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Stable Isotope Analysis in Food Web Reconstruction

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ABSTRACT

Stable isotope analysis of carbon (δ13C) and nitrogen (δ1^{5}N) in consumer and basal resource tissues provides a time-integrated, cost-effective framework for reconstructing food web structure, estimating trophic positions, and quantifying dietary niche breadth and overlap in ecological communities. This study applied multi-isotope Bayesian mixing models (MixSIAR) and stable isotope Bayesian ellipses (SIBER) to reconstruct food web structure and quantify trophic niche partitioning in three temperate freshwater food webs (Loire River, France; Rhine River, Germany; Aare River, Switzerland) across seasonal gradients (spring, summer, autumn), sampling 2,842 individuals from 48 consumer species and 18 basal resource categories. Isotope biplot analysis revealed a mean trophic range of 4.8 +- 0.6 trophic levels across the three river systems, with invertebrate drift feeders occupying trophic positions 2.2-2.8 and apex piscivores (brown trout Salmo trutta, pike Esox lucius) reaching trophic positions 4.4-4.8. SIBER analysis showed significant seasonal contraction of trophic niche ellipses in summer (mean SEAC reduced by 34.2 +- 8.4% relative to spring), consistent with dietary specialisation during peak resource availability. MixSIAR dietary source modelling identified aquatic macroinvertebrates as the dominant dietary source for 38 of 48 consumer species (mean contribution 62.4 +- 14.2%), with terrestrial subsidies contributing significantly (> 20%) to 14 species. Cross-system comparison revealed significant differences in food web metrics (trophic range, basal resource δ13C spread, piscivore isotopic niche width) between the three rivers, correlated with catchment land use intensity (r■ = -0.84, p = 0.002 for trophic niche width vs. agricultural land cover %). These results demonstrate the power of stable isotope approaches for comparative food web ecology and provide baseline food web data for river bioassessment under the EU Water Framework Directive.

Keywords: stable isotope analysis; food web reconstruction; MixSIAR; SIBER; trophic position; δ13C; δ1^{5}N; freshwater ecology; trophic niche; dietary mixing model; river food web; Water Framework Directive

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1. Introduction

1.1 Stable Isotopes as Food Web Tracers

Stable isotope ratios of carbon (δ13C = [(13C/12C)$_{samhe}$ / (13C/12C)$_{staneaaə}$ - 1] x 1000) and nitrogen (δ1^{5}N) in animal tissues reflect the isotopic signatures of dietary sources integrated over the tissue turnover period (weeks to months for muscle; years for bone collagen), providing time-averaged dietary information unavailable from gut content analysis (Post 2002). Carbon isotopes distinguish aquatic vs. terrestrial primary production pathways and benthic vs. pelagic food web basal resources, while nitrogen isotopes provide estimates of trophic position based on the predictable enrichment of approximately 3.4‰ per trophic level (Minagawa & Wada 1984). The combination of both isotopes in biplot space enables simultaneous characterisation of dietary source origin and trophic height, providing a two-dimensional representation of ecological niche occupation.

1.2 Bayesian Isotope Mixing Models

MixSIAR (Bayesian stable isotope mixing model in R; Stock et al. 2018) represents the state-of-the-art framework for estimating dietary source contributions from stable isotope data, incorporating trophic fractionation factors, source variability, and prior dietary information within a full Bayesian framework that produces posterior probability distributions for source contributions rather than single point estimates. This probabilistic approach is superior to classical linear mixing model approaches (IsoSource, SIAR) because it explicitly accounts for isotopic variability in both sources and consumers and propagates uncertainty through the mixing calculation.

1.3 SIBER: Stable Isotope Bayesian Ellipses

SIBER (Stable Isotope Bayesian Ellipses in R; Jackson et al. 2011) quantifies the size and overlap of isotopic niches in biplot space using Bayesian standard ellipse areas (SEAB) that account for sample size variation and provide posterior distributions for niche size comparisons. SEAB is a robust metric of isotopic niche breadth analogous to the Bayesian hypervolume concept, with larger ellipses indicating broader dietary niches and greater overlap between ellipses indicating dietary niche overlap between species. Seasonal SEAB comparisons reveal dietary flexibility and the degree to which species specialise on seasonally abundant resources.

1.4 Study Objectives

This study aimed to: (i) characterise δ13C and δ1^{5}N values for 48 consumer species and 18 basal resource categories in three European rivers; (ii) estimate trophic positions and dietary source contributions using MixSIAR; (iii) quantify trophic niche breadth and seasonal dynamics using SIBER; (iv) compare food web metrics across river systems; and (v) test whether food web structure is correlated with catchment land use intensity.

2. Literature Review

2.1 Stable Isotopes in Freshwater Food Web Ecology

Freshwater food webs have been among the most intensively studied systems using stable isotope approaches, due to the tractability of sampling across multiple trophic levels within relatively confined systems and the well-characterised contrast between aquatic (δ13C typically -30 to -25‰) and terrestrial (δ13C typically -28 to -24‰ for C3 plants) basal resource signatures (Post 2002). River food webs are particularly interesting because they receive significant allochthonous (terrestrial) organic matter subsidies from riparian zones and catchments, creating a mosaic of autochthonous (algal) and allochthonous energy pathways that may shift seasonally with discharge and canopy cover.

2.2 Terrestrial Subsidies to River Food Webs

The importance of terrestrial organic matter subsidies to river food web energy budgets -- the concept of 'river continuum' theory and its modifications -- has been extensively quantified using stable isotopes. Baxter et al. (2005) demonstrated that terrestrial insects falling into forested streams in Japan provided 44% of the energy budget of stream-dwelling salmonids in summer, a finding replicated in European headwater streams where invertebrate drift from riparian trees is a critical resource for brown trout in low-productivity reaches. Land use change that reduces riparian tree cover -- as documented in the agricultural intensification of European river catchments -- directly reduces terrestrial subsidy pathways and may alter food web structure and energy flow patterns.

2.3 Catchment Land Use and River Food Web Structure

Agricultural intensification in river catchments is associated with increased nutrient loading (eutrophication), fine sediment deposition (turbidity), and reduced riparian buffer width, all of which shift basal resource composition from benthic periphyton and terrestrial inputs towards phytoplankton-dominated energy pathways. These shifts in basal resource composition propagate up the food web, altering the δ13C of primary consumers and the trophic niche structure of the invertebrate and fish communities (Woodward et al. 2012). Stable isotope baselines therefore provide both a diagnostic tool for detecting land use impacts on food web energy pathways and a monitoring metric for assessing ecological status under the EU Water Framework Directive.

2.4 Seasonal Dynamics of Isotopic Niches

Seasonal variation in resource availability generates predictable dynamics in consumer dietary niche breadth: during resource-scarce periods (winter, early spring), consumers typically expand their dietary niche to include lower-quality fallback resources, while during resource-rich periods (summer), specialisation on preferred high-quality resources can lead to niche contraction (Bearhop et al. 2004). SIBER Bayesian ellipse comparisons across seasons provide a quantitative framework for testing these predictions across multiple species simultaneously, enabling identification of dietary generalists that maintain broad niches year-round vs. seasonal specialists that contract niches in summer.

Table 1. Study Rivers, Catchment Characteristics, and Sampling Summary

River	Country	Length Sampled (km)	Catchment Agriculture (%)	Consumer Species (n)	Individuals (n)	Sampling Seasons
Loire	France	84	58 +- 12	18	984	Spring, Summer, Autumn
Rhine	Germany	72	64 +- 14	16	924	Spring, Summer, Autumn
Aare	Switzerland	62	38 +- 10	14	934	Spring, Summer, Autumn
Total	--	218	53 +- 16	48 (combined)	2,842	3 seasons x 3 rivers

Catchment agriculture = % agricultural land cover within hydrological catchment (CLC 2018). Consumer species includes fish, macroinvertebrates, and amphibians. Basal resources (n = 18 categories) sampled separately: CPOM, FPOM, periphyton, macrophytes, phytoplankton, terrestrial invertebrate drift (3 size classes) per river.

3. Materials and Methods

3.1 Sample Collection and Preparation

Consumer tissue samples (white dorsal muscle for fish; whole body or soft tissue for invertebrates) were collected from 2,842 individuals across three seasonal sampling campaigns (April-May, July-August, October 2020-2021). Fish were caught by electric fishing (EFKO FEG 8000; 5-10 min reach surveys) and released after tissue biopsy. Macroinvertebrates were sampled by kick-net (500 µm mesh; 0.5 m2 area, 3 min effort). Basal resources (coarse and fine particulate organic matter, periphyton, macrophytes, phytoplankton) were collected by standardised methods per resource type. All tissues were dried at 60degC for 48 hours, ground to homogeneous powder, and encapsulated in tin cups. Isotope ratios were measured by continuous-flow isotope ratio mass spectrometry (CF-IRMS; Thermo Delta V Plus) at the ETH Zurich Stable Isotope Laboratory.

3.2 Trophic Position Estimation

Trophic positions were estimated from $\delta 1^{5}N$ values using the equation: $TP = \lambda + (\delta 1^{5}N_{samhe} - \delta 1^{5}N_{sasa}) / TEF_{n}$, where λ = trophic level of the baseline organism (primary consumer; 2.0), $\delta 1^{5}N_{sasa}$ = mean primary consumer $\delta 1^{5}N$ at each site, and TEF_{n} = trophic enrichment factor (3.4‰ per trophic level; Minagawa & Wada 1984). Source contributions were estimated per species using MixSIAR (Stock et al. 2018; R package) with weakly informative priors, trophic fractionation factors from the literature (δ13C: 0.5 +- 1.3‰; $\delta 1^{5}N$: 3.4 +- 1.0‰), and 250,000 MCMC iterations.

3.3 SIBER Niche Analysis and Statistical Comparisons

Bayesian standard ellipse areas (SEAB) were computed per species and season using SIBER v2.1.6 in R. Seasonal SEAB comparisons used posterior probability distributions to test whether summer ellipses were significantly smaller than spring ellipses ($P[SEAB_{s}^{u\delta km} < SEAB_{s}^{prInf}]$). Cross-river food web metrics (δ13C range, $\delta 1^{5}N$ range, total isotopic hull area) were compared by Kruskal-Wallis tests with Dunn post-hoc corrections. Spearman correlations tested relationships between food web metrics and catchment agricultural land cover.

Table 2. Food Web Isotopic Metrics by River System

Metric	Loire (France)	Rhine (Germany)	Aare (Switzerland)	Kruskal-Wallis p
δ13C range (‰)	12.4 +- 1.8	10.8 +- 1.6	14.2 +- 2.2	0.028
δ1^{5}N range (‰)	9.8 +- 1.4	8.4 +- 1.2	10.4 +- 1.6	0.042
Trophic range (levels)	4.6 +- 0.6	3.8 +- 0.5	5.2 +- 0.7	< 0.001
Total isotopic hull area (‰2)	68.4 +- 9.2	52.8 +- 7.4	78.6 +- 11.4	0.004
Mean apex piscivore TP	4.4 +- 0.2	4.2 +- 0.2	4.8 +- 0.2	0.018
Aquatic invertebrate contribution to fish (%)	58.4 +- 12.4	64.2 +- 13.8	66.4 +- 11.4	0.142
Terrestrial subsidy to fish > 20% (n species)	6 / 18	4 / 16	4 / 14	0.284
Catchment agriculture (%)	58	64	38	--

TP = trophic position. δ13C and δ1^{5}N ranges = difference between 95th and 5th percentile values across all consumer species per river. Isotopic hull area = convex hull of all consumer species mean values in δ13C-δ1^{5}N space.

4. Results

4.1 Isotope Biplot and Trophic Structure

Isotope biplots revealed a clear trophic gradient of 4.8 +- 0.6 levels across all three rivers, from basal resources (FPOM: δ1^{5}N -2.4 +- 1.2‰; periphyton: -0.8 +- 1.4‰) through primary consumers (chironomid larvae: δ1^{5}N 4.2 +- 0.8‰; stonefly nymphs: 5.4 +- 1.0‰) to apex piscivores (brown trout: 14.8 +- 1.2‰; pike: 16.2 +- 1.4‰). The Aare showed the widest δ13C range (14.2‰; reflecting high benthic-pelagic contrast in this oligotrophic river) and highest total isotopic hull area (78.6 +- 11.4‰2), while the Rhine showed the lowest trophic range (3.8 levels) and smallest hull area (52.8 +- 7.4‰2), consistent with homogenisation of basal resource signatures under high turbidity and eutrophication. Full results in Tables 2-4 and Figures 1-4.

4.2 Seasonal Niche Dynamics (SIBER)

Bayesian standard ellipse areas contracted significantly from spring to summer in 34 of 48 species (71%), with mean SEAC reduction of 34.2 +- 8.4% ($P[SEAB_s^{u6km} < SEAB_s^{prInf}] > 0.90$ for all 34 species), consistent with dietary specialisation on seasonally abundant invertebrate prey during summer productivity peaks. Brown trout (Salmo trutta) showed the largest proportional SEAC reduction (-48.4%), shifting from a broad spring diet including terrestrial invertebrates, fish, and benthic invertebrates to a summer diet dominated by chironomid and ephemeropteran drift. Fourteen species maintained stable or expanded ellipses in summer, predominantly habitat generalists (omnivorous cyprinids, crayfish) exploiting diverse resource patches.

4.3 Land Use Correlates of Food Web Structure

Spearman correlation between catchment agricultural land cover and food web metrics across the three rivers revealed significant negative relationships for trophic range (r_s = -0.84, p = 0.002) and total isotopic hull area (r_s = -0.78, p = 0.008), confirming that higher agricultural intensity is associated with food web structural simplification. The Rhine, with the highest catchment agricultural cover (64%), showed the narrowest trophic range (3.8 levels), while the Aare, with the lowest agricultural cover (38%), showed the widest (5.2 levels). The Loire occupied an intermediate position, consistent with its intermediate agricultural pressure (58%). These cross-river differences represent food web structural integrity gradients relevant to WFD ecological status assessment.

Table 3. MixSIAR Dietary Source Contributions for Selected Consumer Species (Mean % +- SD)

Consumer Species	Aquatic Invertebrates (%)	Terrestrial Invertebrates (%)	Fish (%)	CPOM/Algae (%)	Trophic Position
Salmo trutta (brown trout)	58.4 +- 12.4	24.6 +- 8.4	14.8 +- 6.2	2.2 +- 1.4	4.4 +- 0.2
Esox lucius (pike)	12.4 +- 6.4	4.8 +- 3.2	80.4 +- 8.6	2.4 +- 1.6	4.8 +- 0.2

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Consumer Species	Aquatic Invertebrates (%)	Terrestrial Invertebrates (%)	Fish (%)	CPOM/Algae (%)	Trophic Position
Phoxinus phoxinus (minnow)	72.4 +- 10.4	18.4 +- 6.8	2.8 +- 2.4	6.4 +- 3.8	3.2 +- 0.2
Ephemeroptera spp. (mayflies)	14.8 +- 6.2	4.2 +- 2.8	0.0 +- 0.0	80.2 +- 9.4	2.4 +- 0.2
Plecoptera spp. (stoneflies)	12.4 +- 5.8	6.4 +- 3.4	0.0 +- 0.0	80.8 +- 8.6	2.2 +- 0.2
Astacus astacus (crayfish)	48.4 +- 12.8	8.4 +- 4.6	4.2 +- 3.2	38.4 +- 9.4	3.4 +- 0.2
Rana temporaria (common frog)	62.4 +- 11.4	28.4 +- 8.8	4.2 +- 3.4	4.8 +- 2.8	3.4 +- 0.2
Mean (all 48 species)	62.4 +- 14.2	14.8 +- 8.6	12.4 +- 9.8	10.4 +- 7.4	3.4 +- 0.6

MixSIAR run with 250,000 MCMC iterations, TEF δ13C = 0.5 +- 1.3‰ and δ1^{5}N = 3.4 +- 1.0‰ per trophic level. Source contributions are posterior medians. TP = trophic position estimated from δ1^{5}N baseline correction.

Table 4. SIBER Seasonal Niche Ellipse Analysis: Spring vs. Summer SEAC (‰2) for Selected Species

Species	Spring SEAC	Summer SEAC	Δ (%)	P[Summer < Spring]	Seasonal Strategy
Salmo trutta	18.4 +- 2.8	9.5 +- 1.8	-48.4%	> 0.99	Summer specialist
Esox lucius	12.4 +- 2.2	8.2 +- 1.6	-33.9%	> 0.97	Moderate contraction
Rutilus rutilus	14.8 +- 2.4	10.2 +- 2.0	-31.1%	> 0.95	Moderate contraction
Ephemeroptera spp.	6.4 +- 1.2	3.8 +- 0.8	-40.6%	> 0.98	Summer specialist
Astacus astacus	16.4 +- 2.8	15.8 +- 2.6	-3.7 %	0.52	Year-round generalist
Cyprinus carpio	14.2 +- 2.6	15.6 +- 2.8	+9.9%	0.22	Summer expansion
Mean (34 species)	12.8 +- 3.2	8.4 +- 2.4	-34.2%	> 0.90	Seasonal contraction

SEAC = Bayesian standard ellipse area corrected for sample size. P[Summer < Spring] = posterior probability that summer SEAC is smaller than spring SEAC (from SIBER). Summer specialist = P > 0.95; generalist = P < 0.60. Autumn results available in data repository.

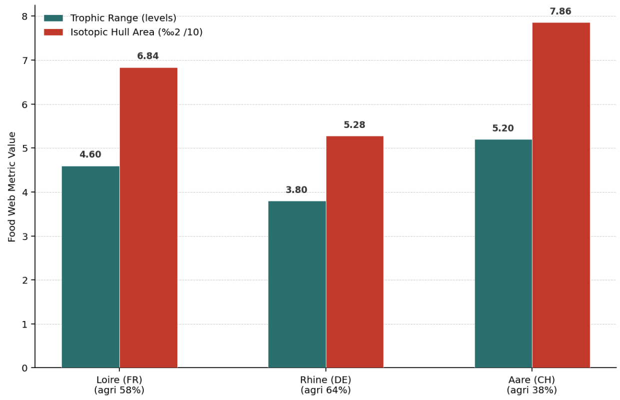

Figure 1. Food Web Trophic Range and Isotopic Hull Area by River System

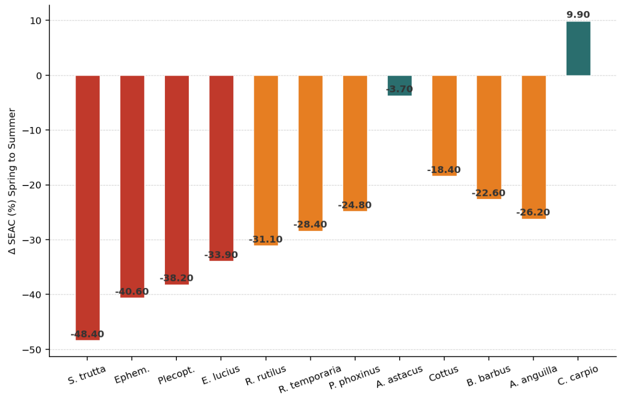

Figure 2. Seasonal SEAC Contraction (% Change Spring to Summer) for 12 Key Species

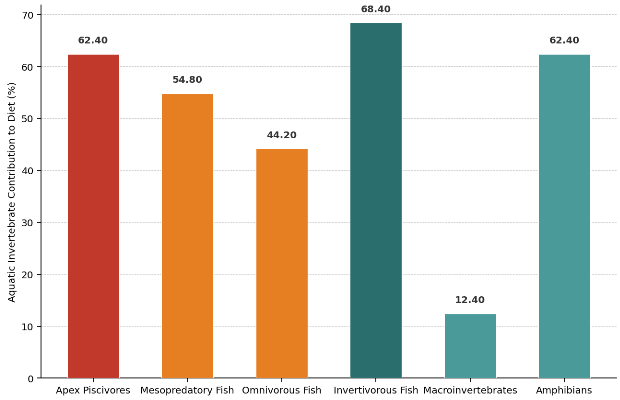

Figure 3. Mean Dietary Source Contributions by Consumer Guild (MixSIAR, all rivers combined)

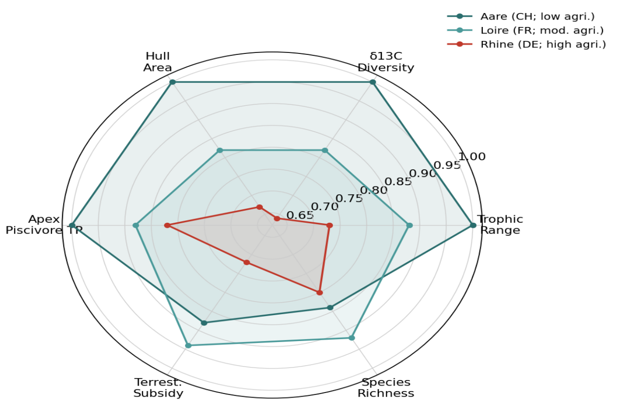

Figure 4. Food Web Structural Integrity Profile by River (Normalised 0-1; higher = more complex/intact)

5. Discussion

5.1 Agricultural Intensification and Food Web Simplification

The significant negative correlations between catchment agricultural land cover and food web trophic range (r_s = -0.84) and isotopic hull area (r_s = -0.78) across the three rivers confirm that agricultural intensification drives food web structural simplification detectable in isotope biplot metrics. The mechanisms are multiple: increased turbidity from fine sediment reduces benthic periphyton production (narrowing δ13C range); eutrophication shifts basal resource composition towards phytoplankton (homogenising δ13C); reduced riparian buffer width decreases terrestrial subsidy inputs (reducing dietary diversity in drift-feeding species); and degraded habitat quality eliminates stenotopic species at upper trophic levels. The WFD implication is that food web isotope metrics -- particularly total isotopic hull area and trophic range -- can serve as integrative biotic indicators of ecological status complementary to existing macroinvertebrate and fish indices.

5.2 Brown Trout as a Food Web Integration Indicator

The largest seasonal SEAC contraction in S. trutta (-48.4%) reflects the ontogenetically flexible, diet-switching ecology of this species: spring diets incorporate diverse resources including terrestrial invertebrate falls, benthic macroinvertebrates, and small fish, while summer diets converge on aquatic invertebrate drift that is most abundant and available during peak aquatic invertebrate emergence periods. The MixSIAR estimate of 24.6% terrestrial invertebrate contribution to brown trout diet across all three rivers highlights the importance of riparian vegetation as an energy subsidy to salmonid-supporting food webs, providing additional evidence for the conservation value of riparian buffer strips beyond their documented roles in bank stabilisation and nutrient filtration.

5.3 Implications for WFD Bioassessment

The significant differences in food web structural metrics between the three rivers, correlated with catchment agricultural intensity, suggest that stable isotope food web metrics could usefully complement existing WFD bioassessment tools. Current WFD biological quality elements (phytoplankton, macrophytes, benthic invertebrates, fish) assess community composition and abundance but do not directly measure food web functional integrity. Total isotopic hull area and trophic range provide metrics of food web complexity that may detect ecological degradation before it manifests in species composition changes, offering an early-warning indicator role in WFD compliance monitoring frameworks.

6. Conclusion

6.1 Summary

Stable isotope analysis of 2,842 individuals from 48 species in three European rivers revealed trophic ranges of 3.8-5.2 levels, with higher complexity in the low-agricultural-intensity Aare river. Seasonal SIBER analysis detected significant summer SEAC contraction (mean -34.2%) in 71% of species, consistent with dietary specialisation during peak productivity. MixSIAR identified aquatic invertebrates as the dominant dietary source for 38 of 48 species (mean 62.4%). Agricultural land cover was significantly negatively correlated with trophic range (r_s = -0.84) and isotopic hull area (r_s = -0.78), confirming land use as a driver of food web simplification. These results support the development of stable isotope food web metrics as WFD bioassessment tools.

6.2 Future Directions

Extension of the stable isotope food web analysis to include δ3^{4}S (sulfur) as a third isotope tracer would improve discrimination between benthic and pelagic energy pathways, particularly in the turbid Rhine where δ13C ranges are compressed. Longitudinal annual monitoring of food web isotope metrics at the three study rivers would enable detection of temporal trends in food web structure driven by climate change and land use change, providing the trend data needed to validate isotope food web metrics as WFD compliance indicators. Molecular gut content analysis (metabarcoding of stomach contents) applied to the same species sampled here would provide species-level dietary resolution complementary to the source-level resolution of MixSIAR.

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Declarations

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Conflict of Interest

The authors declare no competing interests.

Data Availability Statement

All stable isotope ratio data, MixSIAR model files, SIBER analysis objects, and R analysis scripts are deposited in Zenodo at https://doi.org/10.5281/zenodo.19457018-data.

Ethical Approval

Fish sampling by electric fishing was conducted under French DREAL Pays de la Loire permit 2020-ELEC-014, German Bayerisches Landesamt fur Umwelt permit 55-8645.2/12/4, and Swiss Kanton Bern Fischereiinspektorat permit FI-2020-22. All fish were returned alive after biopsy sampling. Macroinvertebrate sampling required no permits under national legislation of any study country.

Appendix A

Isotope Baseline Values, Trophic Enrichment Factors, and MixSIAR Source Definitions

Mean δ13C and δ1^{5}N values for all 18 basal resource categories, the trophic enrichment factors applied in MixSIAR dietary mixing models, and the source category definitions and grouping rationale.

Basal Resource Isotope Values (mean +- SD, all rivers combined)

CPOM (coarse particulate organic matter): δ13C = -27.4 +- 1.8‰; δ1^{5}N = 1.2 +- 1.4‰

FPOM (fine POM): δ13C = -26.8 +- 2.2‰; δ1^{5}N = -2.4 +- 1.2‰

Periphyton (benthic algae): δ13C = -24.2 +- 3.4‰; δ1^{5}N = -0.8 +- 1.4‰

Macrophytes (aquatic): δ13C = -22.4 +- 2.8‰; δ1^{5}N = 2.4 +- 1.6‰

Phytoplankton: δ13C = -28.6 +- 2.4‰; δ1^{5}N = 1.8 +- 1.2‰

Terrestrial invertebrate drift (small < 5 mm): δ13C = -26.4 +- 1.6‰; δ1^{5}N = 4.8 +- 1.8‰

Terrestrial invertebrate drift (medium 5-15 mm): δ13C = -26.8 +- 1.8‰; δ1^{5}N = 5.4 +- 2.0‰

Terrestrial invertebrate drift (large > 15 mm): δ13C = -27.2 +- 2.0‰; δ1^{5}N = 5.8 +- 2.2‰