators (Worischka et al. 2012). Dietary differences among individuals may therefore intensify if individuals occupy distinct habitat spaces. de Camargo et al. 2019 demonstrated this principle in a neotropical marsupial wherein variation in vertical habitat-use was related to between-individual diet variation (nestedness in their study). Habitat conditions that promote spatial isolation among individuals may therefore also promote divergent diets. Because substrate clogging is detrimental and actively avoided by some benthic fishes (Kawanishi et al. 2015), it may present a barrier to movement for *Z. asper* . The strong direct effect of substrate clogging on BIC may therefore also relate to a spatial isolation effect, wherein lower foraging area overlap similarly relates to lower dietary overlap between individuals. Habitat-related effects on trophic ITV are generally associated with hypothetical prey-mediated effects (Layman et al. 2007) and direct habitat effects are rarely considered (but see: Musseau et al. 2015). By simultaneously accounting for habitat and prey community effects, this study demonstrated that habitat conditions may also directly influence trophic ITV, irrespective of prey-mediated processes. In this sense, habitat conditions may constitute a previously hidden dimension of ecological opportunity and actively drive how predators interact with and perceive their prey community.

4.2. Spatial heterogeneity drives trophic ITV

Despite the prominent role of food distribution in OFT models, wherein the spatial heterogeneity of resources is expected to greatly affect consumers' access to their trophic resources (Charnov, 1976; R. H. MacArthur & Pianka, 1966; Stephens & Krebs, 1986; Westoby, 1974), relatively few studies have evaluated how landscape heterogeneity influences individual diet variation in wild populations (Darimont et al. 2009; Robertson et al. 2015, Trevail et al. 2021; Walker et al. 2023).

Especially in foragers with small home ranges, high site fidelity, and limited mobility, diet selection is constrained by spatial variation in the distribution of prey because individuals have only a subset of the population-level resource base available to them. Consequently, in systems where high-quality foods are heterogeneously distributed, individual diets may be both differentiated and "optimal" depending on the distribution of resources in each individual's home range (Stephens & Krebs, 1986). Thanks to our extensive sampling effort of the prey community, we were able to estimate the spatial structure of prey revealing a marked positive effect of the spatial heterogeneity of prey richness on BIC. In fact, spatial heterogeneity variables made up the majority of prey variables that resulted from our variable selection process, further supporting their importance as drivers of BIC and INW. In our study, trophic ITV appeared to mirror the spatial structure of prey resources, in the sense that prey heterogeneity also led to diet heterogeneity at the population-level (i.e. *BIC*) and narrower niches at the individual-level (i.e. INW). As a benthic predator with limited mobility, it is logical that a highly structured prey community would also promote dietary variation in *Z. asper* . Spatially heterogeneity factors appeared to be highly complementary to classic measurements of alpha diversity, in our case prey richness, explaining different aspects of ecological opportunity for *Z. asper* . High ecological opportunity is expected to promote dietary divergence (i.e. BIC) due to a greater capacity to partition resources among individuals (Araújo et al. 2011; Costa-Pereira et al. 2019; Sánchez-Hernández et al. 2017). However, overall alpha diversity poorly represents individual access to resources which will likely vary across spatial scales. This is especially the case of river systems which represent mosaics of diverse habitat types (e.g. runs, riffles, pools) with unique macroinvertebrate communities (Perez Rocha et al. 2018). The high performance of our BIC-model, explaining 55% of the total variance, highlights the importance of quantifying ecological opportunity not only as alpha diversity of prey availability, but also in terms of their spatial distribution.

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We join our voices to Walker et al. 2023 calling for trophic studies to account for spatial heterogeneity in resource conditions across landscapes to better characterise the mechanisms that drive intra-specific trophic variation.

4.3. Ecological opportunity drives seasonal ITV in Zingel asper

Seasonality in ecological opportunity is expected to cause predators to adopt alternative foraging strategies between seasonal contexts, leading to seasonal trophic ITV (e.g. Costa-Pereira et al. 2017). *Zingel asper* is known to undergo a significant seasonal trophic niche shift, wherein individuals appear to specialise on *Baetis* and Heptageniidae mayflies in spring and summer and subsequently diversify their niche in autumn (Villsen et al. 2022a). In this study we confirmed our initial hypothesis that *Z. asper* positively selects *Baetis* and Heptageniidae, and supplements its diet with secondary prey (Orthocladiinae, *Hydropsyche*, *Gammarus* etc.) in autumn when its preferred prey are scarce (Figure 3). Furthermore, we provide evidence that the autumnal niche shift is not simply related to preferred prey abundance, but also their spatial distribution and the richness of the prey community as a whole. The depletion of high-value prey is predicted to lead to dietary diversification by the *optimal foraging theory* (Ivlev, 1961; Perry & Pianka, 1997; Tinker et al. 2008). However, the spatial distribution of the prey community is a key aspect of this prediction, determining the opportunity cost of rejecting a prey and searching for higher value prey (R. H. MacArthur & Pianka, 1966). Our results support this notion as the entire prey community appears to be less rich in autumn, in terms of high quality large preferred prey, their spatial distribution and prey richness. It is likely that the high BIC observed in autumn is indicative of an opportunistic foraging strategy as the opportunity cost of rejecting prey encounters increases for *Z. asper when its prey community is comparatively scarce* .

Our causal analysis approach also provides an explanation for the seasonal decline in the quality of the prey community: two key habitat parameters substrate clogging, and vegetal development show clear seasonal variation. We detected significantly higher degrees of substrate clogging and lower vegetal development in autumn. Substrate clogging is known to negatively impact macroinvertebrate communities with mayflies like *Baetis* sp. being particularly sensitive to clogging (Bo et al. 2007; Kaller & Hartman, 2004; Leitner et al. 2015) and its negative effect on the prey community was strongly supported by casual analyses. Similarly, as the main prey of *Z. asper* are largely scrappers, lower vegetal development in autumn may have contributed to a depauperate prey community in autumn. Our results shed light on how both abiotic and biotic dimensions of ecological opportunity may interact to jointly explain seasonal phenomenon like the autumnal niche expansion in *Z. asper* .

4.4. Towards a more comprehensive view of ecological opportunity

Since the ecological opportunity concept was appropriated from evolutionary theory to explain individual trophic niche variation, it has largely been conceptualised as the abundance or diversity of prey (e.g. Sánchez-Hernández et al. 2021). In this context, greater ecological opportunities has been shown to promote resource partitioning within populations (i.e. higher BIC), as individuals are able to specialise on their preferred prey (e.g. Costa-Pereira et al. 2017; Evangelista et al. 2014). However, in the present study we demonstrated that prey diversity or abundance can be complemented with spatial heterogeneity, preferred prey availability and habitat information to greatly improve the estimation of ecological opportunity. Indeed, in addition to prey abundance and diversity, an individual's access to its resources is expected to have profound effects on how it forages and thus its trophic niche (Schoener, 1971). Accounting for both abiotic and biotic aspects of ecological opportunities is more accordance with one of the original definitions of ecological opportunity in trophic ecology: *interspecific competition is presumed to reduce ecological opportunity, but opportunity also depends on factors such as patch size, microhabitat diversity, resource diversity and environmental stability* (Araújo et al. 2011). Since this original definition, there have been examples of patch-size (Bolnick & Ballare, 2020), habitat structure (Bartels et al. 2012; Lunghi et al. 2020; Musseau et al. 2015) and weather (Cuff et al. 2023) influencing individual diet, but these abiotic factors are rarely conceptualised in the ecological opportunity framework. In this study, we highlighted the importance of (i) prey opportunities (e.g. preferred prey abundance; prey richness), (ii) habitat conditions via its direct effect on foraging behaviour and its prey mediated, indirect effect and (iii) spatial variability in ecological opportunities, as key determinants of BIC.

Moving forward, we recommend that trophic ecologists adopt a holistic approach when defining ecological opportunity. Variation in both abiotic and biotic ecological opportunity should have diverse implications for how individuals acquire nutrients and interact with other organisms in their ecosystem (Bolnick et al. 2011; Schreiber et al. 2011). Our understanding of the mechanisms that drive patterns of individual trophic niche variation would be greatly improved by accounting for these complementary dimensions of ecological opportunity.

4.5. Metabarcoding data for mechanistic analyses

This study serves as a proof of concept for the use of faeces metabarcoding diet data for mechanistic analyses. Initially, molecular-based dietary studies mainly described the presence/absence of prey (Corse et al. 2010; King et al. 2008). Later, thanks to the rise of High Throughput Sequencing (HTS) techniques, molecular approaches gained in popularity through the metabarcoding approach (Pompanon et al. 2012). After a few years of development and validation studies, diet metabarcoding has proven to be very useful for characterising complex interactions in trophic networks (Alberdi et al. 2020; Cuff et al. 2023; Pansu et al. 2019). However, a number of recent articles have highlighted the importance of minimizing the biases that can arise within the metabarcoding workflow (Alberdi et al. 2019; Zinger et al. 2019), which can easily lead to false positive and negative detections and thus spurious ecological interpretations (Dickie et al. 2018). In this study, we implemented a highly validated metabarcoding workflow including both negative and positive ('mock' communities) controls, multiple primer pairs (Corse et al. 2017, 2019) and a stringent filtering procedure (González et al. 2023). To our knowledge this is the first time that a highly validated diet metabarcoding dataset has served to study complex ecological interactions like trophic ITV. In addition to allowing us to obtain a large sample size, our faeces metabarcoding approach accurately captured the diverse range of prey consumed by Z. asper and thus interindividual diet variability (i.e. BIC). In river systems, benthic macroinvertebrate community sampling usually targets specific habitat types (e.g. riffles, runs, pools) to estimate prey availability for predators (e.g. Esnaola et al. 2021; Sánchez-Hernández et al. 2021). However, by delimiting continuous habitat conditions (e.g. water-velocity, depth, slope) into discrete groups, important variation in prey and habitat conditions for benthic predators is likely overlooked. The prey community and habitat sampling design of this study was designed to be highly representative of the different habitat and prey conditions available within each sampling site. A more randomised, representative approach seems immediately more appropriate for Z. asper, as individuals tend to space themselves out within populations, rather than converging on preferred habitats (Labonne & Gaudin, 2005). While this approach necessitate an extensive sampling effort (90, 60 or 45 Surber samples per sampling campaign), it provided fine-scale prey community and habitat information allowing us to accurately test the underlying mechanisms of trophic ITV determinism in Z. asper. Its thanks to the dietary resolution of diet data obtained using metabarcoding coupled with extensive habitat and prey community sampling, that allowed us to accurately model the processes that drive trophic ITV in Z. asper.

4.6. Trophic ecology of Zingel asper and conservation implications

In Villsen et al. 2022a, authors hypothesised that Z. asper specialises on preferred prey in spring and summer and adopts an opportunistic feeding strategy in autumn to maintain its body condition. The present study presents a mechanistic explanation of the processes that underly the shift from specialisation towards opportunism in Z. asper, highlighting the importance of preferred prey availability (abundance and spatial distribution) but also key habitat conditions like substrate clogging that may promote opportunistic foraging. Such insights into diet and habitat-use are often used to guide conservation management in the case of endangered species (Agosta, 2002; Titulaer et al. 2017) and facilitate the selection of habitats to be protected (Arrizabalaga-Escudero et al. 2015; Ramírez et al. 2016). For example, we demonstrated that Z. asper nearly universely positively selected *Baetis* and Heptageniidae across its range and appeared to be particularly sensitive to the availability of large individuals within these taxa. As the availability of high-quality prey can have direct implications for life-history traits like survival, growth and energy reserves (Bagenal, 1978; Elliott & Hurley, 2000; Garvey & Whiles, 2016), estimations of habitat quality for Z. asper (e.g. reintroduction sites, ongoing river management) should account for the availability of preferred prey detailed in this study. Indeed,

a previous study demonstrated that growth in *Z. asper* was strongly influenced by the availability of key prey taxa (Baetidae, Heptageniidae and Chrionomidae) (Monnet et al. 2022). Our results further suggest that *Z. asper* is sensitive to spatial availability of its resources and therefore that any estimate of resource quality for this species must imperitively account for not only the abundance of preferred and secondary prey, but also their spatial distribution in the environment. Lastly we also highlighted the negative effect of substrate clogging which simultaneously favoured heterogeneous prey communities, reduced the abundance of preferred prey and promoted dietary divergence among individuals. It will therefore be important to limit the degree of sediment clogging when possible (e.g. with flushing flows in regulated rivers), to avoid potential negative effects on *Z. asper* and its prey. This is especially important as substrate clogging is an ongoing issue in the Durance river (Cazaubon & Giudicelli, 1999; Corse et al. 2015) and risks to endanger the largest and most genetically diverse *Z. asper* population.

Conclusion

Thanks to an extensive characterisation of habitat and prey conditions coupled with faeces metabarcoding diet data, the present study provides important insights into the drivers of trophic ITV for *Z. asper* . The insights obtained in this study include the decoupling of the direct and indirect habitat effects, and the importance of the spatial structure of prey and habitat conditions in ITV determinism. Our detailed characterisation of both abiotic and biotic conditions allowed us to more broadly conceptualise ecological opportunity in the context of trophic ecology, accounting for within-population variation in access to resources and the role of habitat in modifying predator foraging behaviour. Furthermore, the strong habitat effect revealed in this study highlights the need to additionally account for habitat conditions in studies of trophic ecology, not only for their prey-mediated effect but for their direct effect on trophic ITV.

Conflict of interest:

The authors declare no conflict of interest

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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 faeces; (iv) the final diet dataset used to estimate individual trophic traits; and (iv) the macroinvertebrate and habitat datasets used for statistical analysis and modelling.
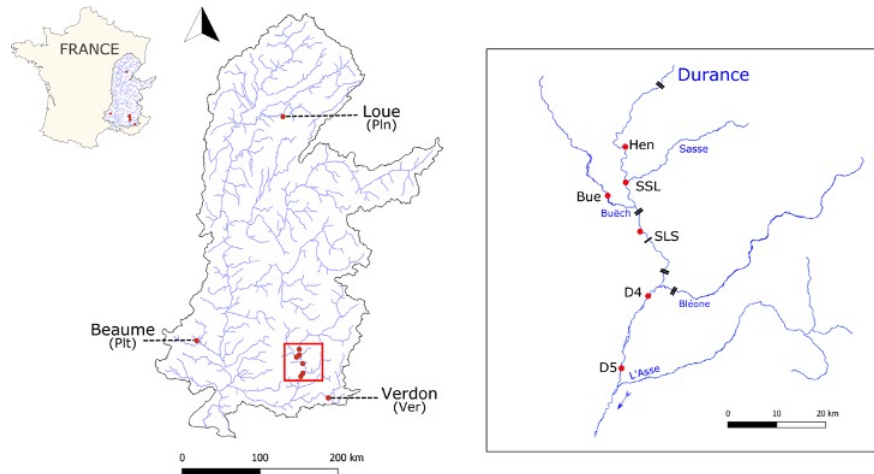
Figure 1. Location of sampling sites *(red dots). Double and single black lines indicate dams and weirs, respectively.*
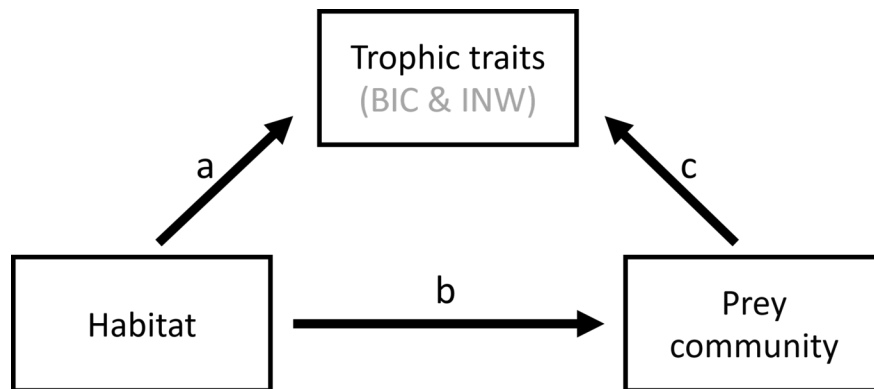

Figure 2. Structural equation model framework . Path "a" relates to the direct habitat effect on individual trophic traits (INW; individual niche width and BIC; between-individual trophic variation). The indirect habitat effect is the product of the "b" and "c" path estimates (i.e. estimate b * estimate c).

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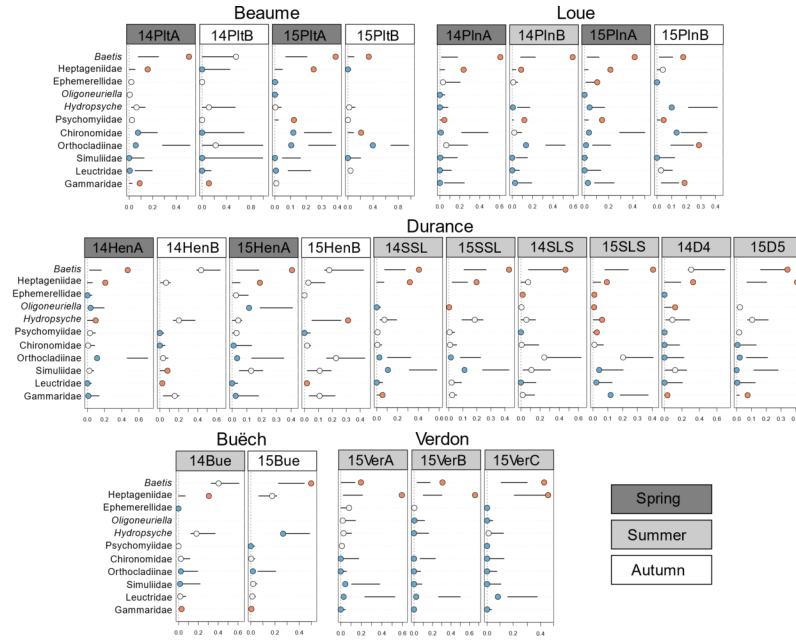
Figure 3. Zingel asper prey preferences. *The position of dots along the x axis indicates observed consumption (dietary proportions; 0-1). The colour of dots indicates deviations from expected frequencies of trophic interactions; Blue, lower consumption than expected; white, as expected (in proportion to relative abundance); red, higher than expected (consumed more frequently than predicted). Horizontal lines denote 95% confidence limits of null model expectations of predation.*
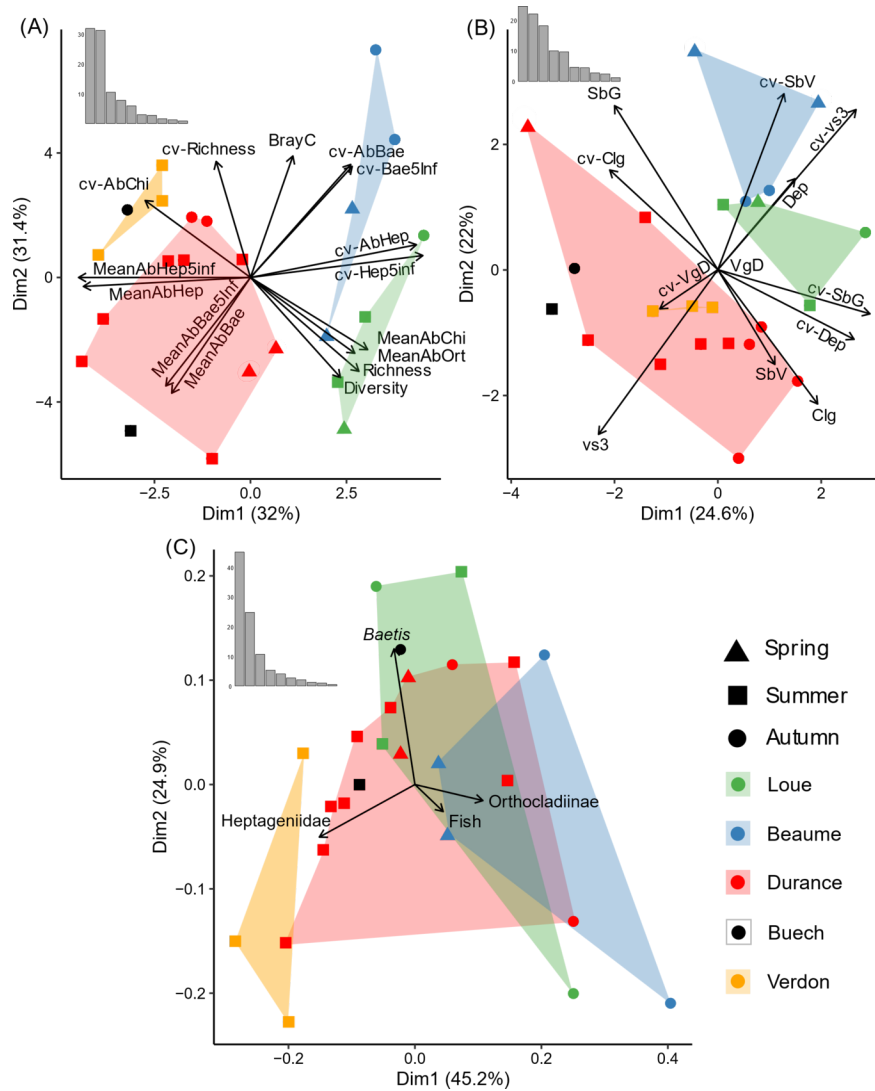
Figure 4. Spatiotemporal variation in (A) prey community, (B) habitat conditions and (C) diet proportions according to Principal Components Analysis . Colours represent river catchments and points indicate sampling campaigns. The prey biplot illustrates the 15 most contributing variables (out of 22 total) while the habitat biplot shows all 12 habitat variables. Prey variable codes relate to: Richness; mean prey richness, mean diversity (Shannon index) BrayC: Mean Bray-Curtis dissimilarity index between sampling points, MeanAbBae; Mean *Baetis* abundance, MeanAbBae5sup; Mean *Baetis* abundance (> 5 mm), MeanAbHep; Mean Heptageniidae abundance, MeanAbHep5sup; Mean Heptageniidae abundance (> 5 mm), MeanAbChi; Mean Chironomidae abundance (without Orthocladiinae), MeanAbOrt; Mean Orthocladiinae abundance. Habitat variable codes relate to: SbV; Mean substrate size-class richness, SbG; Mean of the maximum substrate size-class, Clg; Mean mineral clogging class, Dep; Mean of the water depth (cm), VgD; Mean of the vegetal development class, vs3; Mean current (cm.s-1) at 3cm from the bottom. When a code has the prefix cv- it refers to the coefficient of variation for that variable.
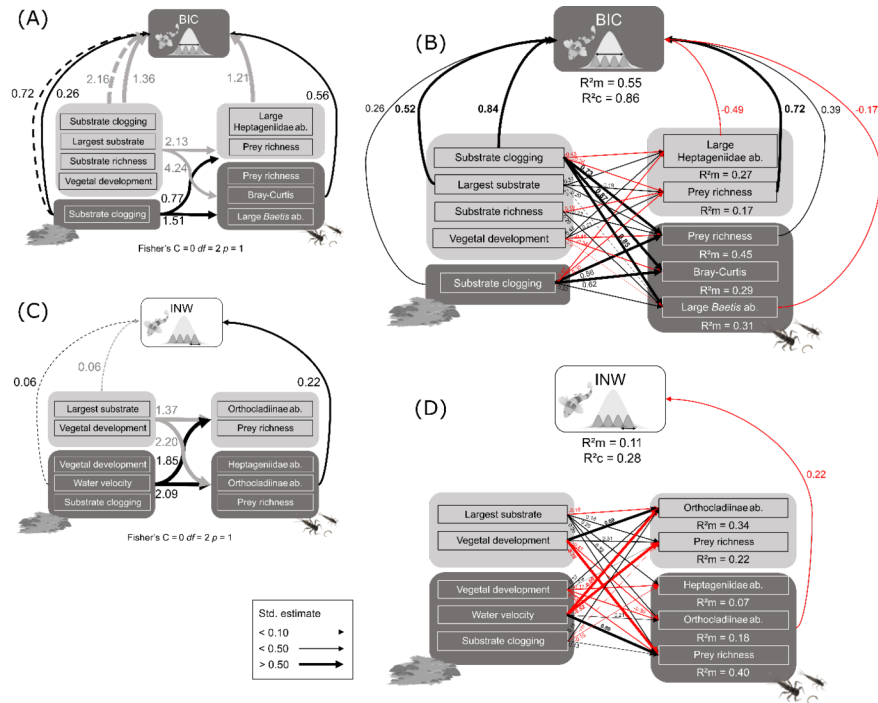
Figure 5 . **Causal relationships between habitat and resource conditions, between-individual trophic variation (BIC) and Individual Niche Width (INW).** Light grey boxes indicate mean habitat and prey variables, while dark grey boxes indicate spatial heterogeneity related variables. Models were constructed using variables with high importance according to randomForest tests (see Supplementary information 1 and 2). Global pSEM models are presented including standardized estimates for causal links (BIC; A, INW; C) only $p < 0.05$ interactions are shown. The weight of causal links (i.e. Std. estimates) is indicated by the thickness of the arrows. Marginal and condition R^2 values are indicated for INW and BIC response variables and R^2m is indicated for prey response variables. Simplified illustrations of causal links between habitat, resource and (A) BIC, and (C) INW indicate the cumulative standardised regression estimates (i.e. the total of estimates for a given casual link irrespective of sign or significance) for habitat (dashed line = indirect effect via the prey community; solid line = direct effect) and resource variables. For full model details see Table S5.
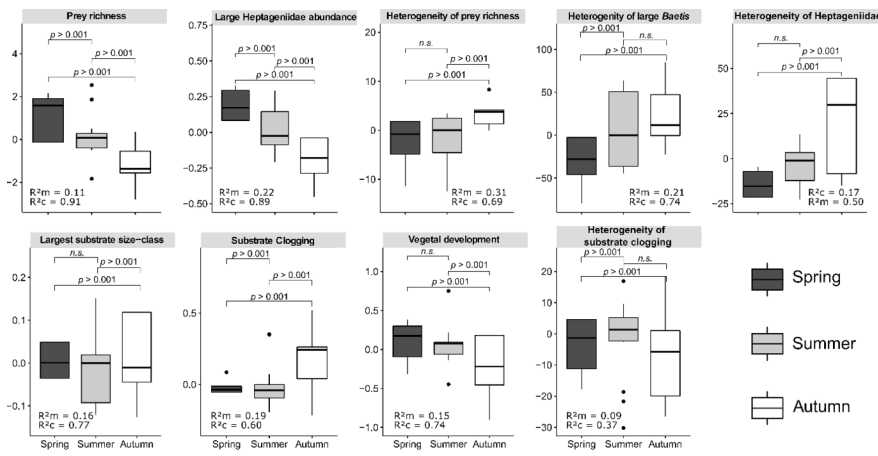
Figure 6. Seasonal variation in estimates of ecological opportunity . Variables that had a significant effect on either between-individual niche variation or individual niche width in causal analyses are included. Boxplots represent residuals extracted from the following linear model: lm(y ~ Site + Year). Statistically significant differences between seasons were determined by Tukey post-hoc *emmeans* tests on linear mixed models y ~ *season* + (1| *site*) + (1| *year*). P values are indicated, ns; not significant.

Table 1. Faeces metabarcoding, prey community and habitat sampling design. Mean and standard deviation (SD) of the Between-Individual Component of the trophic niche (BIC) and Individual Niche Width (INW) by campaign are indicated. Diet samples size corresponds to the number of faeces retained after bioinformatic filtering of faeces metabarcoding data and with at least one occurrence of macroinvertebrate species.

Catchment area	Campaign ID	Coordinates	Campaign date	Dietary sampling size	*BIC* mean (SD)
Durance	14HenA	N 44° 18' 46" E 5° 55' 29"	May-14	33[1]	0.65 (0.17)
	14HenB		Oct-14	34[1]	0.76 (0.15)
	15HenA		May-15	30[1]	0.46 (0.10)
	15HenB		Nov-15	27[1]	0.78 (0.14)
Durance	14SSL	N 44° 14' 50" E 5° 55' 17"	Aug-14	25	0.50 (0.12)
	15SSL		Sep-15	44[2]	0.52 (0.13)
Durance	14SLS	N 44° 8' 47" E 5° 58' 9"	Aug-14	12	0.47 (0.08)
	15SLS		Aug-15	43[2]	0.63 (0.14)
Durance	14D4	N 44° 2' 13" E 5° 57' 56"	Jul-14	11	0.52 (0.10)
Durance	15D5	N 44° 0' 1" E 5° 55' 40"	Jul-15	46	0.47 (0.15)
Buëch	14Bue	N 44° 13' 29" E 5° 52' 27"	Sep-14	24	0.49 (0.11)
	15Bue		Sep-15	39	0.57 (0.14)
Verdon	15VerA	N 43° 44' 15" E 6° 20' 58"	Jul-2015	20[1]	0.59 (0.11)
	15VerB		Jul-2015	30[1]	0.48 (0.15)
	15VerC		Sep-15	29[1]	0.47 (0.09)
Beaume	14PltA	N 44° 27' 18" E 4° 16' 39"	Jun-14	35[1]	0.54 (0.08)
	14PltB		Oct-14	6[1]	0.53 (0.14)
	15PltA		Jun-15	49[1]	0.51 (0.12)
	15PltB		Oct-15	30[1]	0.75 (0.14)
Loue	14PlnA	N 47° 0' 4" E 5° 49' 36"	Jun-14	21[1]	0.40 (0.12)
	14PlnB		Sep-14	49[1]	0.40 (0.10)
	15PlnA		Jul-15	41[1]	0.45 (0.14)
	15PlnB		Sep-15	48[1]	0.75 (0.11)

[1]data from Villsen et al. (2022a); [2]data from Villsen et al. (2022b).