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PRIMARY RESEARCH PAPER

Assessing a standardized method to identify optimal baselines for trophic position estimation in stable isotope studies of stream ecosystems

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Abstract Nitrogen stable isotope ratios ($\delta^{15}N$) are widely used to quantify trophic position in aquatic ecosystems. Comparing trophic position across space requires identifying baselines to account for variation in $\delta^{15}N$ values of basal energy resources, but few standardized methods exist for identifying suitable baselines. We evaluated a standardized method for identifying optimal isotopic baselines in streams spanning the Rocky Mountains–Great Plains ecotone. We assessed candidate taxonomic groups and feeding groups following four criteria: (1) Organisms should be easy to collect and widely distributed, (2) Within-site $\delta^{15}N$ variation should be low (representative of uniform feeding behavior), (3) $\delta^{15}N$ values should be correlated with geographic variability in $\delta^{15}N$ values, and (4) Trophic position of consumers calculated using the baseline should be independent of geographic $\delta^{15}N$ variability when there is no change in diet. Simuliidae (obligate, sestonic filter feeders) met all four criteria for four fishes and produced trophic position estimates consistent with dietary changes for brown trout along a longitudinal stream gradient. The four-criteria screening method is suitable for temperate streams in North America and supports the recommendation to use Simuliidae or potentially grouped filter feeders as baseline organisms for stable isotope studies quantifying trophic position in higher-order consumers.

Keywords Stable isotope analysis · Trophic level · Isotopic baselines · Food chain · Food web

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Introduction

A major goal in ecology is to understand how trophic relationships vary over space and time, which is often done by quantifying an organism's trophic position (TP) on a continuous scale. Stable isotope analysis is widely used to assess trophic position (Post, 2002; Martínez del Rio et al., 2009). In particular, the ratio of stable isotopes of nitrogen (15N:14N, $\delta^{15}N$) is used to estimate TP of consumer organisms because of

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changes in the proportion of light to heavy nitrogen isotopes during trophic transfers (DeNiro & Epstein, 1981; Minagawa & Wada, 1984). Assimilation of ingested food items results in consumers having tissues enriched with the heavy isotope relative to their food resource. Nitrogen isotope analysis can therefore be used to quantify shifts in trophic structure from species invasions (Vander Zanden & Rasmussen, 1999; Hickerson et al., 2019; Kirk et al., 2022a) and across longitudinal gradients (Maitland & Rahel, 2023), or to understand contaminant exposure or biomagnification (McHuron et al., 2018; Lepak et al., 2019). However, in aquatic ecosystems, large geographic variability in nitrogen isotope ratios at the base of local food webs can make comparisons of TP among sites or along longitudinal gradients problematic (Anderson & Cabana, 2005; Barnes et al., 2008).

Variability in $\delta^{15}N$ values arises from natural (e.g., in situ denitrification and nitrogen fixation) and anthropogenic (i.e., land use) processes (Chappuis et al., 2017). Agricultural runoff, sewage effluent, or livestock manure increases denitrified components of nitrogen in the system through bacterial decomposition. During decomposition, bacteria preferentially assimilate ^{14}N over ^{15}N which results in elevated $\delta^{15}N$ values that enter a system and propagate up the food web (Di Lascio et al., 2013). In the Saint Lawrence lowlands in Quebec, Canada, $\delta^{15}N$ values of primary consumers increased up to 15 ‰ with regional nitrogen loading from synthetic fertilizers and manure from local livestock operations (Anderson & Cabana, 2005). In contrast, watersheds in the northeastern USA dominated by natural forest cover exhibit variability in $\delta^{15}N$ of primary consumers on the order of 2 ‰ (Mayer et al., 2002). In streams in northeastern Spain, $\delta^{15}N$ values of basal resources were highest in human-impacted mainstem locations (Pastor et al., 2013). Further, anthropogenic land use practices have increased $\delta^{15}N$ values in lacustrine systems in Rhode Island (Lake et al., 2001) and boreal streams in south-central Sweden (Bergfur et al., 2009). Accordingly, comparing TP estimates without correcting for these baseline differences in $\delta^{15}N$ values among locations can lead to erroneous inferences of food web structure and trophic relationships.

To account for the effects of geographic variation on nitrogen isotope ratios, TP is typically estimated relative to an isotope "baseline" (i.e., material representing geographic variation) using simple formula transformations or statistical analysis (Kjeldgaard et al., 2021). These approaches generally use a proxy organism as a baseline to correct for geographic $\delta^{15}N$ variation (Cabana & Rasmussen, 1996; Jardine et al., 2014; Kristensen et al., 2016). While this proxy approach has been applied in food web studies, careful consideration of the proxy baseline is required. Commonly, researchers collect all potential basal primary resources at a site (i.e., suspended and benthic organic matter, biofilms, filamentous algae, macrophytes, and riparian vegetation in aquatic ecosystems) multiple times during a given time interval (e.g., three times over the growing season) and use the average $\delta^{15}N$ values of the primary producers as a baseline (Vinagre et al., 2008; Govender et al., 2011). However, collecting primary producers in streams is arduous and expensive because it requires many samples over many dates to adequately characterize baseline variation. Therefore, long-lived primary consumers such as bivalves, with tissue turnover rates closer to higher level consumers, are more appropriate than primary producers (Vander Zanden & Rasmussen, 2001; Jardine et al., 2014). Bivalves also exhibit a uniform, specialized filter-feeding strategy that can reduce variation from omnivorous feeding habits. However, bivalves and similar long-lived primary consumers can be sparsely distributed in temperate stream ecosystems, rendering them impractical as baseline proxies.

Only two studies—Anderson & Cabana (2007) and Kristensen et al. (2016)—have evaluated standardized approaches for selecting primary consumer taxonomic groups as baselines in temperate streams where long-lived bivalves are not widely distributed. Both standardized approaches have limitations, and optimal baselines have not been compared to find consensus between regions. Anderson & Cabana (2007) recommended that baselines be widely distributed and use the same basal resources across sites (i.e., low omnivory). Kristensen et al. (2016) included two additional criteria: the baseline's $\delta^{15}N$ values must track geographic variability in $\delta^{15}N$, and the baseline should remove the influence of $\delta^{15}N$ variation on TP estimates for common higher-order consumers (i.e., fish). However, Anderson & Cabana (2007) relied on low mean $\delta^{15}N$ values as indicative of low levels of omnivory. But there is often a large range of $\delta^{15}N$ values within basal resource compartments. Taxonomic

groups that feed on a single resource with high $\delta^{15}N$ values would meet the low omnivory criteria but might not be considered as baseline proxies because they have high $\delta^{15}N$ values relative to omnivorous taxonomic groups that feed on a variety of resources with low $\delta^{15}N$ values. Alternatively, Kristensen et al. (2016) used the mean coefficient of variation (CV) of taxonomic groups, which likely better represents omnivory. However, when determining whether baselines track geographic $\delta^{15}N$ variation, Kristensen et al. (2016) correlated $\delta^{15}N$ only to land use at sample sites (i.e., a gradient form natural land use to human land uses) which may ignore other sources of geographic variation in $\delta^{15}N$ values arising from other sources such as temperature (Maitland et al. 2021). Additionally, when assessing whether a chosen baseline accounted for $\delta^{15}N$ variation on TP estimates of common higher-order consumers, Kristensen et al. (2016) assumed that TP should not change along the land use gradient, which may not be valid for consumers that exhibit diet shifts along longitudinal stream gradients (e.g., high-elevation headwater to low-elevation flood plains). Therefore, updated criteria and regional comparisons are needed to develop a consensus for determining optimal baseline proxies in food web studies.

Our goals were to (1) Build on the standardized method proposed by Kristensen et al. (2016); (2) Select a suitable baseline in streams that span the Rocky Mountain—Great Plains ecotone in western North America, where baseline taxa have not been identified; and (3) Compare our selected baselines to those found in other regions. To build upon the standardized method for selecting baselines, we looked for relationships involving $\delta^{15}N$ values of basal resources, invertebrates and fishes using a synthetic variable that includes several potential sources of $\delta^{15}N$ variation rather than land use changes alone. We also analyzed fish stomach contents to inform our expectation of how TP of common higher-order consumers might change if these consumers exhibit systematic changes in diet composition. Finally, we compared our selected baselines to those selected in Danish Lowland streams (Kristensen et al., 2016), to basal resource compartments, and to common aggregation methods, to look for consensus among regions and among aggregation methods. Improving standardized methods for finding baselines that are applicable across regions should help future studies better identify and understand drivers of trophic structure across space and time.

Methods

Study system and site selection

We surveyed sixteen 2nd-5th order stream sites distributed along the Rocky Mountain—Great Plains ecotone (Fig. 1). Sites spanned the longitudinal (i.e., upstream–downstream) gradient of the three primary tributaries to the North Platte River in Wyoming, USA. The Sweetwater, Medicine Bow, and Laramie rivers are snowmelt dominated with peak flows occurring in the spring and base flows occurring by mid-summer. At higher elevations, streams are dominated by natural forested land, and at low elevations, streams are dominated by agricultural land with some urbanization. Sites were selected to represent this longitudinal gradient which is expected to drive the wide variation of $\delta^{15}N$ values at the base of local food webs making the sites opportune for identifying a widely distributed and reliable baseline indicator for estimating species' trophic positions.

Longitudinal gradient

To quantitatively describe the longitudinal gradient, we used a synthetic index variable for a site's location along the longitudinal stream gradient. The index variable describes environmental changes from headwaters to lowlands, and was previously created using principal component analysis in a larger study assessing food web expansion and trophic redundancy across the Rocky Mountain—Great Plains ecotone (Maitland & Rahel, 2023). The synthetic variable creates an index of longitudinal stream position by combining seven metrics of environmental conditions into one variable (PC1). Input variables to the principal component analysis were elevation (m a.s.l.), stream slope (km/km), downstream distance to North Platte River (km), Strahler stream order, mean August water temperature (°C), upstream drainage area (km^2), and stream width (m). We recorded hourly water temperature at each reach from June to September each year with loggers (Onset Computer Corporation, Bourne, Massachusetts) to quantify mean August water

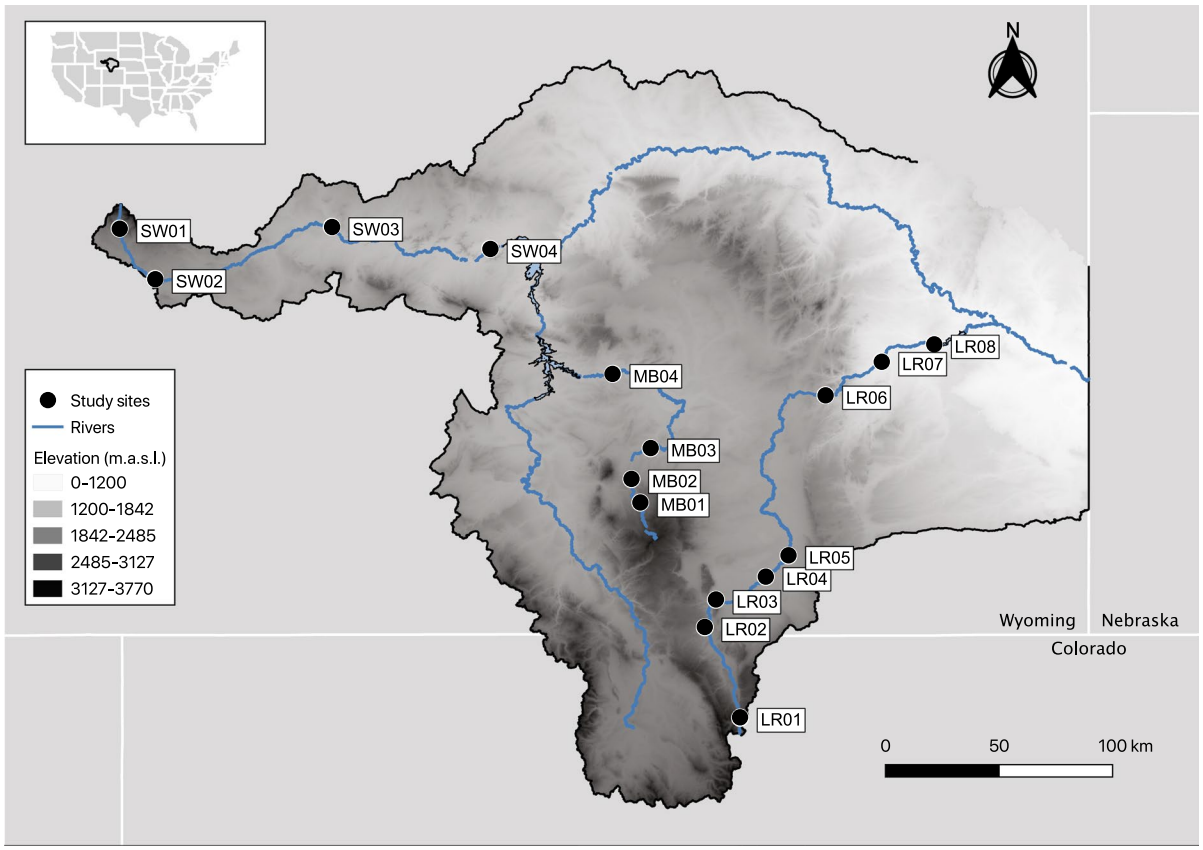


Fig. 1 Map of study site locations within the North Platte River drainage in Wyoming, USA. LR = Laramie River, MB = Medicine Bow River, and SW = Sweetwater River

temperature (August, ℃). Stream slope (km/km), upstream drainage area (km^2), and downstream distance to the North Platte River (km) were estimated using ArcGIS. Channel width (m) was measured at 10 equally spaced transects at each reach in 2016. The longitudinal gradient index (PC1) explained 78% of site-level variation among the 16 study sites and thus is a good description of changes in environmental conditions from upstream, high-elevation, cold water streams to downstream, low-elevation, warm water streams. In the previous study, the index was positively correlated with $\delta^{15}N$ values of basal resources and primary consumers in the system (Maitland & Rahel, 2023), consistent with research elsewhere (Cabana & Rasmussen, 1996; Lake et al., 2001; Kristensen et al., 2016), and indicates a need for baseline correction when describing trophic position along the ecotone. We used the index score (PC1) as a measure of a site's position along the stream gradient for all subsequent analyses (Table S1).

Sample collection and preparation

We sampled basal resources, macroinvertebrates, and fishes during the summer of 2016. Basal resources and macroinvertebrates were sampled once in June, July, and August to account for fine-scale temporal variation in $\delta^{15}N$ (Jardine et al., 2014), but fishes were only sampled in the last sampling event in August. Seston was collected by filtering three replicates of up to 10 L of water onto pre-combusted (550 °C, 4 h) filters (Whatman GF/F, 47 mm O) in the field using a modified portable drill pump (Kelso & Baker, 2016). Biofilm was collected by scraping five cobble-sized rocks within the reach. Nine replicates of fine benthic organic matter (FBOM) were collected using a food baster by sucking material off the streambed from

randomly selected pools at each site. Filamentous algae was collected by hand. We used a D-framed kick net in a variety of habitats (i.e., riffle, pools, macrophytes, and river margins) to collect a representative sample of the macroinvertebrate assemblage. Macroinvertebrates were kept in filtered stream water for 24 h to allow for gut clearance and then frozen. We collected fish using a backpack electrofishing unit (Smith-Root, Vancouver, WA). A muscle biopsy was taken using a 5-mm biopsy punch from fish greater than 300 mm in length to minimize the number of fish euthanized for stable isotope analysis (Maitland & Rahel, 2021). Stomach contents of fish greater than 300 mm were collected using pulsed gastric irrigation (Light et al. 1983), preserved in individual containers with 95% ethanol, and transported to the laboratory for identification. The remaining fish used for stable isotope analysis or stomach content analysis were euthanized with a lethal dose of MS-222, placed on ice, and then frozen upon returning to the laboratory. Fish larger than 300 mm in length were returned to the stream.

We identified macroinvertebrates in the laboratory to the family level using an invertebrate guide (Merritt et al., 2008), and we characterized each taxonomic group into feeding groups using "fuzzy coding" (Chevene et al., 1994). "Fuzzy coding" assigns trait affinity (i.e., feeding strategies) scores from 0 (absent) to 3 (strong affinity) to each taxonomic group using trophic trait data (Chevene et al., 1994; Burdon et al., 2020; Maitland & Rahel, 2023). We obtained invertebrate trophic trait data from the USA Freshwater Biological Traits Database (Vieira et al., 2006). Because larvae and adults have different feeding behaviors, Elmidae were separated by life stage during the assigning of trait affinities. For stable isotope analysis, we used individuals from each taxonomic group whenever possible, but for small taxa, we pooled individuals to ensure sufficient sample sizes for stable isotope analysis.

Muscle filets (from the anterior dorsal portion) and stomachs were removed from fish prior to stable isotope analysis. We focused on brown trout *Salmo trutta* (Linnaeus, 1758), creek chub *Semotilus atromaculatus* (Mitchill, 1818), longnose dace *Rhinichthys cataractae* (Valenciennes, 1842), longnose sucker *Catostomus catostomus* (Forster, 1773), and white sucker *Catostomus commersonii* (Lacépède, 1803) because these species were widely distributed along the stream gradient (Kirk et al., 2020, 2022b; Maitland & Rahel, 2023).

In total, we analyzed 1,391 invertebrate, fish, and basal resource samples which were oven-dried (60 °C, 48 h) and ground into a homogenous powder. Animal samples (~1.0 mg) and basal resources (~2.0 mg) were weighed into 8×5-mm tin capsules and analyzed for $\delta^{15}N$ at the University of Wyoming Stable Isotope Facility using a Delta Plus XP Continuous Flow Stable Isotope Ratio Mass Spectrometer (Thermo Finnigan, Bremen, Germany) coupled to a Costech Analytical 4010 elemental analyzer. Nitrogen (^{15}N:^{14}N) isotope ratios (R) were estimated relative to atmospheric nitrogen. Working internal standards were run as controls throughout the analyses to ensure accurate measurements. Isotopic values are reported in per mill delta notation (Eq. 1):

$$\delta^{15}N = \left[\frac{R_{\text{sample}}}{R_{\text{standard}}} + 1\right] \times 1000$$

where $\delta^{15}N$ is the level of ^{15}N enrichment and R is the ratio of the heavier to lighter isotope. Analytical error (i.e., 1 SD of laboratory standard) of sample runs was estimated at 0.18‰ ($\delta^{15}N$) for the invertebrates and 0.1‰ ($\delta^{15}N$) for fish.

Data analysis

Suitability of various taxonomic groups and feeding groups as baselines was based on the four updated criteria: (1) Organisms should be easy to collect and widely distributed, (2) Within-site $\delta^{15}N$ variation within a potential baseline group should be low, (3) $\delta^{15}N$ values should be correlated with geographic variability in $\delta^{15}N$ values, and (4) Trophic position of consumers calculated from the baseline should be independent of geographic $\delta^{15}N$ variability when there is no dietary change. Although all analyses were performed for all widely distributed taxonomic and feeding groups, selection occurred progressively from each criterion. For example, if a taxonomic group met the first criterion, it would stay in the list of potential baselines and be evaluated for the second criterion, but if it did not meet the first criterion, then it would be removed from the list. Common methods for baseline corrections in the literature are to either use individual basal resource compartments, the average of

all primary consumers, or the averages of all basal resources. We also evaluated if composite averages of primary consumers or basal resources would meet criteria 3 and 4.

The geographic distribution was expressed as the percentage of the 16 sites that contained a given taxonomic or feeding group. We used a cut off value of 75% of sites in the selection process as a reasonable value for identifying a taxonomic or feeding group with a wide distribution. Although 75% was chosen arbitrarily, Kristensen et al. (2016) included two taxa that would fall below this mark, and the optimal baseline identified by Kristensen et al. (2016) was found at 78.9% of sites. This makes our cutoff a more conservative measure of distribution while retaining the distribution levels of the best baselines in other studies.

We compared differences in within-site variation (coefficient of variation = CV) of the mean $\delta^{15}N$ values of single taxonomic and feeding groups with 1-way analysis of variance (ANOVA). We calculated within-site CV as SD/mean at a site. The within-site CV values were log transformed to ensure normality. We used the Tukey HSD post hoc tests for pairwise comparison using an alpha of 0.05.

We used least squares linear regression to assess relationships between stream compartment $\delta^{15}N$ and trophic position values (see below) versus the longitudinal stream gradient (PC1 score). Taxonomic and feeding groups with significant ($P \leq 0.05$) relationships with the longitudinal gradient were considered to sufficiently track geographic variation in $\delta^{15}N$ values.

We compared uncorrected TP of the five fish species to the TP estimates of the fish species after correction by various taxonomic and feeding groups. We calculated trophic positions for brown trout, creek chub, longnose dace, longnose sucker, and white sucker using each taxonomic or feeding group as baselines using the following equation (Post, 2002):

$$TP_{corrected} = \frac{\delta^{15}N_{consumer} - \delta^{15}N_{baseline}}{\Delta^{15}N} + TL_{baseline}$$

where $TP_{corrected}$ is the corrected trophic position of each individual fish at a site, $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ signature of each individual fish, $\delta^{15}N_{baseline}$ is the mean $\delta^{15}N$ signature of the taxonomic or feeding group at each site, $\Delta^{15}N$ is the discrimination factor for each trophic transfer, and $TL_{baseline}$ is the expected trophic level of the baseline. To assess the difference between corrected TP estimates and uncorrected TP estimates of brown trout, creek chub, longnose dace, longnose sucker, and white sucker, we modified the above equation by removing the baseline correction. The resulting formula was:

$$TP_{uncorrected} = \frac{\delta^{15}N_{consumer}}{\Delta^{15}N}$$

where $TP_{uncorrected}$ is the uncorrected trophic position of each individual fish at a site, $\delta^{15}N_{consumer}$ is the $\delta^{15}N$ signature of each individual fish, and $\Delta^{15}N$ is the discrimination factor for each trophic transfer. Discrimination factors were calculated for each species using a conversion factor for fish muscle tissue (Caut et al., 2009).

To inform our expectations of how TP might change across the longitudinal gradient, we examined changes in diet composition for brown trout, creek chub, longnose dace, longnose sucker, and white sucker by using stomach content analysis. The diet items from each stomach sample were split into seven groups: algae, amphibians, aquatic invertebrates, terrestrial invertebrates, fish, crayfish, and debris. The stomach content groups were counted and weighed. To assess the relative importance of the diet items in the stomachs, we used an index of relative importance (%IRI) that considers the proportional number of diet items, the proportional weight of the items, and the frequency of occurrence of the items in diets (Cortés, 1997). We then used analysis of covariance (ANCOVA) to determine if fish changed their diet composition along the longitudinal gradient.

We compared the uncorrected and corrected TP estimates derived from $\delta^{15}N$ values to assess if a given taxonomic or feeding baseline group removed the influence of geographic variability in $\delta^{15}N$. We tested for a geographical gradient in TP by regressing the uncorrected TP estimates and the corrected TP estimates across the longitudinal gradient. If the slopes (β_1) of the corrected TP estimates were lower than the slopes of the uncorrected TP estimates, we inferred that geographic variability was reduced or removed. We then used our stomach content data to infer if fish diet composition changed along the gradient. If fish consumed more fish and crayfish along the longitudinal gradient, then we expected that TP

should increase along the gradient even after baseline correction (i.e., $\beta_1 > 0$). If there were no dietary changes, then we expected the corrected TP to not change along the longitudinal gradient (i.e., $\beta_1 = 0$). Taxonomic or feeding groups that removed or reduced geographic variability *and* gave TP values consistent with stomach content changes were considered suitable baselines.

Results

Criteria 1: geographic distribution

Of the 62 taxonomic groups and 6 feeding groups observed across all sites, 12 taxonomic groups and 5 feeding groups were found at ≥ 75% of sites (Criterion 1; Fig. 2). Elmidae (both adult and larval life stages), Heptaganiidae, and Hydropsychidae were the most common taxa and were found at all sites (Fig. 2).

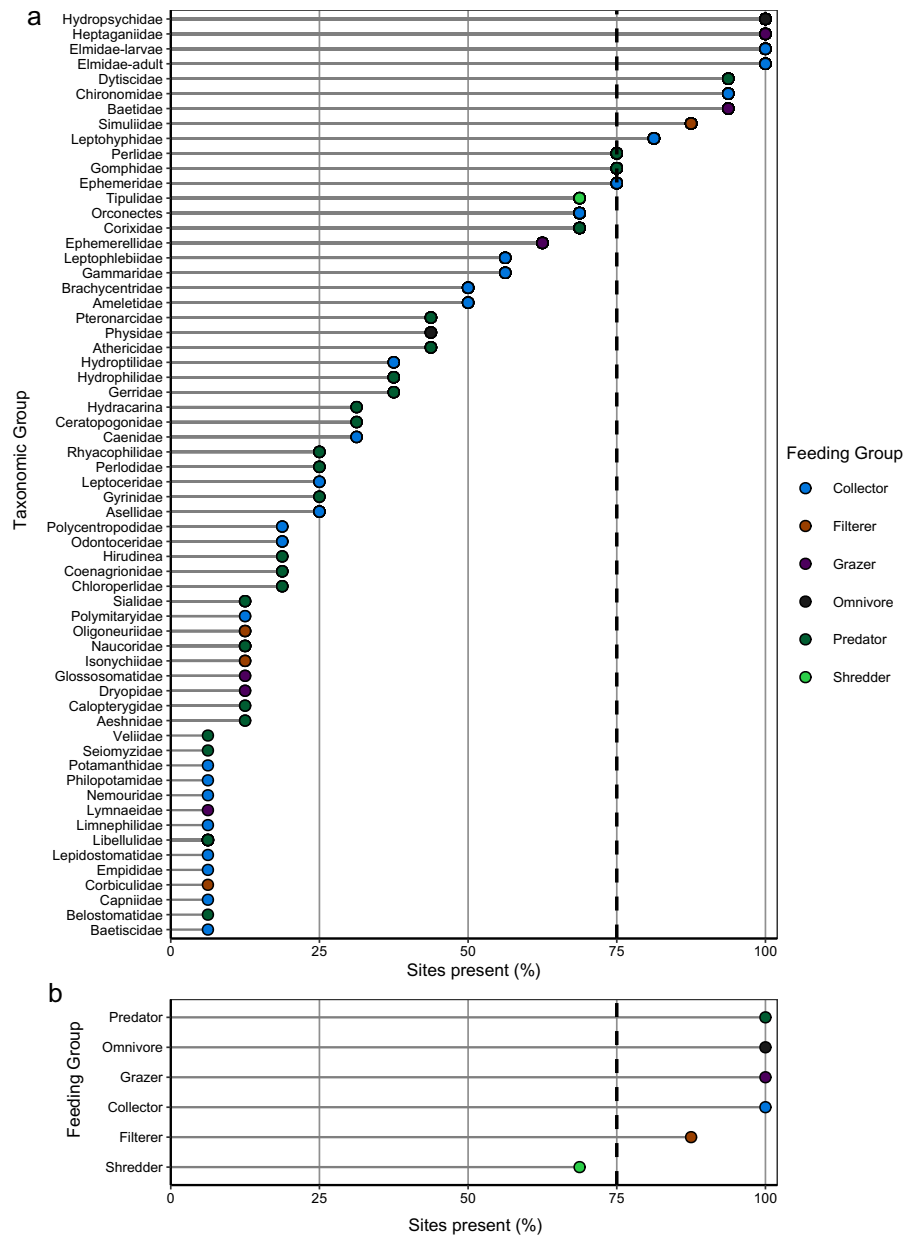


Fig. 2 Geographic distribution of specific taxanomic **a** and feeding groups **b** expressed as the percentage of sites found during the study

Veliidae, Seiomyzidae, Potamanthidae, Philopotamidae, Nemouridae, Lymnaeidae, Limnephilidae, Libellulidae, Lepidostomatidae, Empididae, Corbiculidae, Capnidae, Belostomatidae, and Baetiscidae were the least common taxon and were found at one site. Collectors, grazers, omnivores, and predators were the most common feeding groups and were found at all sites, while shredders were only found at 10 (63%) of the sites. Seventeen potential baselines (12 taxonomic groups, 5 feeding groups) were considered widely distributed and used in subsequent analyses.

Criteria 2: low mean coefficient of variation

Mean CV varied by taxonomic group (ANOVA; $F_{11,152} = 7.2052$, $P < 0.001$; Fig. 3a). Simuliidae had the smallest CV across sites and Elmidae-adult had the largest (Fig. 3a). Simuliidae had statistically lower CVs from Elmidae-adult, Elmidae-larvae, Chironomidae, Dytiscidae, Leptohyphidae, Gomphidae, Hydropsychidae, Perlidae, and Ephemeridae (Tukey HSD test compared to Simuliidae CV; $P < 0.001$ for Elmidae-adult, $P < 0.001$ for Elmidae-larvae, $P < 0.001$ for Chironomidae, $P < 0.001$ for Dytiscidae, $P < 0.001$ for Leptohyphidae, $P < 0.001$ for Gomphidae, $P < 0.001$ for Perlidae, $P = 0.001$ for Ephemeridae, and $P = 0.005$ for Hydropsychidae; Fig. 3). The CV for Simuliidae was not different from Baetidae nor Heptaganiidae (Tukey HSD test, $P = 0.114$; $P = 0.932$, respectively). Overall, Simuliidae, Baetidae, and Heptaganiidae had the lowest mean CV values relative to the other taxonomic groups.

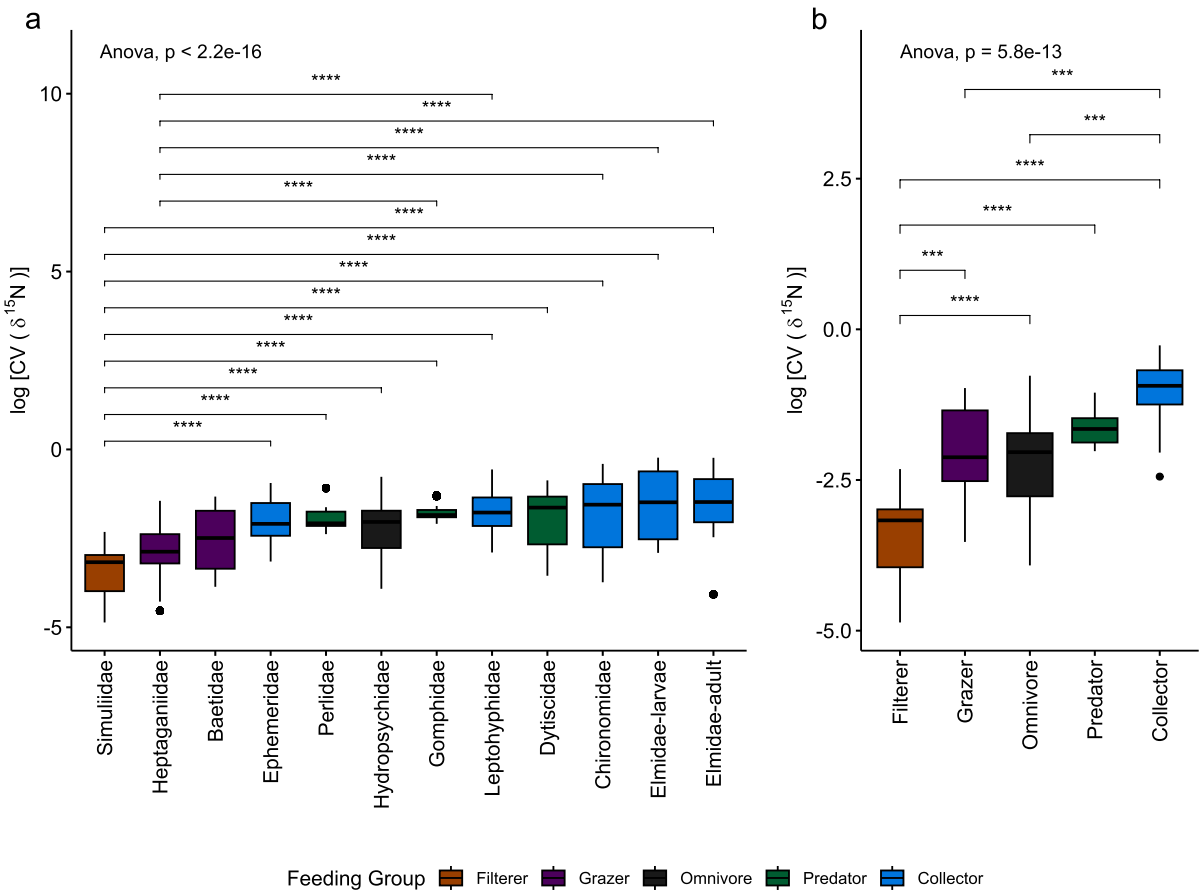


Fig. 3 Log-transformed mean coefficient of variation (CV) of $\delta^{15}N$ across sites for the twelve taxonomic groups that were widely distributed **a** and the five feeding groups **b**. The bars represent significant pairwise comparison at *=0.05, **=0.01, an ***=0.001 level. Bars with non-significant pairwise comparison are not shown

Mean coefficient of variation also varied by feeding group, (ANOVA; $F_{4,72}=24.886$, $P<0.001$; Fig. 3b). Filterers had the smallest CV across sites, and Collectors had the largest. The CV for Filterers was lower than all other feeding groups (Tukey HSD tests; $P<0.001$; Fig. 3b). Overall, Filterers had the lowest mean CV relative to the other feeding groups. Of the 17 widely distributed taxonomic and feeding groups, four (3 taxonomic and 1 feeding group) were considered to have sufficiently low CVs.

Criteria 3: correlation with environmental $\delta^{15}N$

The $\delta^{15}N$ values of all basal resources (i.e., biofilm, seston, filamentous algae, and FBOM) increased along the longitudinal stream gradient (PC1), though the increase was only marginally significant for filamentous algae ($R^2 \geq 0.105$, $P \leq 0.068$; Fig. 4a–b). All 12 of the widely distributed taxonomic groups and all five feeding groups had $\delta^{15}N$ values that increased with PC1 ($R^2 \geq 0.252$; $P<0.001$ Fig. 4c–f). $\delta^{15}N$ values increased with PC1 for all fish species ($R^2 \geq 0.092$; $P<0.001$ Fig. 4g-h).

Criteria 4: trophic position estimates independent of environmental $\delta^{15}N$

Of the five widely distributed fishes, only brown trout showed a statistically significant diet shift along the longitudinal gradient, marginally decreasing their consumption of benthic and terrestrial invertebrates (benthic invertebrates: $R^2=0.205$, $P=0.059$; terrestrial invertebrates: $R^2=0.197$, $P=0.063$; Fig. 5). Brown trout increased their diet of crayfish downstream ($R^2=0.362$, $P=0.014$; Fig. 5). Creek chub appeared to follow a similar pattern to brown trout, but the diet shifts were not statistically significant ($P \geq 0.244$). Longnose dace, longnose sucker, and white sucker did not change their diets along the longitudinal gradient ($R^2 \leq 0.139$, $P \geq 0.233$). For a baseline to be consistent with each species' diet along the longitudinal gradient, correcting by a baseline should produce TP estimates with an increasing slope for brown trout but a slope of zero for the other fishes.

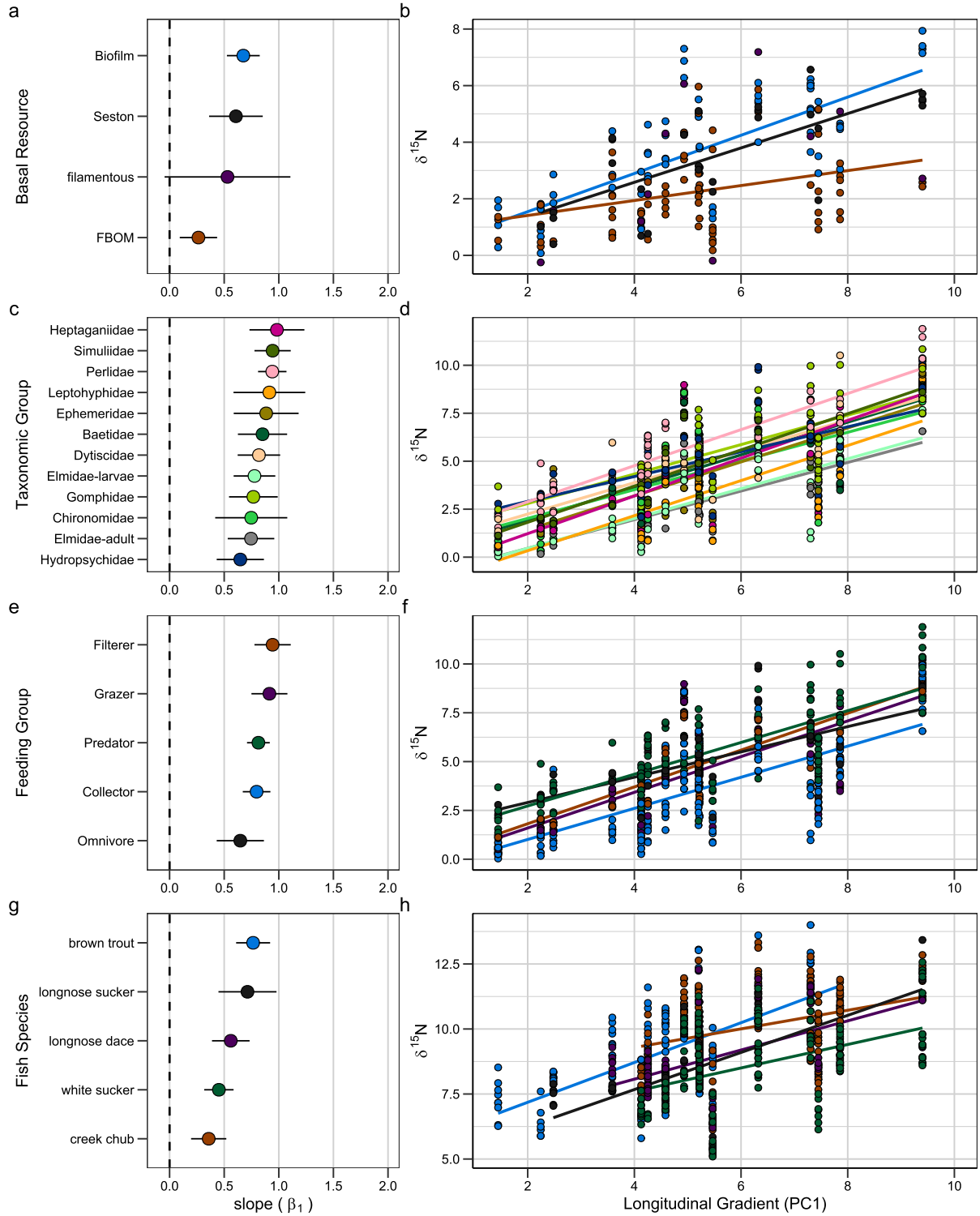
Uncorrected TP estimates for all five fish species were positively correlated with the longitudinal gradient (see uncorrected estimates in all panels in Figs. 6 and 7), which emphasized the need for baseline correction.

For brown trout, the effect of the longitudinal gradient on TP was reduced (i.e., decreased slope) but not completely eliminated when baseline corrections were done using either taxonomic groups (Fig. 6a, b) or feeding groups (Fig. 7a, b). This is consistent with the increased consumption of crayfish in downstream reaches (Fig. 5a). Interestingly, the relationship between TP and PC1 was statistically insignificant when corrected by the taxonomic groups Heptageniidae and Chironomidae (Fig. 6a, b).

For creek chub, the effect of the longitudinal gradient on TP was generally reduced (i.e., decreased slope) when baseline corrections were done using either taxonomic groups (Fig. 6c, d) or feeding groups (Fig. 7c, d). However, the positive correlation between TP and PC1 remained when correcting by the taxonomic groups Baetidae, Hydropsychidae, and Chironomidae. The relationship was reversed when correcting by the taxonomic group Dytiscidae and Gomphidae (Fig. 6c, d). In addition, the positive correlation between TP and PC1 remained when corrected by the feeding group Grazers and Omnivores. The relationship was reversed when correcting by the feeding group Predators (Fig. 7c, d). When correcting by basal resources, positive correlations between TP and PC1 remained when correcting by the basal resource compartments biofilm, filamentous, FBOM, and average of all basal resources (Fig. 6c, d; Fig. 7c, d).

For longnose dace, the correlation between TP and the longitudinal gradient was removed after correction for most of the taxonomic groups and feeding groups (Fig. 6e, f, Fig. 7e, f). The correlation between TP and PC1 was reversed when corrected by the taxonomic Chironomidae and Leptohyphidae. When correcting by filamentous, FBOM, and the average of all basal resources, the correlation between TP and PC1 remained (Fig. 6e, f, Fig. 7e, f).

For longnose sucker, the correlation between TP and the longitudinal gradient was reduced or became insignificant for most taxonomic groups and feeding groups (Fig. 6 g, h, Fig. 7 g, h). However, the positive correlation between TP and PC1 remained when corrected by the taxonomic group Dytiscidae and Gomphidae (Fig. 6 g, h). When correcting by basal resources, the correlation between TP and PC1 remained when baseline corrections were based on individual basal resources (Fig. 6 g, h) or the average of all basal resources (Fig. 7 g, h).


a
Basal Resource
Biofilm
Seston
filamentous
FBOM
0.0
0.5
1.0
1.5
2.0
b
δ15N
0
2
4
6
8
2
4
6
8
10
c
Taxonomic Group
Heptaganiidae
Simuliidae
Perlidae
Leptohyphidae
Ephemeridae
Baetidae
Dytiscidae
Elmidae-larvae
Gomphidae
Chironomidae
Elmidae-adult
Hydropsychidae
d
δ15N
0.0
2.5
5.0
7.5
10.0
e
Feeding Group
Filterer
Grazer
Predator
Collector
Omnivore
f
δ15N
g
Fish Species
brown trout
longnose sucker
longnose dace
white sucker
creek chub
slope (β1)
h
δ15N
5.0
7.5
10.0
12.5
Longitudinal Gradient (PC1)

◂**Fig. 4** Relationships between $\delta^{15}N$ values of food web compartments and PC1, which represents a longitudinal stream gradient. Panels a, c, e, and g show the slopes estimates (±95% CI) of the adjacent regressions in panels b, d, f, and h. Colors in each scatter plot (**b**, **d**, **f**, **h**) correspond to the colors of the adjacent fish species plots (e.g., white sucker are colored dark green in panels g and h). The dashed line in panels a, c, e, and g indicates a slope of zero (no relationship between $\delta^{15}N$ values and PC1). Each point in panels b, d, and f is a mean $\delta^{15}N$ value, while points in panel h are $\delta^{15}N$ values of individual fish. Only significant regression lines are shown in the scatter plots ($P < 0.05$)

For white sucker, the correlation between TP and the longitudinal gradient was reduced or became insignificant after correction by most taxonomic groups and feeding groups (Fig. 6 i, j; Fig. 7 i, j). However, the positive correlation between TP and PC1 was reversed when corrected by the taxonomic groups Elmidae-larvae, Heptaganiidae, Gomphidae, Perlidae, Leptohyphidae, Ephemeridae, and Dytiscidae (Fig. 6 i, j). Also, the positive correlation between TP and PC1 was reversed when correcting by the feeding groups Collectors and Predators (Fig. 7 i, j). The average of all aquatic invertebrates reversed the correlation between TP and PC1 for white sucker (Fig. 7 i, j). When correcting by basal resources, the correlations between TP and PC1 remained for the basal resource compartments filamentous, FBOM, and average of all basal resources (Fig. 6 i, j; Fig. 7 i, j).

Discussion

Obtaining consensus about suitable baseline indicators that account for geographic variation in $\delta^{15}N$ values is a key step toward improving the use of stable isotopes in aquatic ecosystems. We evaluated the effectiveness of a standardized method for identifying optimal isotopic baselines previously developed for Danish lowland temperate streams (Kristensen et al., 2016) by applying it to stream systems spanning the Rocky Mountains–Great Plains ecotone. The four screening criteria appear useful for selecting a suitable baseline for estimating trophic positions of consumer organisms even for species that exhibit dietary shifts across the system. In a recent review of trophic position estimation, most studies used aquatic herbivores as baselines (Kjeldgaard et al., 2021) rather than the ideal longer-lived filter-feeding bivalves (i.e., detritivores, Jardine et al., 2014). Our results from the

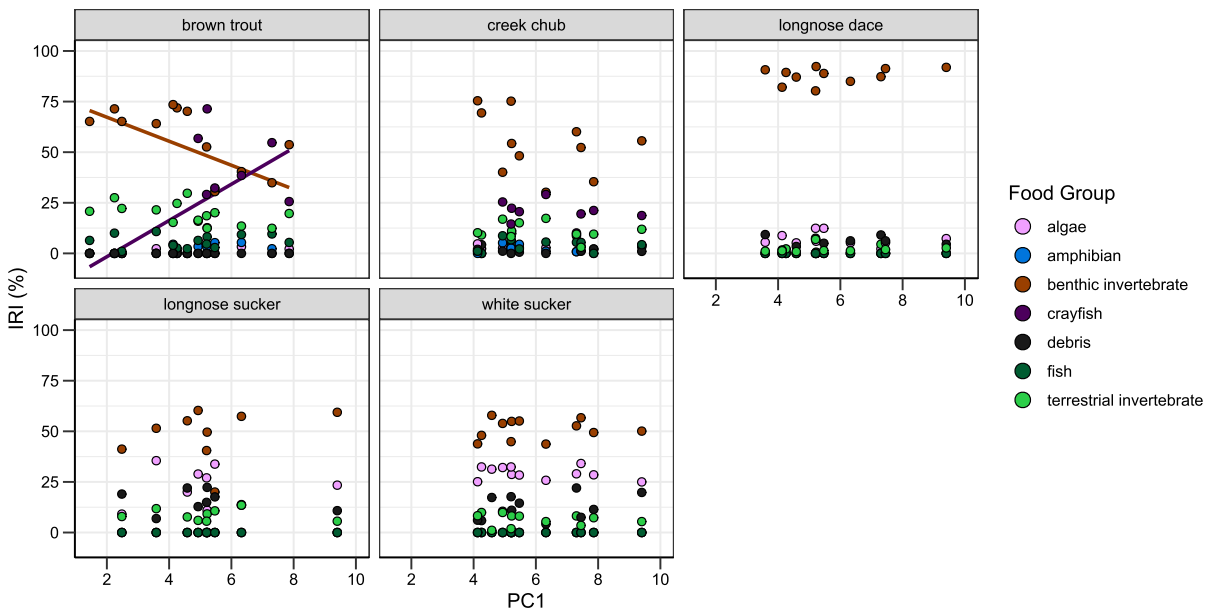
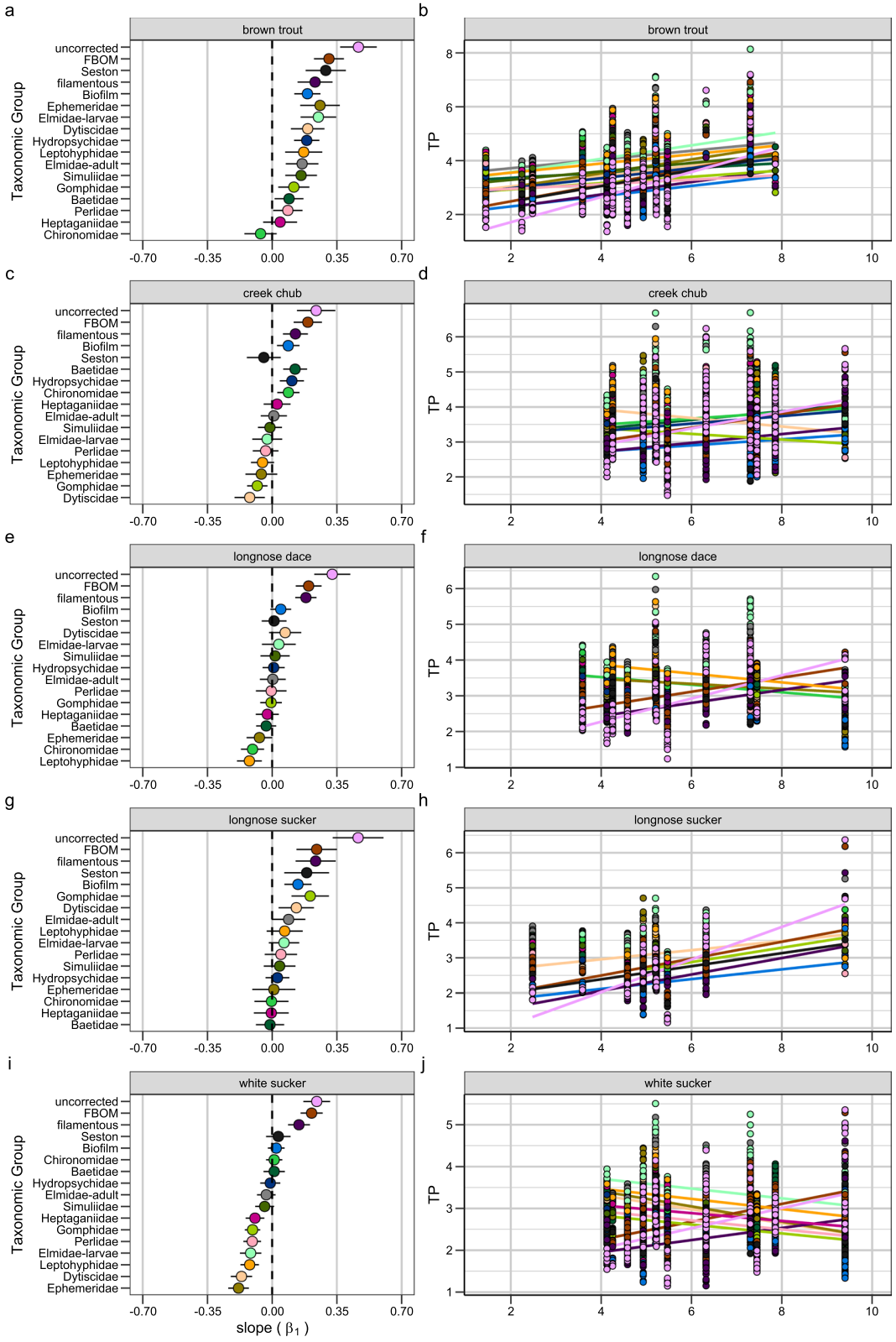


Fig. 5 Relationships between the index of relative importance (IRI) based on stomach content analysis for fish species and the longitudinal gradient (PC1). Each point is the mean IRI index of all the fish captured at that site, and colors correspond to the food group. The lines of best fit are only shown for significant relationships ($P < 0.05$)


a
brown trout
Taxonomic Group
uncorrected
FBOM
Seston
filamentous
Biofilm
Ephemeridae
Elmidae-larvae
Dytiscidae
Hydropsychidae
Leptohyphidae
Elmidae-adult
Simuliidae
Gomphidae
Baetidae
Perlidae
Heptaganiidae
Chironomidae
-0.70
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b
brown trout
TP
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c
creek chub
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uncorrected
FBOM
filamentous
Biofilm
Seston
Baetidae
Hydropsychidae
Chironomidae
Heptaganiidae
Elmidae-adult
Simuliidae
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Taxonomic Group
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FBOM
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Elmidae-larvae
Simuliidae
Hydropsychidae
Elmidae-adult
Perlidae
Gomphidae
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Taxonomic Group
uncorrected
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Seston
Biofilm
Gomphidae
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white sucker
Taxonomic Group
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filamentous
Seston
Biofilm
Chironomidae
Baetidae
Hydropsychidae
Elmidae-adult
Simuliidae
Heptaganiidae
Gomphidae
Perlidae
Elmidae-larvae
Leptohyphidae
Dytiscidae
Ephemeridae
-0.70
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slope (β_1)
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white sucker
TP
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◂**Fig. 6** Relationships between TP of brown trout (**a**, **b**), creek chub (**c**, **d**), longnose dace (e,f), longnose sucker (**g**, **h**), and white sucker (**i**, **j**) in relation to position along the longitudinal stream gradient represented by PC1. Graphs to the left show the slopes (±95% CI) for the corresponding scatter plots to the right. The colors in each scatterplot correspond to the colors of the adjacent plots for the taxonomic group correction. Uncorrected TP estimates are in pink. The dashed line in a, c, e, g, i indicates a zero slope (i.e., no relation between TP and PC1). The lines of best fit are only presented for significant relationships (i.e., 95% CI for the slope does not overlap 0). For the scatterplots, each point is the individual fish TP signature before (uncorrected) or after correction by taxonomic group. Upstream sites are to the left in the scatter plots. Note the differences in the scale of the y-axis in the scatter plots. Only significant regression lines are shown in the scatter plots ($P < 0.05$)

standardized method further support that other taxa in the feeding group filterers (i.e., Simuliidae) may also serve as a reliable baseline when bivalves are not present. Simuliidae were also suggested as a useful baseline for food web studies in Danish lowland streams (Kristensen et al., 2016).

Selecting optimal baselines

We started with 62 candidate taxonomic groups, but only twelve taxonomic groups were present at ≥75% of sites (Fig. 2a). Simuliidae, Baetidae, and Heptaganiidae had the lowest CVs relative to the other nine widely distributed taxonomic groups (Fig. 3a; Table 1). The $\delta^{15}N$ values of Simuliidae, Baetidae, and Heptaganiidae were correlated with the longitudinal gradient (Fig. 4c–d; Table 1). When used as a baseline, all three groups generally removed or reduced the geographic variation associated with the longitudinal gradient. But when estimating TP for the five fish species, Simuliidae was the only taxonomic group that removed or reduced the geographic variation associated with the longitudinal stream gradient *and* retained slopes consistent with changes in diet for brown trout (Fig. 6; Table 1).

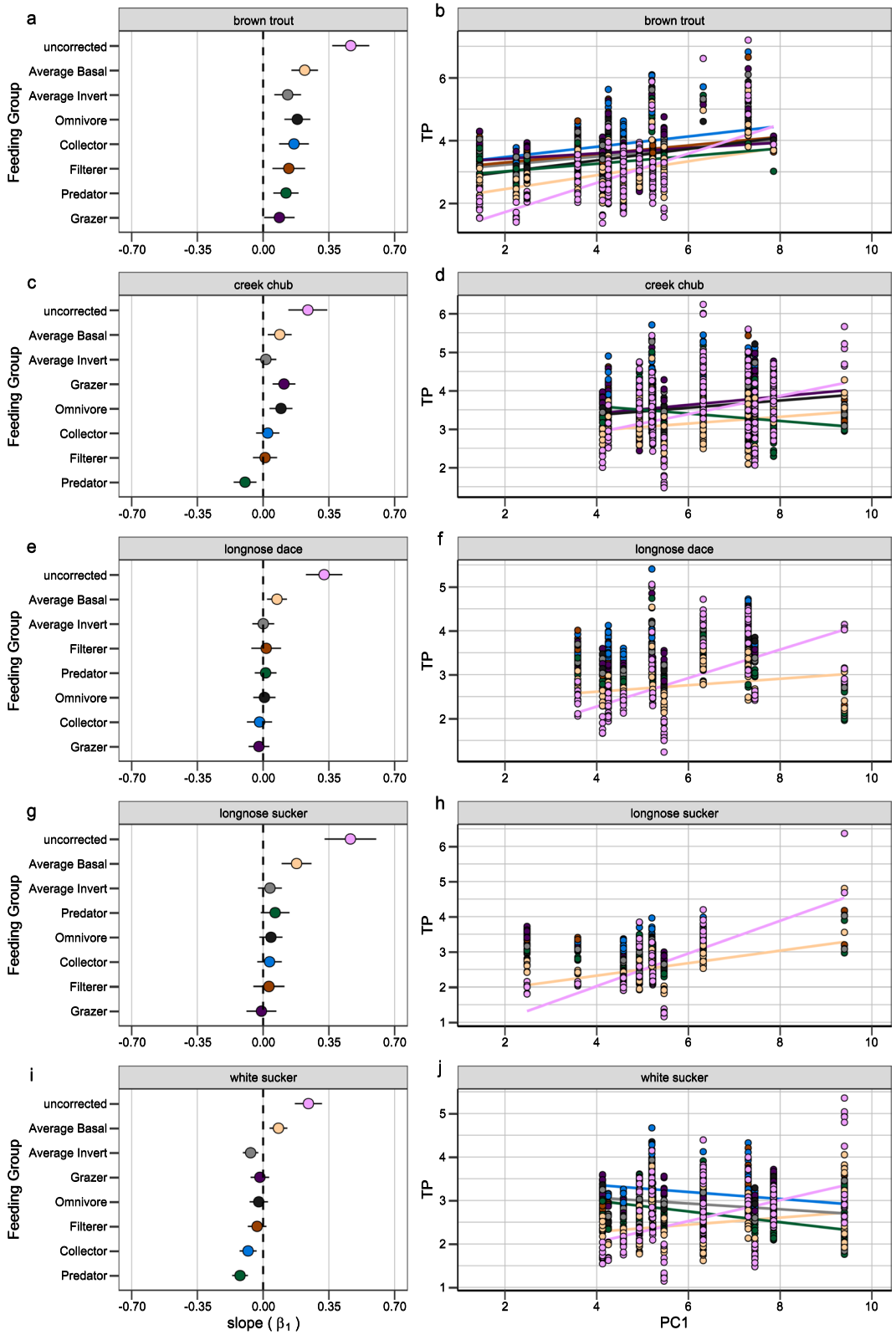
We started with 6 candidate feeding groups, and five were present at ≥75% of sites (Fig. 2b). Filterers had the lowest CV relative to the other 4 feeding groups and had $\delta^{15}N$ values that were correlated with the longitudinal gradient (Fig. 3b; Fig. 4e–f; Table 1). When estimating TP of the five fishes, Filterers also removed or reduced the geographic variation associated with the longitudinal stream gradient *and* retained slopes consistent with changes in diet for brown trout (Fig. 7).

Our results coupled with those in Danish Lowland streams indicate that taxonomic groups that exhibit filter feeding, like Simuliidae, may be particularly good baseline indicators (Kristensen et al., 2016). Although not widely distributed in our system, filter-feeding bivalves have been found to be good baseline indicators (Jardine et al., 2014) which corroborates our conclusion that filter feeders are useful for controlling for geographic variability in $\delta^{15}N$ values. Filter feeders rely on seston, which, interestingly, was the best of the basal resources at reducing or removing the influence of the longitudinal gradient on TP of fishes while also retaining TP relationships to the longitudinal gradient consistent with independent diet data (Table 1). Seston is largely suspended detritus and includes bacteria that denitrify and elevate $\delta^{15}N$ values. By consuming seston and its associated bacteria, filter feeders reflect geographic variability in $\delta^{15}N$ values, making them good baseline indicators.

Most studies in aquatic systems that calculate TP using stable isotopes use herbivores (e.g., Grazers) as baselines indicators (Kjeldgaard et al., 2021). The prevalence of herbivores may reflect previous work that identified grazing coleopterans and snails (Psephenidae, Physidae, and Viviparidae) as optimal baseline groups because they exhibited the lowest within-site mean $\delta^{15}N$ as an indicator of low omnivory (Anderson & Cabana, 2007). However, grazing coleopterans and snails were not widely distributed in our study system, occurring at only 2 of 16 and 5 of 16 study sites, respectively (Fig. 1a). The two grazing taxonomic groups that were widely distributed were Baetidae (15 sites) and Heptaganiidae (16 sites). These two groups may have potential as baseline taxa. Their within-site $\delta^{15}N$ variation was low, but not as low as Simuliidae (Criterion 2, Fig. 3), and they were slightly less effective than Simuliidae at removing geographic variability from TP estimates (Criterion 4; Table 1). The Grazer feeding group also was not as effective a baseline as Simuliidae for Danish lowland streams (Kristensen et al., 2016).

Assessment of criteria

The distribution of aquatic macroinvertebrates poses a limitation when selecting suitable baselines. Widely distributed baselines are preferred to ensure that


a
brown trout
uncorrected
Average Basal
Average Invert
Omnivore
Collector
Filterer
Predator
Grazer
Feeding Group
-0.70
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brown trout
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Average Basal
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Omnivore
Collector
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Predator
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Average Basal
Average Invert
Filterer
Predator
Omnivore
Collector
Grazer
Feeding Group
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Predator
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Grazer
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Filterer
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Predator
Feeding Group
slope (β_1)
j
white sucker
TP
PC1

◂**Fig. 7** Relationships between TP of brown trout (**a**, **b**), creek chub (**c**, **d**), longnose dace (**e**, **f**), longnose sucker (**g**, **h**), and white sucker (**i**, **j**) in relation to position along the longitudinal stream gradient represented by PC1. Graphs to the left show the slopes (±95% CI) for the corresponding scatter plots to the right. The colors in each scatterplot correspond to the colors of the adjacent plots for the feeding group correction. Uncorrected TP estimates are in pink. The dashed line in a, c, e, g, i indicates a zero slope (i.e., no relation between TP and PC1). The lines of best fit are only presented for significant relationships (i.e., 95% CI does not overlap 0). For the scatterplots, each point is the individual fish TP signature before (uncorrected) or after correction by the indicated feeding group. Upstream sites are to the left in the scatter plots. Note the differences in the scale of the y-axis in the scatter plots. Only significant regression lines are shown in the scatter plots ($P < 0.05$)

baselines cover the geographic extent of the study. However, 81% percent of the taxonomic groups found within our region were not present at 75% of our sites. Similarly, 87% of taxonomic groups in Danish Lowland streams were not distributed widely enough to be useful for baseline corrections (Kristensen et al., 2016). Although frustrating for selecting baselines, typical metacommunities consist of many locally distributed taxonomic groups and a few widely distributed taxonomic groups (Rosi-Marshall & Wallace, 2002).

Care must be taken when combining taxonomic groups into feeding groups to increase the spatial coverage of a suitable baseline. Often, studies have used averages of all primary consumers or averages of all basal resources as baselines to increase spatial coverage, but our synthesis indicates that averages of all primary consumers or basal resources do not reliably remove geographic variability of $\delta^{15}N$ on TP estimates (Table 1). In the present study, TP estimates of only 1 out of the 5 fishes were free of geographic variation and were consistent with dietary changes when correcting by the average of all basal resources (Table 1). TP estimates of 4 out 5 fishes in the present study and 0 out of the 2 fishes assessed in Danish lowland streams were free of geographic variation when correcting by the average of all primary consumers (Table 1; Kristensen et al., 2016). Feeding groups (particularly Filterers) present a promising alternative to averaging all the basal resources or primary consumers (Table 1). Filterers correctly reduced or removed the geographic variation associated with the longitudinal gradient in our study and was the only feeding group to remove the geographic variation for one of the fish species in Danish lowland streams (Table 1). Furthermore, collecting and processing samples of filter feeders such as Simuliidae is much easier than collecting and processing samples of basal resources.

Our study was done along the transition between the Rocky Mountains and the Great Plains of central North America. Optimal baselines may differ for other regions with different dominant taxonomic and feeding groups (Fig. 8). The optimal baseline in our study region, Simuliidae, is also the dominant filterer. Other filterers such as bivalves were uncommon in our region (see, Corbiculidae, Fig. 2a). Because of temperature and habitat preference, Simuliidae become less widespread and abundant at lower elevations, whereas bivalves typically become more widespread and abundant (Fig. 8). Similar changes occur with feeding groups (Fig. 8). The River Continuum Concept suggests that feeding ecologies change predictably along the longitudinal gradient based upon the relative amount and type of autochthonous or allochthonous resources (Vannote et al., 1980). Indeed, at our regional scale, Shredders were not widely distributed because they were primarily located in the upper reaches of our region where riparian vegetation cover was highest. In the lower elevation St. Lawrence watershed, the feeding groups Filterers and Shredders were not widely distributed, so Grazers became the most important baseline (Anderson & Cabana, 2007). Because basal resources and feeding groups change along longitudinal gradients, optimal baselines for isotopic food web studies will also likely differ along the longitudinal gradients (Fig. 8).

For suitable baselines to track geographic variation in $\delta^{15}N$, taxonomic groups should exhibit low omnivory which can be estimated by measuring mean within-site variation in $\delta^{15}N$. In our study, Simuliidae, Baetidae, and Heptaganiidae had the lowest mean within-site variability (CV) in $\delta^{15}N$ relative to other taxonomic groups indicating they had the lowest degree of omnivory. Comparing CV values among taxonomic or feeding groups appears to be an effective criterion for identifying suitable baselines of geographic variation in $\delta^{15}N$ values.

The $\delta^{15}N$ values for all taxonomic groups were correlated with the longitudinal gradient which is consistent with results from Danish Lowland streams (Kristensen et al., 2016). This criterion was not used when selecting suitable baselines of geographic

Table 1 Summary of baselines used to correct trophic position (TP) of higher-order consumers (i.e., fish) from Kristensen et al., (2016) and the present study

Group	Study	Mean CV (±SD)	Correlated with Gradient	Variation removed & consistent with diet changes (% Fish Species)
Taxonomic group				
Elmidae-adult	Present	0.294 (±0.226)	Y	(4/5) 80%
Elmidae-larvae	Present	0.328 (±0.284)	Y	(4/5) 80%
Chironomidae	Present	0.246 (±0.191)	Y	(2/5) 40%
Dytiscidae	Present	0.169 (±0.169)	Y	(2/5) 40%
Baetidae	Present	0.110 (±0.080)	Y	(4/5) 80%
	Kristenson et al., (2016)	0.080 (±0.090)	Y	(1/2) 50%[a]
Simuliidae	Present	0.041 (±0.026)	Y	(5/5) 100%
	Kristenson et al., (2016)	0.028 (±0.019)	Y	(2/2) 100%[a]
Hydropsychidae	Present	0.146 (±0.110)	Y	(5/5) 100%
Heptaganiidae	Present	0.070 (±0.057)	Y	(3/5) 60%
Gomphidae	Present	0.172 (±0.053)	Y	(2/5) 40%
Gammarus pulex	Kristenson et al., (2016)	0.094 (±0.061)	Y	(1/2) 50%[a]
Ephemeridae	Present	0.167 (±0.113)	Y	(4/5) 80%
Dytiscidae	Present	0.190 (±0.125)	Y	(2/5) 40%
Leptohyphidae	Present	0.225 (±0.151)	Y	(3/5) 60%
Perlidae	Present	0.153 (±0.067)	Y	(4/5) 80%
Feeding group				
Filterers	Present	0.047 (±0.027)	Y	(5/5) 100%
	Kristenson et al., (2016)	0.030 (±0.018)	Nd	(1/2) 50%[a]
Predator	Present	0.223 (±0.097)	Y	(3/5) 60%
	Kristenson et al., (2016)	0.120 (±0.063)	Nd	(0/2) 0%[a]
Omnivore	Present	0.126 (±0.076)	Y	(4/5) 80%
	Kristenson et al., (2016)	0.063 (±0.035)	Nd	(0/2) 0%[a]
Collector	Present	0.390 (±0.262)	Y	(4/5) 80%
	Kristenson et al., (2016)	0.120 (±0.087)	Nd	(0/2) 0%[a]
Scraper	Kristenson et al., (2016)	0.130 (±0.156)	Nd	(0/2) 50%
Grazer	Present	0.162 (±0.154)	Y	(4/5) 80%
Shredder	Kristenson et al., (2016)	0.120 (±0.065)	Nd	(0/2) 50%[a]
Basal				
Filamentous	Present	nd	N	(1/5) 20%
FBOM	Present	nd	Y	(1/5) 20%
Seston	Present	nd	Y	(4/5) 80%
Biofilm	Present	nd	Y	(3/5) 60%
Average				
Primary producer	Present	nd	Nd	(1/5) 20%
Primary consumer	Present	nd	Nd	(4/5) 80%
	Kristenson et al., (2016)	nd	Nd	(0/2) 0%[a]

[a] Did not assess dietary changes with isotope-independent data along the gradient,

Nd not determined

Only groups that met criteria 1 (i.e., widely distributed) are presented. The percentage of variation removed (Criterion 4) was calculated by dividing the number of fish species that had TP estimates free of the geographic variation and consistent with diet changes by the total number of fish species examined in the study

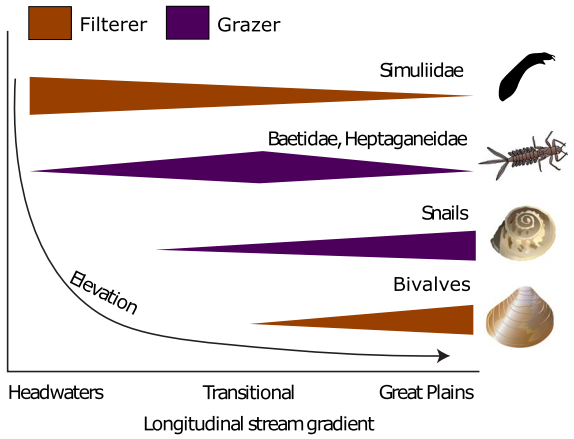

Fig. 8 Distribution and abundance of commonly suggested baseline taxonomic groups are likely to vary along the longitudinal habitat and thermal gradients of streams in mountainous regions. The optimal taxonomic groups will depend on the portions of the longitudinal gradient sampled in each study

variation of $\delta^{15}N$ in the St. Lawrence watershed (Anderson & Cabana, 2007). Correlations of $\delta^{15}N$ values with the longitudinal gradient affirm that the taxonomic or feeding groups are tracking geographic variation in $\delta^{15}N$. Consistent with a large body of literature (Lake et al., 2001; Mayer et al., 2002; Anderson & Cabana, 2005; Bergfur et al., 2009; Pastor et al., 2013), tracking geographic variation is common and confirms the need for baseline corrections when estimating TP based on $\delta^{15}N$ values.

Finally, assessing if baselines give TP estimates independent of environmental influence for higher-order consumers (i.e., fishes) serves as a crucial check that the baseline is working as intended. But interpreting "independent of the longitudinal gradient" is dependent on how TP is (or is not) expected to change with the longitudinal gradient due to dietary shifts by consumers. We echo the call by Kjeldgaard et al. (2021) for the need for independent dietary composition data to inform these expectations. In our study, brown trout exhibited dietary shifts across the longitudinal gradient, eating more crayfish and less benthic invertebrates (Fig. 5). Thus, we concluded that brown trout should exhibit an increasing trophic position along the longitudinal gradient even after correction. Previous methods for selecting suitable baselines have either not used this criterion (Anderson & Cabana, 2007) or have used mixing models that are dependent on stable isotopes to assess if diets changed along the longitudinal gradient (Kristensen et al., 2016).

Conclusions

Studies calculating TP using stable isotopes have used nine major groups of baseline taxa and eight methodological approaches (Kjeldgaard et al., 2021). More work on standardizing procedures will reduce variation in choices of baselines and methodology that complicates across-system syntheses of TP studies. Kjeldgaard et al. (2021) recommended that the first decision when calculating TP should be the selection of an appropriate baseline. The four criteria proposed by Kristensen et al. (2016) combined with our adjustments (i.e., assessments along geographic not just land use gradients, and evaluation of fish diet composition using stomach contents) present a promising standardized approach for selecting a suitable baseline. Using this approach, we found evidence that the taxonomic group Simuliidae (Filterers) may be an adequate baseline for temperate streams where other macroinvertebrate baseline candidates are not widely distributed. Additional studies, particularly those involving geographical gradients further down the stream continuum, are needed to further validate the use of this approach.

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Author contribution Conceptualization was performed by NTB, BMM, FJR; developing methods by NTB, BMM; data analysis by NTB, BMM; preparation of figures and tables by NTB; writing first draft by NTB. All authors contributed critically to the drafts and gave final approval for publication.

Data availability The data and code supporting the results are archived in the GitHub (https://github.com/bmait101/baseline-sia-proxies/tree/v1.0) and in the Zenodo public repository (https://doi.org/10.5281/zenodo.12508779).

Declarations

Conflict of interests The authors declared that they have no conflict of interest.

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