Baseline identification in stable-isotope studies of temperate lotic systems and implications for calculated trophic positions

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Abstract: Stable-isotope analysis is widely used in aquatic ecosystem studies to evaluate trophic structure and resource dynamics. Because $\delta^{15}N$ values vary in freshwater systems, e.g., reflecting variations in land use, suitable baseline indicators must be specified. Few investigators have identified specific baseline organisms based on thorough and methodical screening. We screened for baseline organisms in temperate lotic waters based on 4 criteria: 1) baseline organisms should be easy to collect, 2) within-site variation in $\delta^{15}N$ levels should be low, 3) $\delta^{15}N$ should reflect land use, and 4) trophic position (TP) of consumers calculated from the baseline should be independent of system-specific $\delta^{15}N$ variability as long as no systematic change in food consumption occurred. We investigated individual taxa and bulked groups representing different feeding modes as baselines. We found that Simuliidae, a sestonic filter feeder, fulfilled all criteria. Furthermore, TP estimates of 2 common fishes that were based on the Simuliidae or grouped filterers as baselines were the only estimates in our study that were independent of landuse changes. In addition, the diet of these fishes did not change across land use as based on stable-isotope mixing-model analysis. Simuliidae also had the lowest within-site variation, i.e., the lowest trophic level range, probably a result of uniform feeding behavior. Therefore, Simuliidae and grouped filterers could be suitable baseline indicators in future studies. We recommend minimizing $\delta^{15}N$ variability in and among systems because the precise, complex choice, timing, or proportions of food sources consumed cannot be mimicked. We also promote combining TP estimation and mixing-model analyses as a strong tool in studies of stream food webs.

Key words: foodweb analyses, stream ecosystems, isotope baseline, isotope methods, trophic relations

Stable-isotope analysis (SIA) is a widely used method to evaluate trophic structure and resource dynamics in aquatic ecosystems (e.g., Fry 1983, Cabana and Rasmussen 1996, Vander Zanden et al. 1999, Post 2002, Reid et al. 2008). Elemental transfer of food to consumers through assimilation and excretion processes leads to changes in isotopic composition in a predictable manner and, thus, provides information on the trophic position (TP) and potential food sources of a consumer (Peterson and Fry 1987, Vander Zanden and Rasmussen 2001, Post 2002, Reid et al. 2008). The relationships between heavy and light isotopes of C ($^{13}C/^{12}C$; $\delta^{13}C$) and N ($^{15}N/^{14}N$; $\delta^{15}N$) typically are used (Peterson and Fry 1987, Vander Zanden and Rasmussen

2001). Furthermore, SIA gives a time-integrated picture of trophic structure and consumption rather than the snapshot picture provided by gut-content analysis of consumers (Vander Zanden et al. 1997, Rybczynski et al. 2008).

The degree of fractionation of the isotopes at each consumption step and whether the heavy or the light isotope is favored depend primarily on the organism and tissue investigated and, particularly for C, whether the main process is photosynthesis or respiration (Park and Epstein 1960, Hobson et al. 1996). $\delta^{13}C$ has a relatively small fractionation in freshwater systems at every consumption step (~0.4‰; e.g., McCutchan et al. 2003), whereas the frac-

tionation of $\delta^{15}N$ is usually higher (2–4‰). Therefore, $\delta^{15}N$ is used widely to assess the TP of consumers (Cabana and Rasmussen 1996, Vander Zanden and Rasmussen 1999). However, aquatic systems and their biota can vary greatly in $\delta^{15}N$ values as a result of different land uses (e.g., Cabana and Rasmussen 1996, Lake et al. 2001, Anderson and Cabana 2006, Pastor et al. 2013). Excessive loads of anthropogenic N arising from agricultural production can increase the $\delta^{15}N$ level. Anderson and Cabana (2005) reported up to 15‰ increase, which reflects that the N pool already is fractionated when entering the system (e.g., Anderson and Cabana 2005, Bergfur et al. 2009). In comparison, $\delta^{15}N$ values of 3.5 to 5.5‰ have been reported for more pristine watersheds with limited agricultural production (>80% forest; Mayer et al. 2002).

The large variability in $\delta^{15}N$ values emphasizes the need for identification of baseline indicators that reflect the $\delta^{15}N$ level at the base of the system to permit cross-site comparisons of $\delta^{15}N$. The challenges of identifying and handling isotopic baselines satisfactorily have been addressed in a number of lake and stream systems (e.g., Vander Zanden et al. 1997, Post 2002, Anderson and Cabana 2007, Jardine et al. 2012). Primary consumers usually are used as baselines because they generally show lower $\delta^{15}N$ variability than do primary producers and typically have longer life histories, which improves temporal resolution. Some investigators have argued that baseline indicators should have a broad spatial distribution to allow among-system comparisons (e.g., Anderson and Cabana 2007) and, overall, large temporal and spatial discontinuities in baselines should be avoided (Post 2002). Vander Zanden and Rasmussen (1996) and Vander Zanden et al. (1997) used filterers (unionid mussels) as baseline indicators in lakes because these mussels are large, long-lived primary consumers with a uniform nonomnivorous feeding strategy, i.e., filtration of suspended particulate organic matter, primarily phytoplankton and bacteria, in the water column. However, such long-lived primary consumers are rarely abundant and may be absent in temperate streams and, therefore, unsuitable as baseline indicators.

Choosing organisms with the same feeding mode has been popular because of difficulties in specifying suitable primary consumers as baseline indicators. For example, Anderson and Cabana (2007) used scrapers as their baseline (a mix of members from the macroinvertebrate families Psephenidae, Heptageniidae, Ephemerellidae, Physidae, and Viviparidae) because these organisms had the lowest $\delta^{15}N$ and were the most common primary consumers present. Other widely represented primary consumer functional feeding groups (FFGs) include shredders and sediment feeders. On the other hand, omnivorous organisms foraging at different trophic levels in different systems may yield biased baselines (Vander Zanden and Rasmussen 1999, Anderson and Cabana 2007). In the absence of 1 widely distributed appropriate group or species, a mean primary

consumer $\delta^{15}N$ value also has been used as baseline (e.g., Rybczynski et al. 2008).

Many investigators have treated the difficult and important task of identifying reliable baseline organisms, but only a few comprehensive comparative tests exist of the effect of using different potential baseline organisms or groups of organisms in stable-isotope studies of trophic structure in streams and rivers (but see Post 2002, Anderson and Cabana 2007, Jardine et al. 2012). Consensus about baseline criteria still is needed for reliable comparisons of TP results from different types of systems. We compared and evaluated the applicability of baselines of specific primary consumer organisms and baselines based on various degrees of taxon aggregation (FFGs and the mean of all primary consumers). Specifically, we searched for organisms or organism groups that could be used reliably as baseline indicators in lowland streams with varying landuse characteristics in the watershed.

METHODS
Stream selection

We sampled 21 sites in 12 Danish lowland streams. We selected the sites to represent an anthropogenic stressor gradient based on nutrient loads and land use. We collected samples during summer 2007 and 2010 (June–August) and conducted sampling in a given stream within the same year to avoid temporally induced effects on stable-isotope signatures.

Land use and catchments

We defined the catchment areas of each sampling site with a national digital topographic map (1:25,000 scale). We obtained information on land use in the catchment areas from a national land-cover raster map (25-m grid) with 12 land-cover classes. We reclassified the land-cover classes into: intensive agriculture, permanent grass (e.g., pasture), forest, natural dryland areas (e.g., heath and moorland fields), natural wetland areas (e.g., bogs, marshes, and wetlands), artificial areas (e.g., built-up areas and roads), and lakes. For each site, we expressed land cover as % coverage. Following reclassification, we categorized land use into: 1) % arable land, calculated as the sum of intensive agricultural areas in the catchment, and 2) % natural land cover, calculated as the sum of all remaining land uses except artificial areas.

We found that both landuse categories (% arable land and % natural land cover) were correlated with $\delta^{15}N$ of most primary and secondary consumers. $\delta^{15}N$ increased with an increasing % arable landuse cover and decreased with increasing % natural landuse cover, as was expected from previous findings (Cabana and Rasmussen 1996, Lake et al. 2001, Vander Zanden et al. 2005, Anderson and Cabana 2006). However, slightly better correlations were observed for % natural land cover, so we used this category

for establishing further correlations. In addition, we measured total N (TN) of stream water on a Shimadzu TOC-V$_{CPH}$ (model UV21 160/Shimadzu, UV-1700; Shimadzu, Kyoto, Japan).

SIA

We sampled fishes by electrofishing and netting. We analyzed fish dorsal muscle tissue from up to 20 individuals/species/stream for isotopic composition. We measured all fish to total length (TL; 0.1 cm) and standard length (SL; 0.1 cm) and weighed them (0.01 g). We freeze-dried fish <25 mm whole to obtain sufficient sample material; the dorsal muscle remained the predominant sample material. We used only the dominant taxa of Brown Trout (*Salmo trutta*) and Three-spined Stickleback (*Gasterosteus aculeatus*), which occurred at ≥10 sites, in further analyses of TP. We excluded a range of organisms because they were rare, including macroinvertebrate taxa such as Asellidae (Isopoda), Hydropsychidae and Brachycentridae (Trichoptera), and Heptageniidae (Ephemeroptera), and various chironomids and plecopterans. We also excluded 13 fish species: European Eel (*Anguilla anguilla*), Northern Pike (*Esox lucius*), European Perch (*Perca fluviatilis*), Crucian Carp (*Carassius carassius*), Gudgeon (*Gobio gobio*), Common Dace (*Leuciscus leuciscus*), Burbot (*Lota lota*), Rainbow Trout (*Oncorhynchus mykiss*), Roach (*Rutilus rutilus*), European Flounder (*Platichtys flesus*), Nine-spined Stickleback (*Pungitius pungitius*), Atlantic Salmon (*Salmo salar*), and Grayling (*Thymallus thymallus*), and 1 specimen of the relatively rare Sea Lamprey (*Petromyzon marinus*). However, in bulked samples of consumers, we included these more rarely represented species.

We sampled macroinvertebrates from all main habitats (dominant macrophytes, soft and hard substratum), classified them to family level following Dall and Lindegaard (1995), and assigned them to an FFG according to Wallace and Webster (1996) and Skriver (1982). Before freeze-drying, we removed exoskeleton parts from arthropods and mollusks from their shells. For small-bodied taxa, we often pooled many individuals to reach the recommended mass for analysis. We also sampled the major primary uptake compartments, defined as the compartments that take up nutrients directly from the water column (e.g., von Schiller et al. 2009): periphyton (from gravel and rocks), macrophytes, and fine and coarse benthic organic matter. For the dominant macrophytes in each stream, we collected up to 5 samples and cleaned off periphyton before analysis to avoid mixing isotope signals from the 2 compartments. We collected fine and coarse benthic organic matter as bulked samples from 5 different locations along the stream reach. We sampled periphyton assemblages from gravel and rocks. We filtered these samples on a GF-C filter (precombusted glass-fiber filter) and cleaned them under magnification to remove particles, such as zooplankton and macrophyte leaf remains, before freeze-drying.

In total we analyzed 1337 samples for $\delta^{15}N$. We sorted samples in the field, when possible, or immediately after returning from the field. All samples were kept frozen at −22°C until further processing. After freeze-drying, we homogenized the samples, weighed them into Sn capsules, and had them analyzed at UC Davis Stable Isotope Facility (Davis, California) with a PDZ Europa ANCA-GSL elemental analyzer in conjunction with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon, Cheshire, UK).

The ratios of heavy to light N isotopes are expressed with δ notation as $\delta^{15}N$ ($^{15}N/^{14}N$) and defined as per mil (‰) deviation from a standard material:

$$\delta^{15}N = [(^{15}N/^{14}N_{sample}/^{15}N/^{14}N_{standard}) - 1] \times 1000,$$
(Eq. 1)

where N_{sample} is the ratio of heavy and light isotopes in the analyzed element, and $N_{standard}$ is the ratio of heavy and light isotopes in standard material, which for $\delta^{15}N$ is atmospheric N.

Baseline and TP

We selected the baseline organisms according to 4 criteria: 1) they should be generally ubiquitous and geographically widespread to have broad applicability (e.g., Landolt and Sartori 1995, Bilton et al. 2001, Gooderham and Tsyrlin 2002, Merritt et al. 2008); 2) they should demonstrate low within-site variation in $\delta^{15}N$ values and thus reflect a uniform nonomnivorous feeding behavior; 3) they should display correlation between $\delta^{15}N$ values and % natural land use and, thus, reveal baseline properties reflecting background $\delta^{15}N$ levels; and 4) TP of secondary consumers calculated from the baseline organisms should be independent of system-specific $\delta^{15}N$ variability as long as no systematic change in food consumption occurs with landuse change. Based on the strong evidence of correlation between stream organism $\delta^{15}N$ values and % natural land use (Cabana and Rasmussen 1996, Lake et al. 2001, Vander Zanden et al. 2005, Anderson and Cabana 2006), land use was selected a priori as the environmental variable determining background $\delta^{15}N$ levels, although other environmental variables (e.g., catchment size and soil or bedrock composition) potentially could produce system-specific ^{15}N variability.

To assess whether the different potential baselines responded similarly to landuse changes, we compared the slopes of the regressions between $\delta^{15}N$ and % natural landuse cover in the catchment among the frequently occurring taxa. We analyzed the implications of the different baselines for the TP estimates of fishes by comparing TP estimates of 2 common Danish fish species, *S. trutta* and *G. aculeatus* (Kristensen et al. 2012, Teixeira-de Mello et al. 2012), based on standard procedures (Vander Zanden et al. 1997, Post 2002):

$$TP = [(\delta^{15}N_{consumer} - \delta^{15}N_{baseline})/3.4] + 2,$$ (Eq. 2)

where the fractionation value of 3.4 represents the mean enrichment of heavy to light isotopes (δ^{15}N) from one trophic level to the next of freshwater biota (Post 2002). To test whether the δ^{15}N$_{baselines}$ were independent of % natural land use, we evaluated whether regression lines for TP vs % natural land use had slopes = 0, which should be the case if the consumers had not changed their diet systematically with % natural land use. To assess whether food resources changed, we conducted gut-content analysis and isotope mixing-model analysis.

Fish diet preferences

For each stream, we used the means of isotopic signatures to calculate the proportions of food sources present in the diets of each consumer (single taxa and bulked FFGs) with a mixing-model analysis in the R-based software SIAR (Parnell and Jackson 2015). We included only data from streams where all resources (single taxa or FFG) and both fish were present in the mixing model, which resulted in 7 streams for the single-taxa model and 6 streams for the FFG model. The model applies Bayesian inference to yield output data that provide the most probable dietary proportions of possible sources to a consumer based on C and N isotope ratios of sources and consumers (Parnell et al. 2010). The SIAR mixing model allows correction of trophic enrichment factors, and we corrected these using literature values from Post (2002). We calculated the potential contribution of each of the resources for the 2 fish species analyzed (*S. trutta* and *G. aculeatus*) as a range reported as 5th to 95th percentiles.

Statistics

We used Statgraphics software (Centurion XVI.I; Statgraphics, Warrenton, Virginia) for statistical analyses. When data were distributed normally according to standard skewness and kurtosis, Shapiro–Wilk test, and visual exploration of boxplots and histograms, we performed parametric tests. When data did not exhibit a normal distribution and could not be transformed to normality, we used nonparametric tests.

We compared differences in within-site variation (coefficient of variation = CV) of the mean δ^{15}N values of single taxa and FFGs with 1-way analysis of variance (ANOVA). We calculated CV as SD/mean.

We applied linear regression analysis to identify correlations between stream compartment δ^{15}N and TP estimates vs % natural land use. To investigate whether the potential baselines responded differently to a change in % natural land use, we used analysis of covariance (ANCOVA) to test for differences in the δ^{15}N slopes of the different baseline organisms relative to % natural land use (comparison of regression lines). We also tested for a potential correlation between stream biota δ^{15}N and TN and found no significant correlation (not shown). We used ANCOVA to determine whether the slopes of TP estimates were different from 0 (i.e., absence of relationship signifies TP independent of % natural land use).

We compared TP estimations calculated with different baselines by means of nonparametric tests: Kruskal–Wallis for a multiple-sample comparison of medians plot, followed by a Mann–Whitney–(Wilcoxon) 2-sample U-test for identifying differences between individual samples.

We evaluated whether *G. aculeatus* and *S. trutta* changed their diet along a landuse gradient by examining the relationships between the proportion of the most dominant invertebrate species in the fish diets based on the mixing models vs land use by means of linear and nonlinear regression analyses. The regressions of diet item proportions vs % natural land use were based on 7 stream sites for which all the baseline taxa included in our study were present.

RESULTS

Mean δ^{15}N values and variance of specific taxa and FFGs are summarized in Tables S1, S2. The lowest within-site variation in δ^{15}N for specific taxa was for Simuliidae (t-test, $p < 0.05$; Table 1). Among the FFGs, filterers had the lowest within-site variation, and scrapers exhibited the highest variation (Mann–Whitney U-test, $p < 0.05$; Table 1).

In general, δ^{15}N of the various organisms was negatively correlated with % natural landuse cover in the catchment (Figs 1A–G, S1). Of all primary-producer uptake compartments, only macrophyte δ^{15}N was correlated significantly with % natural landuse cover, but the correlation

Table 1. Mean coefficient of variation (CV ± SD) of δ^{15}N for the most abundant taxa and functional feeding groups (FFGs) in the 12 study streams. Detailed data from each stream are found in Tables S1, S2. Mean δ^{15}N of taxa or FFGs with the same superscripted letter are not significantly different (1-way analysis of variance, $p < 0.05$).

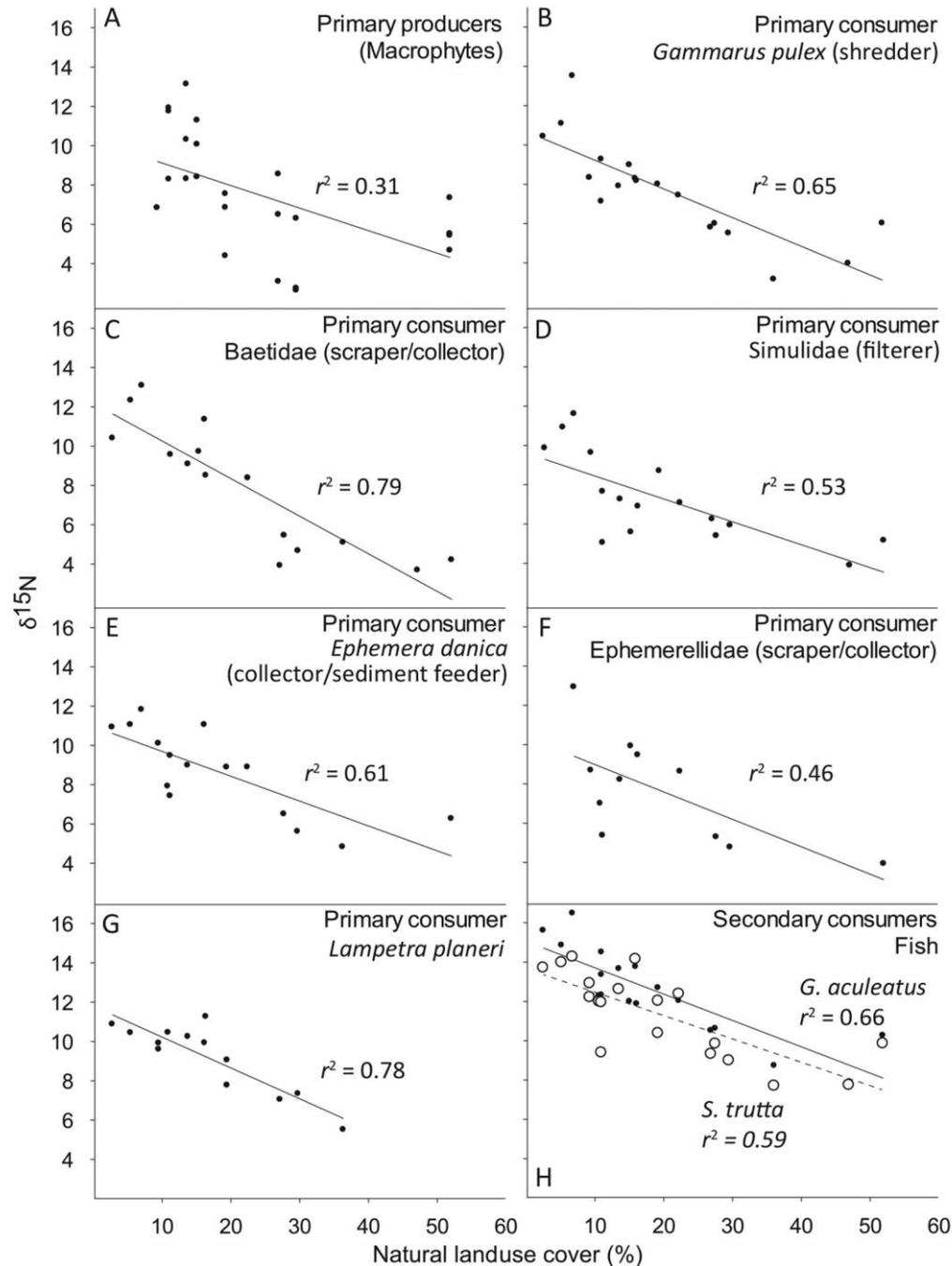| Taxon or group	δ^{15}N mean CV
Most abundant taxa	
Simuliidae	0.028[a] ± 0.019
Baetidae	0.080[b] ± 0.09
Gammarus pulex	0.094[b] ± 0.061
Ephemera danica	0.056[b] ± 0.031
Lampetra planeri	0.090[b] ± 0.051
Functional feeding groups	
Filterer	0.03[a] ± 0.018
Scraper	0.13[c] ± 0.156
Shredder	0.12[bc] ± 0.065
Sediment feeder	0.12[bc] ± 0.087
Omnivore	0.063[ab] ± 0.035
Predator	0.12[bc] ± 0.063

Figure 1. Significant linear regressions ($p < 0.05$) of δ^{15}N values of stream primary producers: macrophytes (A), primary consumers: *Gammarus pulex* (shredder) (B), Baetidae (scraper/collector) (C), Simuliidae (filterer) (D), *Ephemera danica* (collector/sediment feeder) (E), Ephemerellidae (scraper/collector) (F), *Lampetra planeri* (filterer/sediment feeder) (G), and the secondary consumers: fishes (*Salmo trutta* as open circles) and *Gasterosteus aculeatus* as closed circles) (H) vs % natural landuse cover in 12 Danish lowland streams. r^2 given for significant relationships.

coefficient was low (Fig. 1A). For the primary consumers, δ^{15}N was correlated significantly with % natural landuse cover for most groups (Fig. 1B–G) with the exception of the trichopteran Limnephilidae, which belongs to the shredder group, and the coleopteran Elmidae, which belongs to the scraper/collector group (linear regression, $p > 0.05$,

data not shown). For the secondary consumers, δ^{15}N of *G. aculeatus* and *S. trutta* also was correlated with % natural landuse cover (Fig. 1H). Furthermore, mean δ^{15}N of all invertebrate FFGs and all primary consumers was correlated with % natural landuse cover, and predatory invertebrates showed the highest correlation coefficient (Fig. S1).

A comparison of regressions among all combinations of consumers yielded marginally significant differences in slopes ($p < 0.1$) between Baetidae and *S. trutta* (ANCOVA, $p < 0.059$; Fig. 1C, H) and between Baetidae and Simuliidae (ANCOVA, $p < 0.00001$; Fig. 1C, D); i.e., their $\delta^{15}N$ values did not respond in a similar manner to a change in % natural land use. No other comparison of regression lines showed significant differences ($p > 0.1$).

For all sites, TP estimates for *G. aculeatus* were generally higher than for *S. trutta*, whether based on specific taxa (Fig. 2A, B) or FFGs (Fig. 2C, D) as baselines. The TP estimates for both species based on taxon-specific baselines were marginally highest when using Simuliidae as baseline and lowest when using Baetidae (Fig. 2A, B). This result reflected a higher mean baseline $\delta^{15}N$ value for Baetidae than for Simuliidae in the most anthropogenically affected streams. Likewise for both fish species, the TP estimates calculated using FFG as baseline were highest when based on filterers (including the Simuliidae) as baseline (Fig. 2C, D). Invertebrate predator-based estimates gave the lowest TP of both fishes (Fig. 2C, D).

Only the regressions of *G. aculeatus* TP estimates based on Simuliidae, *Gammarus pulex*, and filterer (ANCOVA, $p > 0.1$; Figs 3A–D, S2) and *S. trutta* TP estimates based on Simuliidae (ANCOVA, $p > 0.05$, Figs 4A–D, S3) vs % natural landuse cover had slopes that were not significantly different from 0, i.e., independent of % natural land use. All other regressions between TP of the 2 fishes based on taxon-specific and bulked groups vs % natural land use were significant ($p < 0.05$; Figs 3A–D, 4A–D, S2, S3). The TP estimates based on taxon-specific baselines revealed the steepest slopes for both fish species when based on Baetidae (Figs 3A–D, 4A–D). When based on FFG baselines, the slopes of *G. aculeatus* and *S. trutta* based on omnivores were steepest (Table 2,), but these results were driven by a particularly high omnivore-based TP at 1 site.
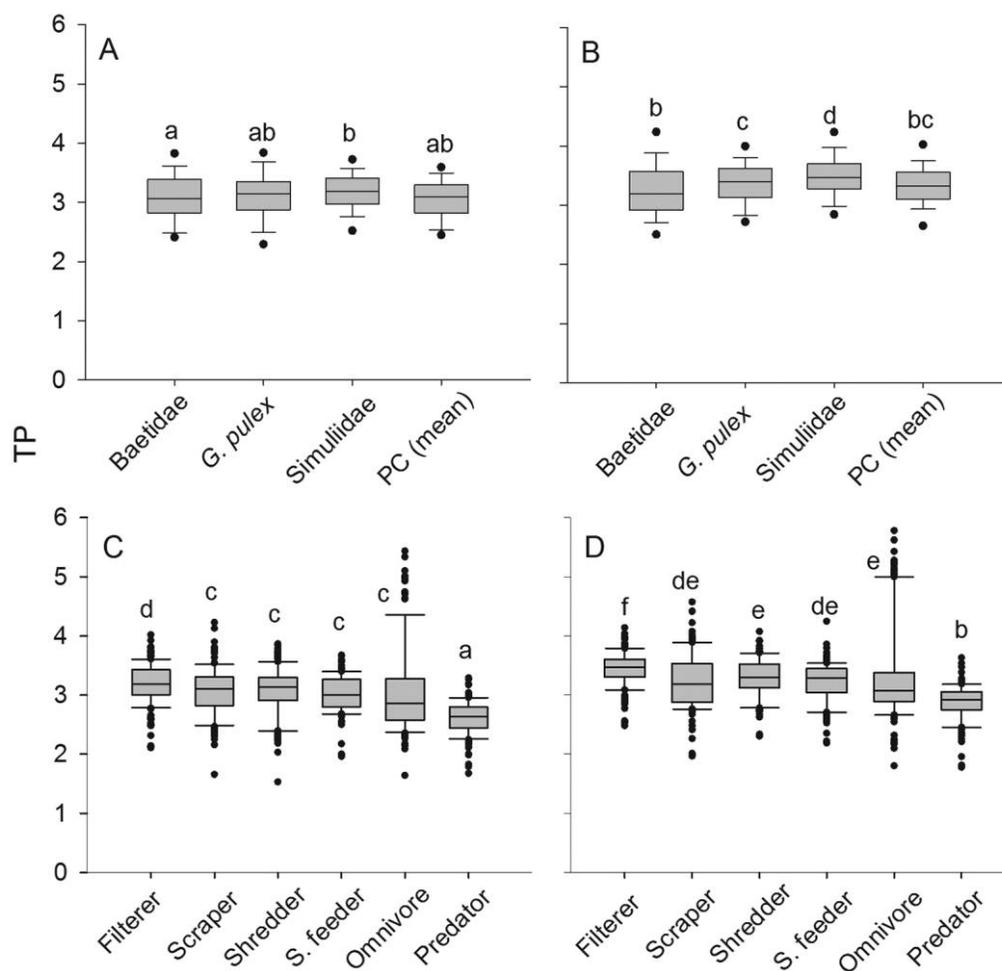

Figure 2. Box-and-whisker plots of trophic position (TP) estimates for *Salmo trutta* (A, C) and *Gasterosteus aculeatus* (B, D) based on taxon-specific baseline indicators (A, B) and functional feeding group baseline indicators (C, D). Lines in boxes are medians, box ends are 25th and 75th percentiles, whiskers are 5th and 95th percentiles, and circles show outliers. Boxes with the same letters are not significantly different ($p < 0.05$). G. = *Gammarus*, PC = primary consumers, S. feeder = sediment feeder.
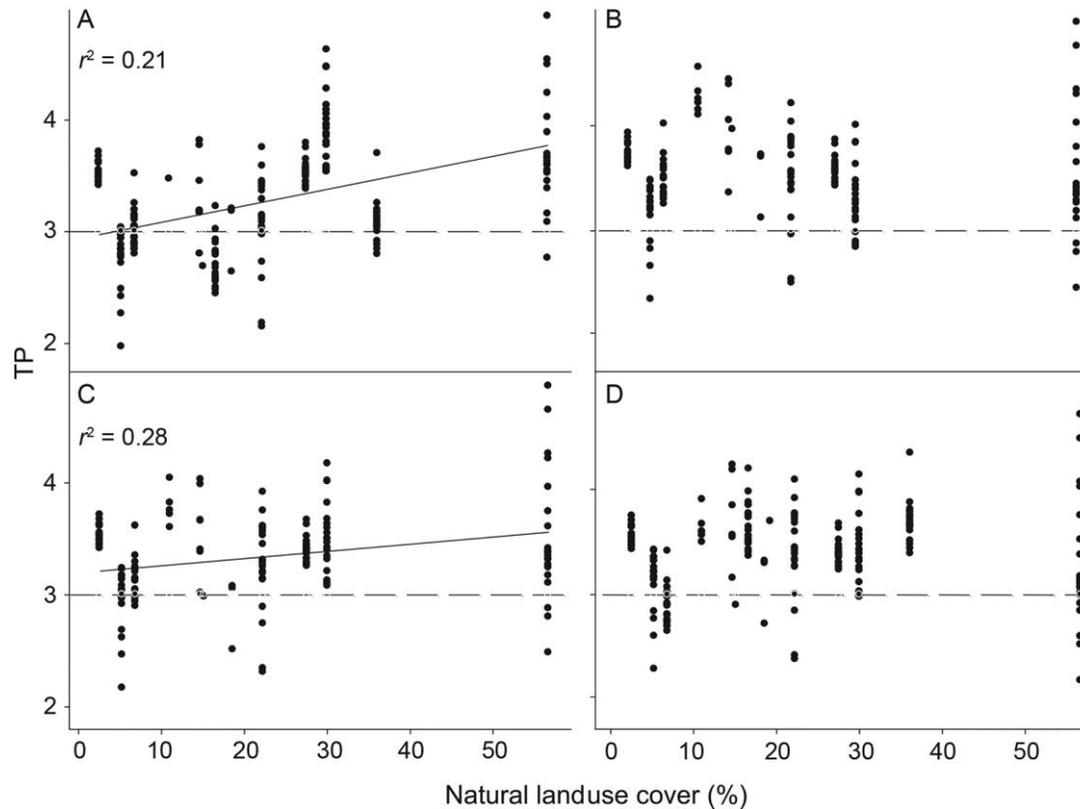
Figure 3. Regressions (from analysis of covariance) of trophic position (TP) estimates vs % natural landuse cover for *Gasterosteus aculeatus*. δ^{15}N baseline for TP based on Baetidae (A), Simuliidae (B), mean of primary consumers (C), and *Gammarus pulex* (D). Regressions were tested against the constant theoretical TP of 3 (i.e., slope = 0): r^2 given for significant relationships ($p < 0.05$).

We compared regressions of all TP estimates based on taxon-specific and bulked group baselines vs % natural land use (Tables 3, 4). Comparison of *S. trutta* TP estimates based on specific taxa and the mean of all primary consumers regressed against % natural land use (Table 3) demonstrated differences in slopes between all combinations except the slopes between mean primary consumer-based TP and Simuliidae-based TP as well as *G. pulex*-based TP. For *G. aculeatus*, the pattern was the same, but also the slopes of TP based on Simuliidae and *G. pulex* did not differ (Table 3). For both fishes, using scrapers or omnivores as baselines for TP resulted in slopes of TP estimates vs % natural land use that differed from all other combinations of FFG baselines used (Table 4). The only other regressions that differed significantly were filterer-based *G. aculeatus* TP from the estimates based on shredders and predators (Table 4).

We evaluated whether the fishes changed their diets with a change in % natural land use by relating means of mixing models with TP estimates based on Baetidae, *G. pulex* (for *S. trutta*) and FFGs except for Simuliidae, *G. pulex* (for *G. aculeatus*) and grouped filterers. We related the results from the mixing-model analysis (Table 5) for each stream to % natural land use. No systematic change occurred in the diet of either *S. trutta* or *G. aculeatus*

when regressing the proportions of the food items against % natural land use (linear and nonlinear regressions, $p > 0.05$, data not shown).

DISCUSSION

Initially, we established 4 criteria for baseline screening. Organisms should: 1) be widely distributed in the streams, 2) show low within-site variation in δ^{15}N values, 3) have δ^{15}N values correlated with % natural land use, and 4) show no systematic change in TP estimates or diet with % natural land use. The 1st criterion limited the number of taxa markedly, from 45 to 6. Of the remaining taxa, we decided to evaluate Simuliidae, Baetidae, and *G. pulex* as taxon-specific baseline descriptors because they were widespread and abundant and represented the filterer (Simuliidae) and scraper (Baetidae) FFGs, which are described in the literature as good baseline indicators (Vander Zanden and Rasmussen 1996, Vander Zanden et al. 1997, Anderson and Cabana 2007).

Of all possible taxon-specific and bulked group baselines, we found that Simuliidae, as a single taxon, and filterers, as an FFG, best fulfilled the 3 remaining criteria. We identified Simuliidae, a sestonic filter feeder (Currie and Craig 1988) that filters small fractions of suspended
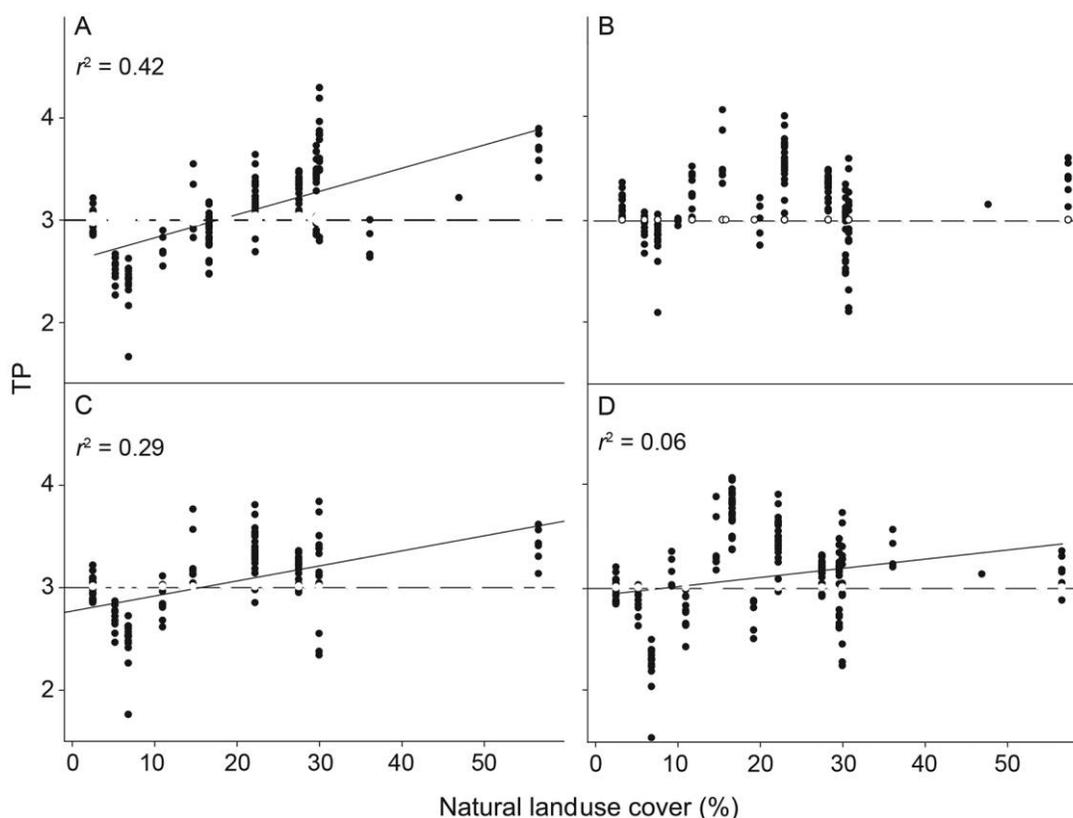
Figure 4. Regressions (from analysis of covariance) of trophic position (TP) estimates vs % natural landuse cover for *Salmo trutta*. δ^{15}N baseline for TP based on Baetidae (A), Simuliidae (B), mean of primary consumers (C), and *Gammarus pulex* (D). Regressions were tested against the constant theoretical TP of 3 (i.e., slope = 0): r^2 given for significant relationships ($p < 0.05$).

particulate organic matter from the water column (e.g., Silverman et al. 1995), as the specific taxon that was most appropriate of the evaluated baselines in our study. Simuliidae alone, but also bulked with other filterers, also exhibited low within-site variation (criterion 2) in δ^{15}N values, probably a result of its uniform feeding behavior, which ensured that the differences in δ^{15}N between systems were not affected by different food sources. In contrast, *G. pulex*, a shredder that is widely distributed and abundant in many temperate streams (Holdich and Pöckl 2007), showed high within-site variation, probably reflecting its occasionally omnivorous feeding behavior (Skriver 1982, Moog 1995). Anderson and Cabana (2007) also found that shredders had relatively variable δ^{15}N levels. Another widely distributed taxon, Baetidae, with 9 Danish species (Jensen 1984b), also showed higher within-site variation than Simuliidae, probably because it forages on mixed sources. Baetidae is a scraper that feeds on bulked sources of periphyton assemblages on macrophytes and rocks (Wallace and Webster 1996), i.e., algae, bacteria, and smaller animals such as protozoa.

All 3 taxon-specific and bulked group baselines showed significant relationships with % natural land use (criterion 3).

When Simuliidae was used as a baseline for calculating TP in *G. aculeatus* and *S. trutta* and when bulked filterers were used for calculating TP in *G. aculeatus*, the resulting TP was independent of % natural landuse changes (criterion 4). This result was supported by the mixing-model analysis, which showed that no significant change in diet took place with changes in % natural land use. Therefore, criterion 4 became the most important gauge separating the best baseline organisms from the others. The small difference in TP estimates among different taxa or FFGs (Fig. 2A–D) reveals low ecological difference based on, Simuliidae or *G. pulex*, filterers or scrapers, shredders or sediment feeders. However, because we found that criterion 4 is highly important in validating the baseline organisms, we argue that Simuliidae and bulked filterers are preferred over other baseline organisms when calculating TP for fishes in lowland streams.

Different approaches have been used when choosing baseline indicators, and bulked primary consumers is a frequent choice. It may seem reasonable to use all likely prey organisms and not just one taxon or FFG as baseline when the ultimate goal is to quantify fish TP because fish, such as those of our study, eat many different

Table 2. Slopes and r^2 values for significant linear relationships between trophic position estimates for *Gasterosteus aculeatus* and *Salmo trutta* based on functional feeding group (FFG) baselines vs % natural landuse cover in the catchment. Regressions for FFGs are shown in Fig. S1.

Fish taxa and FFGs	Slope	r^2
Gasterosteus aculeatus		
Filterers	–	–
Scrapers	−0.010	0.094
Shredders	−0.050	0.037
Sediment feeders	−0.004	0.020
Omnivores	−0.050	0.331
Predators	−0.004	0.029
Salmo trutta		
Filterers	−0.005	0.026
Scrapers	−0.020	0.226
Shredders	−0.008	0.062
Sediment feeders	−0.008	0.085
Omnivores	−0.040	0.259
Predators	−0.007	0.089

invertebrate and fish prey. However, such an approach may be inappropriate. We do not know which food items fish have selected at different times or in what proportions. Consequently, a baseline mimicking the exact complex diet of, for instance, *S. trutta*, cannot be established. By choosing 1 or a few taxa or an FFG as baseline indicators, errors resulting from different feeding patterns in different systems can be minimized. However, to ensure that the resultant TP estimates are reliable, ideally mixing analysis or gut-content analysis should be undertaken to elucidate whether a systematic landuse-dependent change in fish diet has occurred.

In Denmark, the family Simuliidae includes 23 species (Jensen 1984a), and it emerged as the most appropriate

baseline in our study. This family is distributed worldwide (Currie and Adler 2008) and may serve as baseline in future international studies. However, use of Simuliidae larvae or other filterers, such as Psephenidae, as a baseline carries a challenge. Most Simuliidae are univoltine, and the larval stage is usually relatively short, typically a couple of months, although longer larval stages of varying lengths occur for overwintering individuals in temperate regions (Rubtsov 1990). Short larval stage or generation time is a general problem when studying larger, long-lived consumers such as *S. trutta*. In the absence of long-lived primary consumers similar to the unionid mussels used by Vander Zanden et al. (1997), relevant time-series data on baseline organisms (Post 2002) may be needed. However, prioritizing long-lived primary consumers (Cabana and Rasmussen 1996, Post 2002) may not always be appropriate. If, for instance, the end consumer is short lived or has arrived recently (e.g., fish fry), the baseline organisms should ideally have lived in a comparable environment and integrated the isotope signals over a similarly short lifespan.

Habitat-specific variability is another important factor when considering baselines (Post 2002). We observed a >2-unit $\delta^{15}N$ difference between limnephilids inhabiting the same stream but collected on 2 different substrates, macrophyte and soft substratum (Mann–Whitney U-test, $p < 0.05$, not shown). Thus, limnephilids did not appear to be a reliable baseline indicator, and it is likely that other groups also differ in $\delta^{15}N$ between habitats, not least among the shredders, which are likely to reflect their substrate. If used, shredders should be collected from the same habitats, even in small streams, especially at sites with abundant or diverse macrophyte beds or differing in riparian vegetation characteristics. A baseline difference of 2 $\delta^{15}N$ units between 2 baseline organisms, as we observed for limnephilids, would result in a 0.6 difference in TP.

Mixing-model analysis offers new possibilities in understanding energy flow through ecosystems without the

Table 3. Comparison of regressions from analysis of covariance (ANCOVA) based on taxon-specific $\delta^{15}N$ baselines for trophic position of *Salmo trutta* and *Gasterosteus aculeatus* vs % natural landuse cover. Significant ANCOVA results ($p < 0.10$) are bolded. PC = primary consumers.

Fish and baseline taxa	Simuliidae	Baetidae	*Gammarus pulex*
S. trutta			
Baetidae	**<0.00001**		
Gammarus pulex	**0.0013**	**0.0006**	
Mean PC	0.0554	**<0.00001**	0.1068
G. aculeatus			
Baetidae	**<0.00001**		
Gammarus pulex	0.3686	**<0.00001**	
Mean PC	0.2193	**<0.00001**	0.7521

Table 4. Comparison of regression lines from analysis of covariance (ANCOVA) based on functional feeding group (FFG) δ^{15}N baselines for trophic position of *Salmo trutta* and *Gasterosteus aculeatus* vs % natural landuse cover. Significant ANCOVA results ($p < 0.10$) are bolded.

Fish and baseline FFG	Scraper	Filterer	Shredder	Sediment feeder	Omnivore
S. trutta					
Filterer	**0.0015**				
Shredder	**0.0091**	0.3109			
Sediment feeder	**0.0014**	0.3902	0.7823		
Omnivore	**0.0003**	**<0.00001**	**<0.00001**	**<0.00001**	
Predator	**0.0011**	0.4447	0.7111	0.9120	**<0.00001**
G. aculeatus					
Filterer	**0.0007**				
Shredder	**0.0875**	**0.0481**			
Sediment feeder	**0.0310**	0.1403	0.6051		
Omnivore	**<0.00001**	**<0.00001**	**<0.00001**	**<0.00001**	
Predator	**0.0434**	**0.0882**	0.7439	0.8400	**<0.00001**

use of baseline organisms in TP methodology. However, mixing-model analyses are not without challenges (Phillips 2001, Moore and Semmens 2008, Parnell et al. 2013, Brett 2014). Results are sensitive to input data and to how multiple and, perhaps, overlapping isotopic sources are handled. The most serious challenge is, perhaps, the risk of biased interpretations of food sources in the model based on the assumption that these sources serve as food for the consumer. The inability to sample accurately the food sources assimilated by consumers, as highlighted by Dodds et al. (2014), may be responsible for much of the uncertainty in natural stable-isotope studies. Even so, the model output data provide some evidence of the food sources potentially consumed. With careful consideration,

Table 5. Results from SIAR (Parnell and Jackson 2015) mixing model. Contributions (5th–95th percentile range) of each of the resources to the diet of *Gasterosteus aculeatus* (G) and *Salmo trutta* (S) in each of the study streams. Missing values indicate that not enough data were present to run the model. Diet resources are Simuliidae (Simulii), Baetidae (Baetid), *Gammarus pulex* (*G. pul*), *Ephemera danica* (*E. dan*), filter feeders (filter), scrapers (scrap), shredders (shred), sediment feeders (sed), omnivores (omni), and predators (pred).

Stream	Fish	Diet resource									
		Simulii	Baetid	*G. pul*	*E. dan*	Filter	Scrap	Shred	Sed	Omni	Pred
Guden 3	G	1.2–3.7	0.0–43.7	0.2–52.9	6.0–53.4	0.2–28.2	0.2–43.1	0.0–29.9	0.8–38.6	0.0–27.4	0.0–32.0
	S	27.4–52.4	0.0–11.0	0.02–44.8	19.3–42.4	26.6–46.0	0.3–22.5	0.0–23.2	14.0–44.7	0.0–13.7	0.0–18.6
Alling 1	G	3.3–58.3	0.0–26.2	0.5–53.7	0.4–52.1	0.0–20.1	0.0–30.3	10.7–56.5	0.0–38.3	0.0–18.0	0.0–36.0
	S	0.0–39.6	0.0–27.6	0.0–45.6	15.9–90.6	0.0–8.0	0.0–17.5	51.5–94.6	0.0–19.4	0.0–8.8	0.0–19.2
Alling 2	G	0.0–37.8	0.0–19.8	13.9–83.9	0.0–58.3	0.0–15.1	0.0–11.5	0.0–16.5	20.7–69.0	0.0–22.4	4.1–58.3
	S	0.6–44.0	1.4–51.4	0.0–37.3	0.8–53.2	0.0–22.3	0.0–18.5	0.0–21.9	16.2–80.1	0.0–24.1	0.7–32.7
Alling 3	G	0.0–24.3	0.0–13.4	48.1–86.5	0.1–37.2	–	–	–	–	–	–
	S	0.4–56.3	0.0–19.8	0.4–36.6	10.5–80.9	–	–	–	–	–	–
StorkF	G	0.0–49.5	0.0–47.6	0.0–48.6	0.0–47.9	–	–	–	–	–	–
	S	0.0–37.5	6.9–26.6	1.5–48.6	11.3–68.2	–	–	–	–	–	–
Lille	G	0.0–35.4	0.0–11.5	27.2–88.8	0.1–50.2	0.0–38.9	0.0–13.1	0.0–40.4	5.5–67.9	0.0–21.7	0.0–32.0
	S	0.0–40.2	0.0–28.0	2.3–67.3	2.0–70.2	0.0–30.3	0.0–24.2	0.0–34.5	0.3–41.7	0.0–31.2	0.3–42.0
Aakaer	G	0.0–45.7	0.3–7.6	0.0–57.7	12.4–83.4	0.0–27.9	0.0–31.1	0.0–35.0	0.0–31.5	0.0–31.4	1.5–44.9
	S	0.0–41.2	0.0–4.8	0.0–48.1	28.4–96.6	0.0–24.9	0.0–34.6	0.0–32.2	0.0–32.4	0.0–32.0	1.0–46.4
Karstoft	G	–	–	–	–	0.0–23.7	0.0–27.8	0.0–34.3	6.7–63.2	0.0–30.4	0.0–34.3
	S	–	–	–	–	–	–	–	–	–	–

the 2 approaches, TP estimation and mixing-model analysis, can support each other.

Conclusions

Overall, the general demands for a baseline indicator—that it be widely represented within and among the systems, abundant, easy to collect, and a good descriptor of system-specific $\delta^{15}N$ background values—are typically met by Simuliidae. Most important, perhaps, is that Simuliidae in our study showed the lowest within-site variation, i.e., the lowest trophic level range. For both fish species, TP estimates calculated with the Simuliidae baseline were the only TP estimates independent of % natural landuse changes and, concurrently, with no change in fish diet.

Based on these findings, we conclude that Simuliidae species and, second, all filterers bulked can be used as baseline indicators in future studies, taking into account, as for all baselines, the generation times of the species. For Simuliidae species, with their relatively short generation times, sampling of time series may be necessary to cover the relevant lifespan of higher-level consumers. We recommend that when determining a baseline among the abundant and widely distributed primary consumer taxa, the aim should be to minimize $\delta^{15}N$ variability in and among systems as the complexity of choice, timing, quality, and proportions of food sources consumed by the consumer cannot be mimicked easily.

We suggest that future investigators follow a similar trial-and-error method to identify potential baselines in other types of freshwater systems and biomes. A set of reliable baselines that could be used readily would facilitate studies of freshwater food webs. Last, we recommend a combined approach based on both TP estimation and mixing-model analysis in studies of stream food webs.

LITERATURE CITED

Anderson, C., and G. Cabana. 2005. $\delta^{15}N$ in riverine food webs: effects of N inputs from agricultural watersheds. Canadian Journal of Fisheries and Aquatic Sciences 62:333–340.

Anderson, C., and G. Cabana. 2006. Does $\delta^{15}N$ in river food webs reflect the intensity and origin of N loads from the watershed? Science of the Total Environment 367:968–978.

Anderson, C., and G. Cabana. 2007. Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. Journal of the North American Benthological Society 26:273–285.

Bergfur, J., R. K. Johnson, L. Sandin, and W. Goedkoop. 2009. Effects of nutrient enrichment on C and N stable isotope ratios of invertebrates, fish and their food resources in boreal streams. Hydrobiologia 628:67–79.

Bilton, D. T., J. R. Freeland, and B. Okamura. 2001. Dispersal in freshwater invertebrates. Annual Review of Ecology and Systematics 32:159–181.

Brett, M. T. 2014. Resource polygon geometry predicts Bayesian stable isotope mixing model bias. Marine Ecology Progress Series 514:1–12.

Cabana, G., and J. B. Rasmussen. 1996. Comparison of aquatic food chains using nitrogen isotopes. Proceedings of the National Academy of Sciences of the United States of America 93:10844–10847.

Currie, D. C., and P. H. Adler. 2008. Global diversity of black flies (Diptera: Simuliidae) in freshwater. Hydrobiologia 595:469–475.

Currie, D. C., and D. A. Craig. 1988. Feeding strategies of larval black flies. Pages 155–170 in K. C. Kim and R. W. Merritt (editors). Black flies: ecology, population management, and annotated world list. Pennsylvania State University Press, University Park, Pennsylvania.

Dall, P., and C. Lindegaard. 1995. En oversigt over danske ferskvandsinvertebrater til brug ved bedømmelse af forurening i søer og vandløb. Freshwater Biological Laboratory, University of Copenhagen, Copenhagen, Denmark (in Danish). (Available from: Department of Biology, University of Copenhagen, Nørregade 10, 1165 Copenhagen, Denmark.)

Dodds, W. K., S. M. Collins, S. K. Hamilton, J. L. Tank, S. Johnson, J. R. Webster, K. S. Simon, M. R. Whiles, H. M. Rantala, W. H. McDowell, S. D. Peterson, T. Riis, C. L. Crenshaw, S. A. Thomas, P. B. Kristensen, B. M. Cheever, A. S. Flecker, N. A. Griffiths, T. Crowl, E. J. Rosi-Marshall, R. El-Sabaawi, and E. Martí. 2014. You are not always what we think you eat: selective assimilation across multiple whole-stream isotopic tracer studies. Ecology 95:2757–2767.

Fry, B. 1983. Fish and shrimp migrations in the northern Gulf of Mexico analyzed using stable C, N, and S isotope ratios. Fishery Bulletin 81:789–801.

Gooderham, J., and E. Tsyrlin (editors). 2002. The waterbug book. A guide to the freshwater invertebrates of temperate Australia. CSIRO Publishing, Collingwood, Australia.

Hobson, K. A., D. M. Schell, D. Renouf, and E. Noseworthy. 1996. Stable carbon and nitrogen isotopic fractionation between diet and tissues of captive seals: implications for die-

tary reconstructions involving marine mammals. Canadian Journal of Fisheries and Aquatic Sciences 53:528–533.

Holdich, D. M., and M. Pöckl. 2007. Invasive crustaceans in European inland waters. Pages 29–76 *in* F. Gherardi (editor). Invading nature. Volume 2. Biological invaders in inland waters: profiles, distribution and threats. Springer, Dordrecht, The Netherlands.

Jardine, T. D., W. L. Hadwen, S. K. Hamilton, S. Hladyz, S. M. Mitrovic, K. A. Kidd, W. Y. Tsoi, M. Spears, D. P. Westhorpe, V. M. Fry, F. Sheldon, and S. E. Bunn. 2012. Understanding and overcoming baseline isotopic variability in running waters. River Research and Applications 30:155–165.

Jensen, C. F. 1984a. A revision of the taxonomy and distribution of the Danish black-flies (Diptera: Simuliidae), with keys to the larval and pupal stages. Natura Jutlandica 21:69–116.

Jensen, C. F. 1984b. De Danske *Baetis*-arter (Ephemeroptera: Baetidae). Flora og Fauna 90:97–102.

Kristensen, E. A., A. Baattrup-Pedersen, and H. E. Andersen. 2012. Prediction of stream fish assemblages from land use characteristics: implications for design of cost-effective monitoring programmes. Environmental Monitoring and Assessment 184:1435–1448.

Lake, J. L., R. A. McKinney, F. A. Osterman, R. J. Pruell, J. Kiddon, S. A. Ryba, and A. D. Libby. 2001. Stable nitrogen isotopes as indicators of anthropogenic activities in small freshwater systems. Canadian Journal of Fisheries and Aquatic Sciences 58:870–878.

Landolt, P., and M. Sartori (editors). 1995. Ephemeroptera and Plecoptera: biology, ecology, systematics. Mauron+Tinguely and Lachat, Fribourg, Switzerland.

Mayer, B., E. W. Boyer, C. Goodale, N. A. Jaworski, N. van Breemen, R. W. Howarth, S. Seitzinger, G. Billen, K. Lajtha, K. Nadelhoffer, D. Van Dam, L. J. Hetling, M. Nosal, and K. Paustian. 2002. Sources of nitrate in rivers draining sixteen watersheds in the northeastern U.S.: isotopic constraints. Biogeochemistry 57/58:171–197.

McCutchan, J. H., W. M. Lewis, Jr, C. Kendall, and C. C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen and sulfur. Oikos 102:378–390.

Merritt, R. W., K. W. Cummins, and M. B. Berg (editors). 2008. An introduction to the aquatic insects of North America. 4th edition. Kendall/Hunt, Dubuque, Iowa.

Moog, O. 1995. Fauna aquatica Austriaca. A comprehensive species inventory of Austrian aquatic organisms with ecological notes. Bundesministerium für Land- und Forstwirtschaft, Vienna, Austria. (Available from: https://www.researchgate.net /profile/Otto_Moog/publication/268521260_Fauna_Aquatica _Austriaca_-_Catalogue_for_autecological_classification_of _Austrian_aquatic_organisms/links/547046ba0cf24af340c09b62 .pdf)

Moore, J. W., and B. X. Semmens. 2008. Incorporating uncertainty and prior information into stable isotope mixing models. Ecology Letters 11:470–480.

Park, R., and S. Epstein. 1960. Carbon isotope fractionation during photosynthesis. Geochimica et Cosmochimica Acta 21:110–126.

Parnell, A. and A. Jackson. 2015. Stable isotope analysis in R (SIAR). Version 4.2. R Project for Statistical Computing, Vienna, Austria. (Available from: https://cran.rstudio.com /web/packages/siar/index.html)

Parnell, A. C., R. Inger, S. Bearhop, and A. L. Jackson. 2010. Source partitioning using stable isotopes: coping with too much variation. PLoS ONE 5(3):e9672.

Parnell, A. C., D. L. Phillips, S. Bearhop, B. X. Semmens, E. J. Ward, J. W. Moore, A. L. Jackson, J. Grey, D. J. Kelly, and R. Inger. 2013. Bayesian stable isotope mixing models. Environmetrics 24:387–399.

Pastor, A., M. Peipoch, L. Can~as, E. Chappuis, M. Ribot, E. Gacia, J. L. Riera, E. Martí, and F. Sabater. 2013. Nitrogen stable isotopes in primary uptake compartments across streams differing in nutrient availability. Environmental Science and Technology 47:10155–10162.

Peterson, B. J., and B. Fry. 1987. Stable isotopes in ecosystem studies. Annual Review of Ecology and Systematics 18:293–320.

Phillips, D. L. 2001. Mixing models in analyses of diet using multiple stable isotopes: a critique. Oecologia (Berlin) 127:166–170.

Post, D. M. 2002. Using stable isotopes to estimate trophic position: models, methods and assumptions. Ecology 83:703–718.

Reid, D. J., G. P. Quinn, P. S. Lake, and P. Reich. 2008. Terrestrial detritus supports the food webs in lowland intermittent streams of south-eastern Australia: a stable isotope study. Freshwater Biology 53:2036–2050.

Rubtsov, I. A. 1990. Blackflies (Simuliidae). Fauna of the USSR, Diptera. Volume 6, part 6. 2nd edition. Brill Academic Publishers, Leiden, The Netherlands.

Rybczynski, S. M., D. M. Walters, K. M. Fritz, and B. R. Johnson. 2008. Comparing trophic position of stream fishes using stable isotope and gut contents analyses. Ecology of Freshwater Fish 17:199–206.

Silverman, H., E. C. Achberger, J. W. Lynn, and T. H. Dietz. 1995. Filtration and utilization of laboratory-cultured bacteria by *Dreissena polymorpha*, *Corbicula fluminea*, and *Carunculina texasensis*. Biological Bulletin 189:308–319.

Skriver, J. 1982. Fødesammensætning hos *Gammarus pulex* L. vækst i relation til fødens partikelstørrelse, samt artens betydning for bladomsætningen i Rold Kilde. MSc Thesis, University of Copenhagen, Copenhagen, Denmark.

Teixeira-de Mello, F., M. Meerhoff, A. Baattrup-Pedersen, T. Maigaard, P. B. Kristensen, T. K. Andersen, J. M. Clemente, C. Fosalba, E. A. Kristensen, M. Masdeu, T. Riis, N. Mazzeo, and E. Jeppesen. 2012. Community structure of fish in lowland streams differ substantially between subtropical and temperate climates. Hydrobiologia 684:143–160.

Vander Zanden, M. J., G. Cabana, and J. B. Rasmussen. 1997. Comparing trophic position of freshwater fish using stable nitrogen isotope ratios ($\delta^{15}N$) and literature dietary data. Canadian Journal of Fisheries and Aquatic Sciences 54:1142–1158.

Vander Zanden, M. J., J. M. Casselman, and J. B. Rasmussen. 1999. Stable isotope evidence for the food web consequences of species invasions in lakes. Nature 401:464–467.

Vander Zanden, M. J., and J. B. Rasmussen. 1996. A trophic position model of pelagic food webs: impact on contaminant bioaccumulation in lake trout. Ecological Monographs 66:451–477.

Vander Zanden, M. J., and J. B. Rasmussen. 1999. Primary consumer δ^{15}N and δ^{13}C and the trophic position of aquatic consumers. Ecology 80:1395–1404.

Vander Zanden, M. J., and J. B. Rasmussen. 2001. Variation in δ^{15}N and δ^{13}C trophic fractionation: implications for aquatic food web studies. Limnology and Oceanography 46:2061–2066.

Vander Zanden, M. J., Y. Vadeboncoeur, M. W. Diebel, and E. Jeppesen. 2005. Primary consumer stable nitrogen isotopes as indicators of nutrient source. Environmental Science and Technology 39:7509–7515.

von Schiller, D., E. Martí, and J. L. Riera. 2009. Nitrate retention and removal in Mediterranean streams bordered by contrasting land uses: a ^{15}N tracer study. Biogeosciences 6: 181–196.

Wallace, J. B., and J. R. Webster. 1996. The role of macroinvertebrates in stream ecosystem function. Annual Review of Entomology 41:115–139.