



Variability of isotopic partitioning between sympatric brown trout (*Salmo trutta*) and European grayling (*Thymallus thymallus*) in the Loue River (France)

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Abstract

Trophic partitioning, defining how individuals or populations differ in their resource use, is expected to promote coexistence of sympatric species by reducing resource overlap. We used stable isotope measurements ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) to address niche characteristics ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranges, isotope convex hull and standard ellipse areas, mean nearest neighbour distances and trophic positions) and trophic partitioning of sympatric brown trout (*Salmo trutta*, $n = 110$) and European grayling (*Thymallus thymallus*, $n = 63$) in two sampling locations of a French river. Aquatic resources predominantly fuelled both species, yet both terrestrial resources (TER) and trophic positions (TP) were higher for trout ($\sim 36 \pm 13\%$ TER, 3.6 ± 0.7 TP) than grayling ($\sim 26 \pm 9\%$ TER, 2.7 ± 0.6 TP) supporting difference in their trophic niches. Isotope analyses showed that trout had a larger isotope niche than grayling suggesting more opportunistic trophic behaviour. Their isotopic overlapping was higher at the upstream site (isotopic nestedness = 0.8 ± 0.1) than that at the downstream site (isotopic nestedness = 0.4 ± 0.2). Euclidian distances of stable isotopes and TP for the two species increased with salmonid size, while aquatic resource use decreased with salmonid size. These results demonstrate an increase in isotopic niche partitioning and change in trophic attributes with ontogeny. Our study showed that despite relying on similar resources, these two sympatric salmonids exhibited clear trophic differences that were amplified with ontogeny. The consideration of fish ontogenetic dietary shift would hence be a determinant driver of the trophic niche partitioning for these sympatric salmonids.

KEYWORDS

brown trout, European grayling, niche partitioning, ontogeny, stable isotopes, sympatry

1 | INTRODUCTION

Sympatry, the co-occurrence of related species within the same ecosystem exhibiting reproductive isolation, is widespread among salmonids (Bernatchez et al., 1996; Ferguson & Taggart, 2008). Niche

theory predicts that sympatric species should exhibit trophic niche partitioning to reduce inter-specific competition for resources and sustain their coexistence (Dieckmann & Doebeli, 1999; Levin, 1970; Schoener, 1974, 1989). Trophic niche partitioning is driven by different and not exclusive mechanisms related to both consumer

and prey characteristics. First, density and diversity of consumers and preys have been shown to be significant components of trophic niche partitioning (Nakano et al., 1999; Northfield et al., 2010; Sánchez-Hernández et al., 2017, 2020). Additionally, among consumers, differences in morphology (e.g. body and mouth shapes) and behaviour (e.g. surface or bottom feeding behaviour) involve the use of different habitats and prey exploitation, which ultimately reduce trophic overlapping (Crow et al., 2010; Sánchez-Hernández et al., 2016). Finally, inter-specific differences in diel activity patterns can also act as a way to reduce food competition as foraging activities can peak at different instances of the day among related species (Conallin et al., 2012; Ingram et al., 1999; Toobaie et al., 2013). Fish commonly undergo ontogenetic habitat shifts due to change in competition and predation risk, which, in turn, drive ontogenetic dietary shifts (Sánchez-Hernández et al., 2019). Consequently, ontogeny (i.e. fish size) can also act as an important driver of trophic niche partitioning at both intra and inter-specific levels (Olson, 1996; Sánchez-Hernández & Cobo, 2016; Schellekens et al., 2010).

The co-occurrence of brown trout (*Salmo trutta* L., 1758, hereafter trout) and European grayling (*Thymallus thymallus*, L., 1758, hereafter grayling) is confined to restricted geographical areas of Western Europe. In both lakes (Amundsen et al., 2010; Haugen & Rygg, 1996) and rivers (Degerman et al., 2000; Greendale, 1975; Woolland, 1988), their differences in habitat use and feeding behaviour suggest a limited diet overlap between these two competing species. These results may be mostly explained by morphological and behavioural differences; trout has a wide mouth and generally considered as a territorial and opportunistic predator (Elliott 1994, Oscoz et al., 2005) preferring shallower waters (Blanck et al., 2007; Greenberg et al., 1996), while grayling have a small mouth downward-oriented usually forming schools in deeper waters in rivers (Blanck et al., 2007; Ingram et al., 1999).

In this study, we used stable isotopes of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) to address the isotopic niche characteristics (i.e. trophic positions and isotope ranges, areas and dispersions) of trout and grayling living in sympatry in the Loue River (France). Stable isotopes allow tracking resource subsidies assimilated by fish (Eloranta et al., 2013; Fry, 2006) providing relevant metrics to characterise the isotopic niche of fish populations (Jackson et al., 2011; Layman, 2007) and the role of individuals within ecosystems (e.g. trophic position, Alves et al., 2020; Pacioglu et al., 2019). Our working hypotheses regarding these two sympatric salmonids were threefold. First, we characterised their isotopic niches expecting that trout would exhibit a larger isotopic niche than grayling due to its opportunistic behaviour and a low isotopic overlapping between the two species. Second, we quantified the relative contributions of aquatic and terrestrial resources fuelling the two salmonids and computed their trophic positions, expecting that trout would be more subsidised by terrestrial resources and have higher trophic position than grayling. Indeed, trout may preferentially occupy the river edges and may be prompt to piscivory, while grayling may be more associated with the river channel. Third, we suspected a prominent role of ontogeny in driving trophic attributes and niche partitioning

of these two salmonids expecting that aquatic contribution would decrease while trophic position and isotopic distances between the two salmonids would increase with salmonid size.

2 | MATERIAL AND METHODS

2.1 | Study area and salmonids

The Loue River is the second largest French karst river of 125 km long sheltered in the calcareous Jura Mountains feeding the Rhône drainage area within the Mediterranean basin. Climate is characterised by high precipitations (~ 1,300 mm per year) and a marked seasonal variability with warm summer (max. air temperature 35°C) and cold winter (min. air temperature -10°C). The karst nature of the river watershed provides highly mineralised water that exhibits relatively low and seasonally buffered temperatures with annual water temperatures spanning from ~10°C to 18°C throughout the studied area (Frossard, 2006).

The two studied sites are located in the upmost river section (~300 m a.s.l.) and distant of ~10 km in a section where topography significantly change triggering hydromorphological changes in the river (Appendix 1). At the uppermost site, Lods (47.047497 N; 6.255237 E), the valley is incised constraining the river to a limited width of ~20 m with a slope ~3%–4% leading to the dominance of riffles and runs. At the downstream site, Ornans (47.110748 N, 6.119055 E), the valley is significantly widen and the slope consistently decreases to ~1%–2% enabling the river width to reach 30 m to 40 m and the dominance of runs, pools and glides.

2.2 | Sampling

Salmonid caudal fins were used as nonlethal surrogates for salmonid muscles due to the strong correlations among their carbon and nitrogen isotope values (Jardine et al. 2011; Graham et al., 2013; Hette-Tronquart et al., 2012) and their similar isotope half-lives (McIntyre and Flecker 2006, Busst and Britton 2018). Clips of ~1 cm² of the upper section of the caudal fin were sampled from trout ($n = 110$, body size = 346 ± 69 mm, range = 42–500 mm) and grayling ($n = 63$, body size = 366 ± 57 mm, range = 22–450 mm) caught by volunteer fishermen from April to September (i.e. fishing period) in 2013 and 2014 and during the electrofishing survey of the Fédération de Pêche et de Protection des Milieux Aquatiques du Doubs in August 2012. All fin clips were conditioned in 10-ml vials filled with 70% ethanol.

Aquatic macroinvertebrates were collected in the two sites using Sürber net of 500- μm mesh in spring and summer 2013 and 2014, sorted in the field and conditioned into 70% ethanol vials (Table 1). Those represented the dominant invertebrate families occurring at the two sites (Bacchi, 1994). Terrestrial invertebrates were collected in the riparian vegetation surrounding sites concomitantly to aquatic macroinvertebrate sampling and comprised chafer, grasshoppers,

TABLE 1 Summary of the sampled organisms at the two study sites

	Lods	Ornans
Salmonidae	Trout (64)	Trout (46)
	Grayling (32)	Grayling (31)
Ephemeroptera	Ephemerellidae (3)	Ephemerellidae (3)
	Baetidae (1)	Caenidae (1)
	Ephemeridae (2)	Ephemeridae (6)
	Heptageniidae (4)	Heptageniidae (4)
Plecoptera		Chloroperlidae (1)
	Perlidae (8)	Perlidae (2)
Trichoptera	Goeridae (2)	Goeridae (7)
	Brachycentridae (4)	Brachycentridae (3)
	Glossosomatidae (2)	
Crustacea	Gammaridae (4)	Gammaridae (2)
Diptera	Chironomidae (2)	Chironomidae (2)
	Simuliidae (3)	Simuliidae (4)
Mollusc	Lymnaeidae (5)	Lymnaeidae (4)
	Ancylidae (4)	
	Oligochaeta (4)	Acheta (7)
Terrestrial invertebrate	grasshopper (2), chafer (2), beetle (3), spider (2), syrphid (2)	
Primary producers	<i>Fontinalis</i> sp. (2), <i>Ranunculus</i> sp. (1), <i>Batrachospermum</i> sp. (2), <i>Cladophora</i> sp. (3), <i>Diatoma</i> spp. (5), <i>Hydrurus</i> sp. (1), <i>Vaucheria</i> sp. (10) <i>Ulothrix</i> sp. (4)	<i>Ranunculus</i> sp. (2), <i>Fontinalis</i> sp. (2), <i>Cladophora</i> sp. (2), <i>Diatoma</i> spp. (3), <i>Tetraspora</i> sp. (1), <i>Vaucheria</i> sp. (7)

Note: Numbers in brackets indicate the number of isotope replicates. Primary producers support a global representation of the aquatic food webs and are not involved in the analyses.

beetles and spiders. In order to provide a comprehensive isotopic picture of the aquatic organisms at the two sites, a bunch of aquatic primary producers was also gathered concomitantly to invertebrates by gently racking cobbles or cutting leaves and conditioned in 70% ethanol vials (Table 1).

2.3 | Isotope analyses

Salmonid fins were extracted from the ethanol vials, abundantly washed using demineralised water and dried at 40°C for 48h prior conditioning ~1 mg of fins within tin cups for isotope analyses. The same procedure was applied for aquatic and terrestrial invertebrates

and aquatic primary producers. Isotope measurements were performed at the Technical Platform of Functional Ecology (OC 081, INRA1137, Champenoux), using an Isotope Ratio Mass Spectrometer interface coupled to an Elemental Analyzer (EA-IRMS). The analytical precisions ($\pm 1SD$) associated with isotope measurements, estimated using internal standards of the technical platform ($n = 21$, CEA18: $\delta^{13}C = -31.59 \text{ ‰}$, $\delta^{15}N = -4.74 \text{ ‰}$; Chx: $\delta^{13}C = -28.02 \text{ ‰}$, $\delta^{15}N = -3.44 \text{ ‰}$), were 0.06 and 0.29 for $\delta^{13}C$ and $\delta^{15}N$ respectively. Isotope ratios are expressed as $\delta^{13}C$ or $\delta^{15}N$ according to the following equation:

$$\delta^{13}C \text{ or } \delta^{15}N (\text{‰}) = \left[\left(\frac{R_{\text{sample}}}{R_{\text{standard}}} \right) - 1 \right] \times 1000.$$

where $R = {}^{13}C/{}^{12}C$ or ${}^{15}N/{}^{14}N$.

Different corrections were applied to raw isotope measurements to cope with tissue conversion and preservation effect. The fin-to-muscle relationships can vary among species, and we considered for both species a correction factor ($\epsilon_{\text{fin_to_muscle}}$) equal to the mean enrichment found by Graham et al. (2013) for trout of 1.2 ‰ for $\delta^{13}C$ and 1.3 ‰ for $\delta^{15}N$ for both species. Sample preservation can affect isotope values and vary among species and time although patterns have shown limited consistency over different studies. Yet, fish tissue preservation in alcohol has been often reported to induce a limited enrichment on carbon and nitrogen isotopes (i.e. <1 ‰) and we accounted for alcohol preservation on fish fin by applying a correction factor ($\epsilon_{\text{alcohol_preservation}}$) of 0.5 ‰ for both stable isotopes (Kelly et al., 2006; Kische-Machumu et al., 2017; Xu et al., 2011). For macroinvertebrates, Syväranta et al. (2008) indicated that, over a 12-month experiment, alcohol had minor influence on carbon and nitrogen isotope values. Nonetheless, after 12 months of alcohol exposure, the $\delta^{13}C$ values of macroinvertebrates were enriched of ~1.5 ‰, while their $\delta^{15}N$ values were depleted of ~0.8 ‰. The effect of alcohol preservation has seldom been investigated on primary producers, but Kaehler and Pakhomov (2001) reported very similar changes in the isotopes values of the marine macroalgae (*Ecklonia radiata*) to those of Syväranta (2008) for macroinvertebrates after 12 weeks of alcohol exposure. We therefore applied the same correction ($\epsilon_{\text{alcohol_preservation}}$) for these two organism groups set at + 1.5 ‰ for $\delta^{13}C$ values and - 0.8 ‰ for $\delta^{15}N$ values. The carbon- and nitrogen-corrected isotope values of salmonids, macroinvertebrates and primary producers were hence corrected as follows:

$$\delta^n X_{\text{salmo_corrected}} = \delta^n X_{\text{salmo_measured}} - \epsilon_{\text{fin_to_muscle}} - \epsilon_{\text{alcohol_preservation}}$$

$$\delta^n X_{\text{macroinv_algae_corrected}} = \delta^n X_{\text{macroinv_algae_measured}} - \epsilon_{\text{alcohol_preservation}}$$

where $\delta^n X_{\text{salmo_corrected}} / \delta^n X_{\text{macroinv_algae_corrected}}$ and $\delta^n X_{\text{salmo_measured}} / \delta^n X_{\text{macroinv_algae_measured}}$ are the corrected and raw isotope values for carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) respectively, while $\epsilon_{\text{fin_to_muscle}}$ is the correction factor to account for differences in isotope values between caudal fins and dorsal muscles, and $\epsilon_{\text{alcohol_preservation}}$ is the preservation effect of alcohol on isotope values.

2.4 | Data analyses

At each study site, diverse metrics were computed from stable isotope measurements to inform of the trophic characteristics of the two salmonid species at both population and individual levels. At the population level, the isotopic niches were analysed by considering different metrics from Layman et al. (2007): 1) the $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ ranges (CR and NR) that can reflect population variability in basal resources and trophic positions, 2) the total area of the isotope convex hull (TA) that characterises the maximal isotope niche space occupied by a population and informs on the total isotope diversity, and 3) the mean nearest neighbour distance (MNND) that is the mean of the Euclidean distances to each individual nearest neighbour in the isotope space highlighting population packing or trophic redundancy. For robust estimations of these metrics, a bootstrap procedure involving 10^4 iterations based on random sampling of 15 individuals of each species among each site was conducted and no statistical tests were applied as their significance is correlated with sample size (Krzywinski & Altman, 2013). Standard ellipse areas were also computed in a Bayesian framework (SEA_b ; Jackson et al., 2011) that inform of the isotope niche each species at the two study sites as TA may still be sensitive to sample size and outliers despite the bootstrap procedure implemented (Sv aranta et al., 2013). The isotope overlapping of the salmonid populations at each site was estimated using two metrics developed by Cucherousset and Villegier (2015): 1) isotopic nestedness that quantifies the proportion of isotope overlapping relatively to the species that exhibits the smallest TA among the two species; and 2) isotopic similarity that accounts for the proportion of isotope overlapping relatively to the total isotope area covered by the two salmonid species. For these two overlapping metrics, the same bootstrap procedure than for the Layman's metrics was applied.

The Euclidian distance among pairs of isotope measurements of the two species ($d_{\text{euclid_iso}}$) was computed to address isotope partitioning with salmonid size as follows:

$$d_{\text{euclid_iso}} = \sqrt{(\delta^{13}\text{C}_{\text{trout}} - \delta^{13}\text{C}_{\text{grayling}})^2 + (\delta^{15}\text{N}_{\text{trout}} - \delta^{15}\text{N}_{\text{grayling}})^2}$$

Five size classes were retained to ensure measuring isotope distances between individuals of similar sizes while conserving a reasonable sampling size: < 100 mm (8 trout, 7 grayling), 100–200 mm (5 trout, 5 grayling), 200–300 mm (20 trout, 6 grayling), 300–400 mm (50 trout, 25 grayling) and 400–500 mm (18 trout, 12 grayling). A generalised additive model (GAM, Wood, 2006) was fitted to the Euclidian distance ($d_{\text{euclid_iso}}$) against salmonid size classes to quantify isotope partitioning along ontogeny. GAM allows coping with nonlinear relationships between response variables (here $d_{\text{euclid_iso}}$) that are smoothly fitted against explanatory variables (here the salmonid size classes). The extent of nonlinearity for the relationships between the response and the explanatory variables is quantified by the estimated degree of freedom (edf) with nonlinear patterns characterised by edf higher than 1, and the amount of variance explained

by the model was estimated through adjusted correlation coefficient (R^2_{adj}).

At the individual level, the relative proportions of aquatic and terrestrial resources fuelling each individual salmonid were inferred using Bayesian mixing models (BMM; Parnell et al., 2012). Trophic fractionations for carbon and nitrogen were set at: $\Delta\delta^{13}\text{C} = 0.8 \pm 1$ and $\Delta\delta^{15}\text{N} = 3.4 \pm 1$ (Post, 2002; Vander Zanden et al., 1997). The isotope values of each salmonid were considered as an isotope mixture, and the isotope values of aquatic and terrestrial macroinvertebrate were retained as the possible resources. The different families of aquatic macroinvertebrate did not exhibit clear differences according to their feeding groups (Appendix 2) justifying the relevance of their consideration as a global resource (i.e. aquatic resource). The BMM provides density distributions of the relative contributions for the two possible resources, and we extract their mean values available in the summary statistics of the BMM to characterise the assimilated diet for each individual salmonid. Non parametric tests (Mann & Whitney, 1947) were then used to estimate possible differences among the contributions of assimilated resources between salmonid species and between sites for each salmonid species.

Salmonid trophic positions were computed at both population and individual levels. At the population level, trophic positions for trout and grayling at each site were computed using the Bayesian framework proposed by Quezada-Romegialli et al. (2017) considering the same trophic fractionation than those of the BMM. At the individual level, the trophic position of each individual salmonid (TP_{salmo}) was based on the difference of their $\delta^{15}\text{N}$ values relatively to the mean $\delta^{15}\text{N}$ values of the aquatic ($\delta^{15}\text{N}_{\text{inv_aqua}}$) and terrestrial ($\delta^{15}\text{N}_{\text{inv_ter}}$) macroinvertebrates weighted by their mean contributions to the salmonid assimilated diet previously inferred by the BMM ($\alpha_{\text{inv_aqua}}$ or $\alpha_{\text{inv_ter}}$) to account for omnivory according to Post (2002) as follows:

$$\text{TP}_{\text{salmo}} = [\delta^{15}\text{N}_{\text{fish_corrected}} - (\alpha_{\text{inv_aqua}} \times \delta^{15}\text{N}_{\text{inv_aqua}} + \alpha_{\text{inv_ter}} \times \delta^{15}\text{N}_{\text{inv_ter}})] / 3.4 + 2.$$

The denominator 3.4 allows converting the difference of $\delta^{15}\text{N}$ values between salmonids and macroinvertebrates on a scale of trophic position where an increase in 3.4 ‰ in $\delta^{15}\text{N}$ values is related to an increase of 1 for trophic position. The term + 2 is inserted in the equation to explicitly assume that macroinvertebrate are primary consumers with a trophic position of 2.

GAM was also used to assess how salmonid size, species and study sites could explain the variability of the proportions of both aquatic resources assimilated and individual trophic positions. The absence of pattern between the residuals and the fitted values was checked to ensure the validity of all GAM.

All analyses were performed using R 3.6.0 (R Core Team, 2020) and the following packages: *ggplot2* (Wickham, 2009), *tidyr* (Wickham & Henry, 2019), *simmr* (Parnell, 2016), *siar* (Parnell & Jackson, 2013), *cowplot* (Wilke, 2019), *gridExtra* (Auguie, 2017), *dplyr* (Wickham et al., 2019), *vegan* (Oksanen et al., 2019), *SIBER* (Jackson et al., 2011), *tRophicPosition* (Quezada-Romegialli et al., 2018), *rjags* (Plummer, 2019), *mgcv* (Wood, 2006) and the R codes provided by

Cucherousset and Villeger (2015) for quantifying isotopic nestedness and isotopic similarity.

3 | RESULTS

The potential food resources for salmonids clearly differed in their isotope values. The $\delta^{13}\text{C}$ values of aquatic macroinvertebrate were particularly depleted at Lods (-40.3 ± 2.6 ‰) and more ^{13}C -enriched at Ornans (-35.5 ± 2.3 ‰) while clearly differing from those of terrestrial macroinvertebrates (-28.4 ± 2.9 ‰) at both sites (Figure 1). The $\delta^{15}\text{N}$ values of aquatic macroinvertebrates were ^{15}N -enriched at Ornans (5.5 ± 2 ‰) compared with Lods (3.3 ± 1.7 ‰) while those of terrestrial macroinvertebrates exhibit intermediate $\delta^{15}\text{N}$ values (4.6 ± 1.9 ‰) (Figure 1).

Salmonids isotope values were typically ^{15}N -enriched compared with their putative resources at both study sites and exhibited clearer inter-specific isotope segregation at Ornans than at Lods (Figure 1). At both sites, trout were ^{13}C - and ^{15}N -enriched compared with grayling with a mean ^{15}N -enrichment similar at the two sites (i.e. 1.8 ‰ at Ornans and 1.5 ‰ at Lods), whereas the ^{13}C -enrichment was more pronounced at Ornans (~ 2.3 ‰) than at Lods (~ 1.2 ‰) (Figure 1, Table 2).

The two salmonid species also differed according to the different isotope metrics considered. The CRs and NRs were higher at both sites for trout than grayling, and these differences were more pronounced at Ornans than at Lods (Figure 2a,b). TAs of the two salmonids exhibited different patterns among sites with higher TA for trout than grayling at Lods, while it was the reverse at Ornans (Figure 2c). SEA_b indicated clear differences in the isotope niches of the two salmonids with larger isotope niches for trout than grayling at both study sites although SEA_b tended to slightly decrease in the downstream site (Ornans) compared with the upstream site (Lods) (Figure 2d). The MNND mostly differed at Lods with higher values for trout than grayling, while those were very similar at Ornans (Figure 2e).

The isotope overlapping of trout and grayling was more pronounced at Lods than at Ornans (Figure 2f,g). At Lods, trout had consistently larger TA and SEA_b than grayling indicating that isotopic nestedness (median value = 79%) mostly represented an isotope overlapping of grayling by trout at this site. At Ornans, because TA differences between species were minor (1.03 times larger) while SEA_b still indicated larger isotope niche for trout than grayling, the isotopic nestedness (median value of 42%) indicates that less than half of their isotopic niches overlap at this site (Figure 2f). The isotopic similarity confirmed the general non-negligible isotope

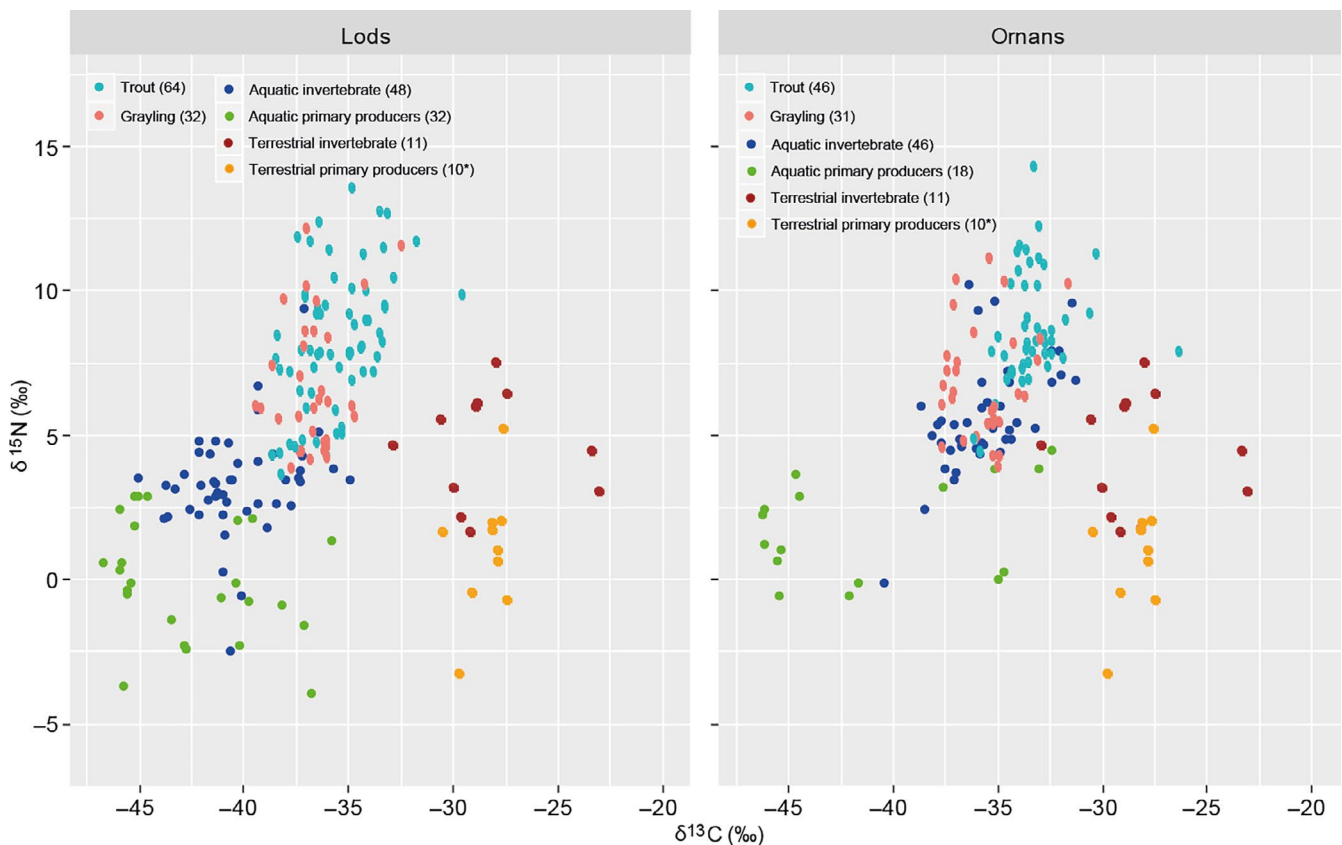


FIGURE 1 $\delta^{13}\text{C}$ - $\delta^{15}\text{N}$ biplots showing the isotope values of the different organism groups at the two studied sites. The numbers of isotope measurements for each organism group are indicated in brackets. The terrestrial invertebrate isotope measurements are pooled for the two sites. Isotope values of primary producers are shown to provide a comprehensive representation of the food webs of the two study sites. *Isotope values of terrestrial primary producers ($n = 10$) were simulated assuming normal distributions for $\delta^{13}\text{C}$ at -28 ± 1 ‰ according to Finlay (2001) and France (1995) and for $\delta^{15}\text{N}$ at 0 ± 2 according to Cloern et al. (2002) and Solomon et al. (2011)

	Lods		Ornans	
	Trout	Grayling	Trout	Grayling
Size (mm)	300 ± 117	307 ± 156	341 ± 76	310 ± 89
$\delta^{13}\text{C}$ (‰)	-35.6 ± 1.8	-36.8 ± 1.4	-33.4 ± 1.6	-35.7 ± 1.6
$\delta^{15}\text{N}$ (‰)	8.3 ± 2.4	6.8 ± 2.3	8.7 ± 1.9	6.9 ± 2

TABLE 2 Summary of salmonid sizes and isotope values for the two study sites

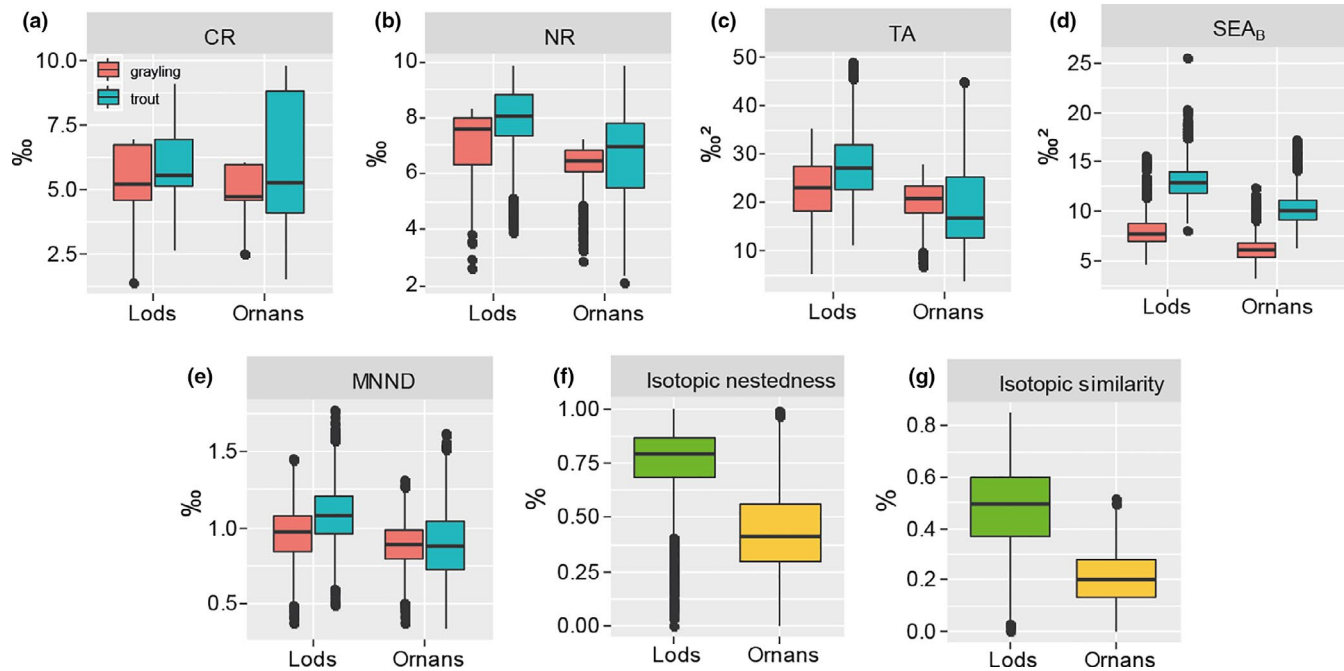


FIGURE 2 Isotope metrics characterising the salmonid populations among the two sites. (a) $\delta^{13}\text{C}$ range (CR), (b) $\delta^{15}\text{N}$ range (NR), (c) total area of the convex hull (TA), (d) standard ellipse area estimated in a Bayesian framework (SEA_B), (e) mean nearest neighbour distance (MNND). The extent of isotopic overlapping is quantified by (f) isotopic nestedness and (g) isotopic similarity. All metrics except SEA_B were obtained following a bootstrap procedure of 10^4 trials of random samples ($n = 15$) for each species among sites. The horizontal lines within boxes represent the median values, the boxes delineate the 25th and 75th percentiles, and vertical lines represent 1.5 times the distance between the first and third quartiles, values beyond this distance represent outliers and are visualised as black points

overlapping between the two species at Lods (median value ~ 50%) while it was clearly lower at Ornans (median of ~ 20%) (Figure 2g). The Euclidian distances between isotope measurements ($d_{\text{euclid_iso}}$) of trout and grayling varied significantly and nonlinearly among the different size classes and plateaued for the two largest size classes ($\text{edf} = 2.3$, $R^2_{\text{adj}} = 10.4\%$, $p < .001$; Figure 3). Those were minimal and below 2.5 ‰ for the smaller salmonids (i.e. <100 mm) and increased up to 10 ‰ for the larger salmonids while becoming more variable.

The two salmonid species mainly relied on aquatic resources according to the BMM (Figure 4a,b). Grayling assimilated significantly more aquatic resources than trout (Mann-Whitney, $p < .001$) with median values of ~75% and ~66%, respectively. This pattern was consistent among sites for trout (Mann-Whitney, $p = .75$), while grayling assimilated slightly but significantly more aquatic reliance at Ornans than at Lods (Mann-Whitney, $p = .04$). Salmonid species, study sites and individual size significantly explained the variability of aquatic resource assimilated by salmonids (GAM, $R^2_{\text{adj}} = 0.26$).

Specifically, aquatic resources significantly and nonlinearly decreased with salmonid body size of both species ($\text{edf} = 2.7$), while grayling rely consistently more on aquatic resources than trout (Figure 4c) as previously indicated.

The TP of trout computed individually were very similar among sites (3.7 ± 0.8 at Lods and 3.6 ± 0.6 at Ornans) and were higher than those computed using Bayesian inferences that suggested generally higher TP at Lods (3.4 ± 0.2) than at Ornans (3.0 ± 0.1) (Figure 5). The TP of grayling computed using the two methods were concordant and higher at Lods (2.9 ± 0.7 for individual computation and 3.1 ± 0.1 for Bayesian computation) than at Ornans (2.4 ± 0.6 for individual computation and 2.3 ± 0.1 for Bayesian computation). As a consequence, TP of grayling were consistently lower than those of trout. A GAM underlined the significant effects of salmonid species, sites and individual sizes on the TP variability ($R^2_{\text{adj}} = 0.5$) and indicated a positive and nonlinear relationship between individual TP and salmonid size ($\text{edf} = 2.6$) that flatten for the larger salmonid sizes (Figure 5b).

4 | DISCUSSION

In sympatry, trout and grayling can be suspected to compete under extensive resource limitation (Mann et al., 1989; Northcote, 1995) and their feeding similarities can vary among ecosystems and cohorts (Degerman et al., 2000; Greendale, 1975; Woolland, 1988). Our isotopic approach indicated non-negligible isotope overlapping

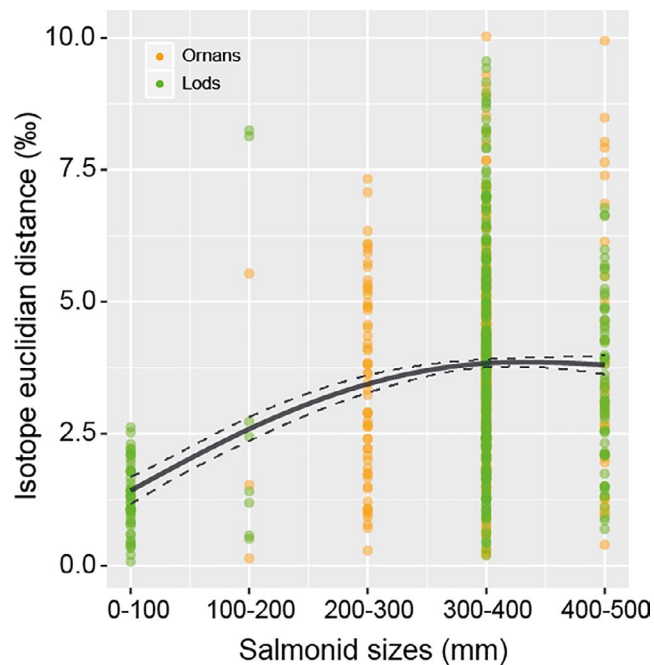


FIGURE 3 Euclidian distances among isotopes measurements of trouts and graylings according to five size classes. The black curve corresponds to the fitted values of a generalised additive model and dashed curves to the standard errors

between these two species at both sites and especially large at the upstream site. This result refutes our initial hypothesis of low isotopic overlapping between these two sympatric species. Trout had wider isotopic niche than grayling in line with our initial expectation of a more opportunistic feeding behaviour of trout than grayling (Greenberg et al., 1996; Jonsson & Jonsson, 2011; Mann et al., 1989). The wider isotope niche of trout may also be involved in isotopic niche partitioning due to the exploitation of complementary habitat and/or preys leading to nonoverlapping regions of the isotope spaces.

Aquatic invertebrates were particularly ^{13}C -depleted in the Loue River likely due to current velocity fostering isotope fractionation of aquatic primary producers that exhibited $\delta^{13}\text{C}$ values as low as -45‰ (Figure 1; Finlay et al., 1999, 2002; Trudeau & Rasmussen, 2003). This feature allowed a clear distinction between aquatic and terrestrial resources supporting robust inference from the BMM (Phillips et al., 2014). Both salmonid species appeared to rely primarily on aquatic macroinvertebrate contrasting with other studies on salmonids in streams where terrestrial subsidies often represent a substantial fraction of prey items and annual energy budget (Baxter et al., 2007; Kraus et al., 2016; Nakano & Murakami, 2001; Sweka & Hartman, 2008; Wilson et al., 2014; Wipfli & Baxter, 2010). Nonetheless, trout was significantly more fuelled by terrestrial subsidies than grayling ($36 \pm 13\%$ against $26 \pm 9\%$) supporting our initial expectation of differences in feeding behaviour and habitat use likely expressing through a spatial segregation in their feeding habitat as reported for other salmonids (Sánchez-Hernández et al., 2016). Specifically, grayling are considered as benthic feeder occupying the main river channel, while trout would be more associated with shallow water, closer to the river edges where terrestrial preys would be preferentially available (Blanck et al., 2007; Greenberg et al., 1996; Ingram et al., 1999; Mann et al., 1989).

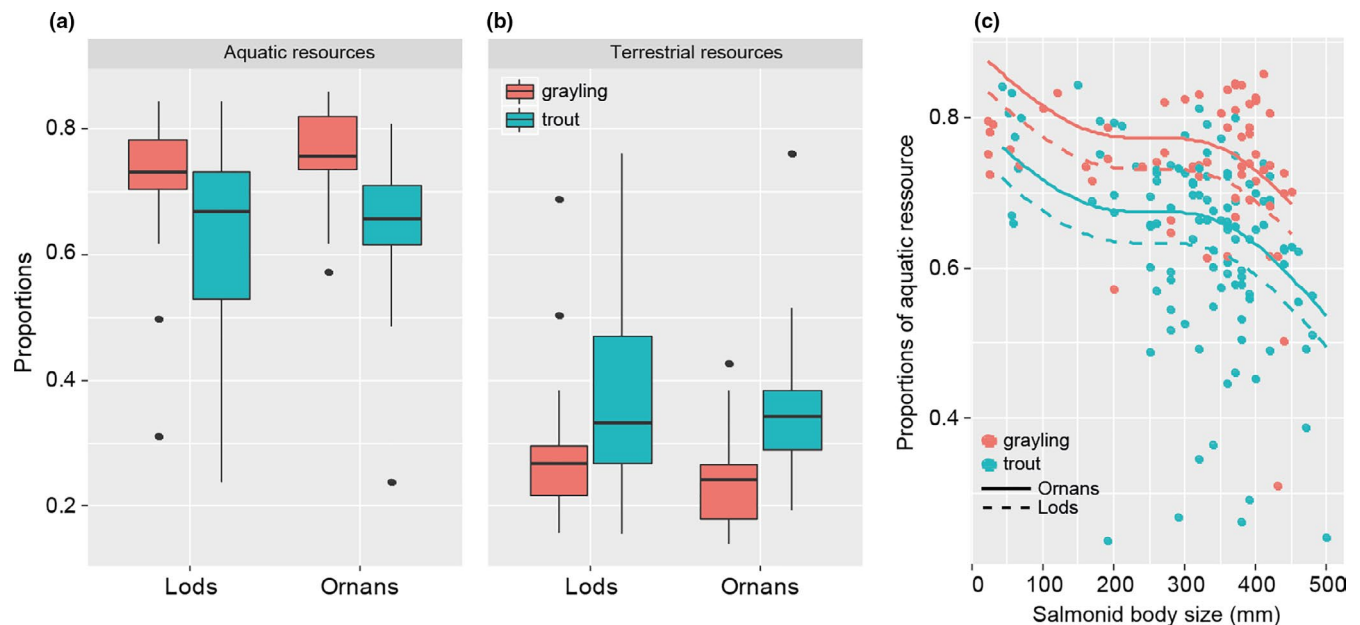


FIGURE 4 Estimated proportions of (a) aquatic and (b) terrestrial resources assimilated by trout and grayling at the two study sites inferred by the Bayesian mixing models. (c) Changes in aquatic resources assimilated along salmonid size. Blue and red curves represent the fitted values of generalised additive models for trout and grayling respectively at Lods (dashed curves) and Ornans (solid curves)

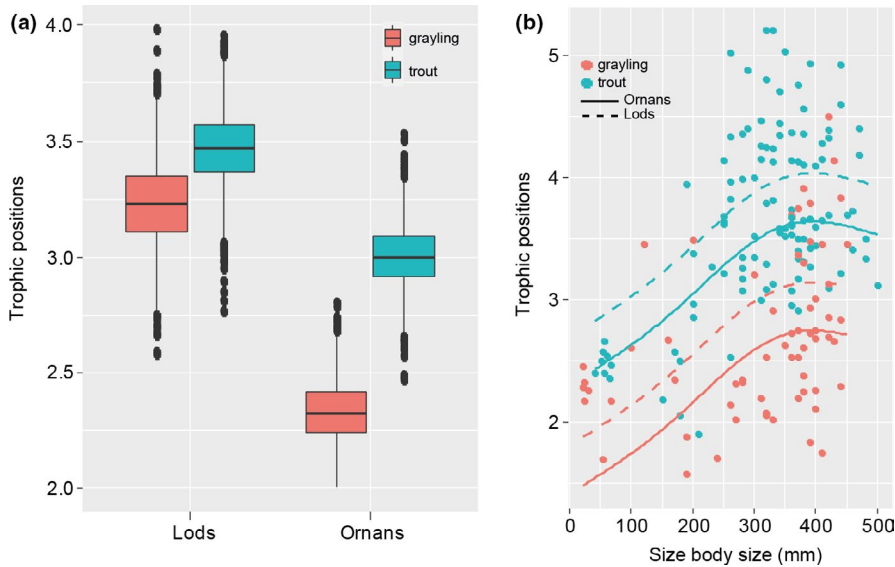


FIGURE 5 Bayesian estimates of trophic positions for the two salmonid species at the two sites (a). Changes in trophic position with salmonid size (b). Blue and red curves represent the fitted values of generalised additive models for trout and grayling respectively at Lods (dashed curves) and Ormans (solid curves)

A significant implication of ontogeny on the trophic characteristics and isotopic niches of these two sympatric salmonids could be highlighted as initially postulated through changes in both resource use (i.e. aquatic resource) and trophic position with salmonid size. Ontogeny was hence a major component of the trophic behaviour of these salmonids as previously reported (Ingram et al., 1999; Mann et al., 1989; Sánchez-Hernández et al., 2019). The decrease in aquatic resource use may indicate ontogenetic changes in habitat use and changes in food resources that were likely driving the increase of TP with salmonid size. Grayling had consistently lower TP than trout confirming that this latter would tend to behave as apex predator. Several trouts could reach TP up to ~5, similar to lake trouts in Ontario and Quebec lakes (Vander Zander et al., 2000), strongly suggesting piscivory as a diet characteristic for the larger individuals (Mann et al., 1989). The increasing isotope distances with salmonid size further indicated that the isotopic niche partitioning between these two salmonids would increase with ontogeny. This result is in accordance with their habitat requirements as 0+ individuals are drastically constrained by current velocity and will be constrained to similar habitats, while adults will tend to exploit more distinct river habitats and prey items (Degerman et al., 2000; Jonsson & Jonsson, 2011; Mallet et al., 2000).

5 | CONCLUSION

This study reported isotope characteristics of two sympatric salmonids and highlighted isotopic differences that could be associated with differences in trophic attributes and habitat use. The strong reliance on aquatic resources suggests that these two salmonids are highly dependent on aquatic secondary production, and mitigation efforts to preserve and enhance aquatic secondary production (aquatic invertebrates) should be of primary interest for their long-term conservation. The strong implication of individual size regarding trophic attributes (i.e. resource use and trophic position) further

indicated that the consideration of ontogeny can help clarifying the trophic niche partitioning of these two sympatric salmonid species where the strongest trophic competition may mostly occur for early life stages.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

VF and AC conceived the study. VF and CR conducted the field investigations. VF and CV analysed the data. VF, AC, CV and CR wrote the paper.

DATA AVAILABILITY STATEMENT

Isotope raw data are available on request from the corresponding author.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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