Stable isotopes reveal the importance of terrestrially derived resources for the diet of the freshwater pearl mussel (*Margaritifera margaritifera*)

Mario Brauns¹ | Thomas Berendonk² | Sina Berg² | Felix Grunicke² | David Kneis¹,² | Sascha Krenek¹,²,³ | Thomas Schiller²,⁴ | Jana Schneider² | Annekatrin Wagner² | Markus Weitere¹,²

¹Dept. of River Ecology, Helmholtz Centre for Environmental Research – UFZ, Magdeburg, Germany
²Institute of Hydrobiology, Technische Universität Dresden, Germany
³German Federal Institute of Hydrology, Koblenz, Germany
⁴Saxon State Ministry for Energy, Climate protection, Environment and Agriculture, Dresden, Germany

Correspondence
Mario Brauns, Dept. River Ecology, Helmholtz Centre for Environmental Research - UFZ, Magdeburg, Germany.
Email: mario.brauns@ufz.de

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Abstract

1. The freshwater pearl mussel (FPM) is among the most endangered freshwater species worldwide. The few remaining populations suffer from low recruitment rates and are subject to habitat fragmentation, pollution, siltation, decline or loss of host fish populations, and climate change.

2. Successful conservation strategies for FPM require a holistic understanding of its ecological requirements, life history, population dynamics, and habitat prerequisites. Although habitat requirements are well described, food requirements at different life stages have received less attention.

3. Stable isotope analyses of FPM and potential food resources in three German streams were combined with mixing model analysis to quantify organic matter resources assimilated by juvenile (first year after encystment from host fish) and semi-adult (10 years old, immature) individuals.

4. There were only slight differences in dietary contributions between the two life stages, and terrestrial particulate organic matter and benthic organic matter contributed substantially to the diet. Tissue type was more important in explaining variation in dietary contributions than individual variation for semi-adult FPM. The strong reliance on terrestrial resources sheds new light on the functional role of unionid mussels and the connection of streams to their riparian area.

5. The dependence of FPM on terrestrial resources also emphasizes the need for a stronger focus on the restoration and protection of intact riparian areas, including wetlands with their specific vegetation, when planning conservation and management strategies for threatened FPM populations.

Keywords
aquatic–terrestrial coupling, conservation, feeding ecology, mixing models, species conservation, stable isotope analysis
Freshwater mussels (Bivalvia: Unionoida) rank among the most endangered freshwater species worldwide (Strayer et al., 2004; Bogan, 2015; Lopes-Lima et al., 2017). The freshwater pearl mussel (FPM, *Margaritifera margaritifera*) was widely distributed across northern and central Europe, but at the beginning of the 20th century, populations collapsed nearly everywhere owing to a lack of recruitment (Lopes-Lima et al., 2017; Stoeckle et al., 2017). FPM is now globally endangered and classified as Critically Endangered in Europe (IUCN, 2020). Furthermore, it is listed on Annexes II and V of the European Habitats Directive (Council of the European Communities, 1992). Annex II requires EU Member States to designate ‘special areas of conservation’ for the species listed, whereas for those on Annex V ‘taking in the wild and exploitation may be subject to management measures’. In Germany, FPM is included in the Red List Germany under category 1 (threatened with extinction) (Jungbluth & Knorre, 2011) and is a national priority target species of the German National Biodiversity Strategy and Action Plan (BMUB, 2007).

There are several causes for the decline in FPM populations. FPM is a highly specialized freshwater organism inhabiting cold, oligotrophic streams in the northern hemisphere (Hastie, Boon & Young, 2000; Geist & Auerswald, 2007; Geist, 2010). Such ecosystems are degraded by habitat fragmentation, pollution, siltation, decline or loss of host fish populations, and climate change (Lydeard et al., 2004; Österling, Arvidsson & Greenberg, 2010; Jung et al., 2013). Losing vital FPM populations not only reduces local biodiversity but also alters stream ecosystem functioning (Vaughn & Taylor, 1999; Vaughan & Hakenkamp, 2001; Boeker et al., 2016). Freshwater mussels play a crucial role in freshwater ecosystems, performing valuable ecosystem services such as water purification by removing suspended particles in the water column, nutrient recycling by depositing faeces or pseudofaeces, and sediment stabilization (Lummer, Auerswald & Geist, 2016; Richter et al., 2016; Vaughn, 2018).

Conservation efforts aim at the protection and restoration of habitats and the release of artificially infected host fish. Moreover, supportive breeding programmes aim to release captive-bred individuals back into natural habitats (Hastie & Young, 2003; Bolland et al., 2010; Schmidt & Vandré, 2010). The re-establishment of FPM populations requires knowledge of the occurrence, distribution, and habitat requirements. Although these aspects of FPM ecology are well understood, dietary requirements have not been taken into account during the substantial conservation efforts. Most research has focused on identifying suitable food resources for juvenile FPM (mussels within their first or second year after encystment from host fish) held in captive breeding facilities. Hruska (1999) was the first to establish a rearing method for FPM in the 1980s, proposing that juvenile FPM reared in small containers should be fed with fine particulate organic matter (FPOM) derived mainly from species of Poaceae growing along stream margins. This captive-rearing method of juvenile FPM was successfully adopted and subsequently modified by other rearing programmes all over Europe. In Germany, ground chironomids were added to the detritus for rearing juvenile mussels (Gum, Lange & Geist, 2011). Similarly, Eybe et al. (2013) used mixtures of organic matter, ground chironomids, and different algae in a rearing facility in Luxembourg. At present, most captive breeding programmes use detritus collected from wet meadows or marsh sites combined with algae (usually *Nannochloropsis* sp. or algae from the shellfish diet 1800® microalgaee mixture) as food for the first months of the post-parasitic stage after encystment from the host fish (Gum, Lange & Geist, 2011).

Despite the knowledge of the food requirements of juvenile, captive-held FPM, only a few studies have addressed the food requirements of adult FPM from field populations. Hruska (1999) described organic matter from the terrestrial surroundings as most important and algae to be of minor importance in the diet of FPM. Geist, Auerswald & Boom (2005) analysed a population in a German headwater stream and found that suspended particles were the most likely food source of the individuals studied. Although both studies provided valuable insights on the dietary requirements of FPM, a better understanding of the feeding ecology of juvenile and adult FPM from field populations is needed for effective habitat management and improved conservation approaches.

In this study, stable isotope analysis ($\delta^{13}C$, $\delta^{15}N$) of FPM and their potential food sources from three German streams were combined with mixing model analysis to quantify the organic matter resources assimilated by juvenile and semi-adult (10 years old, immature) individuals. Based on previous studies, we expected that terrestrial-derived organic matter would be more important than autochthonous resources. The tissue turnover time of juvenile FPM transferred from breeding stations to the field was quantified as an indicator of the time needed until captive-bred individuals are adapted to field conditions. In addition, the contribution of organic matter in the diet was assessed to test whether resource use varies individually, with the type of body tissue, or between populations.

## METHODS

### 2.1 Study area

This study is part of a larger project dealing with implementing regional protective measures and developing a new national conservation programme (ArKoNaVera) for FPM and painter's mussel (*Unio pictorum*). The study was conducted in three second-order (Strahler, 1952) mountain streams (referred to here as S1, S2, and S3) located in the Vogtland region (Saxony, Germany; Table 1). Until the 1960s, large numbers of FPM were present in S2 and S3 (Baer, 1995), but populations declined to a few hundred mussels in both streams. From 2002 to 2018, S1 served as a rearing stream for young mussels within the Saxonian rearing programme. FPM were collected in 2007 after encystment from host fish and kept in small plastic boxes (500 mL) filled with stream water and a mixture of detritus and *Nannochloropsis* sp. for the first 3–4 months. They were then...
TABLE 1  Characterization of the streams studied

| Stream width (m) | Water depth (m) | Flow velocity (m s⁻¹) | Dominant substrate | Shore vegetation | Canopy cover |
|-----------------|----------------|-----------------------|-------------------|-----------------|-------------|
| S1              | 0.90           | 0.11                  | 0.21              | Gravel          | Wet meadows | unshaded    |
| S2              | 1.85           | 0.13                  | 0.57              | Gravel          | Wet meadows, Salix sp., Alnus glutinosa | shaded |
| S3              | 0.45           | 0.16                  | 0.60              | Sand, fine gravel | Wet meadows, Salix sp. | unshaded |

Note: Means are given for stream width, water depth, and flow velocity.

transferred into Buddensiek cages (Buddensiek, 1995) and placed in S1. After 3–4 years, mussels reached a size of approximately 5–8 mm. Subsequently, 16–25 semi-adult mussels were transferred into eight 1-L plastic boxes. Each box was half filled with gravel and covered with gauze (500 μm mesh) at the top and all four sides to ensure a constant food supply, following Gum, Lange & Geist (2011). In May 2017, two boxes were transferred to S2 and S3, respectively, and four boxes remained in S1. Growth rates were monitored by repeatedly measuring shell length, height, and thickness to the nearest 10 μm at the end of May and the beginning of September in 2017.

Juvenile FPM studied here originated from fish infected as part of a captive breeding programme in Bavaria and were collected after excystment in June 2017. After rearing juveniles in plastic boxes for 1 month, 1200 individuals were separated into six Buddensiek cages and two cages were installed in each of the three streams in July 2017.

2.2  Sampling and sample preparation

Sampling for stable isotopes of FPM and putative organic matter resources was conducted in S1 in August and November 2017 and in S2 and S3 in November 2017. Samples of organic matter comprised epilithon (biofilm), benthic organic matter (BOM) from Buddensiek cages, boxes, and the stream bed, submerged macrophytes, and terrestrial particulate organic matter (t-POM), i.e. herbaceous vegetation and leaves from riparian trees. Biofilm samples for stable isotope analysis were collected by brushing at least five stones per stream. The resulting slurry was filtered over 100 μm to remove coarse particles and was immediately frozen for later analysis. BOM from the stream bed was sampled using a sediment corer (Uwitec, Mondsee, Austria) with three replicates. BOM from boxes and cages was sampled concurrently with samples of FPM. Cages were rinsed with stream water, and BOM samples were sieved over 30 μm. The fraction <30 μm was stored on ice for later processing. Terrestrial particulate organic matter was collected by hand. The rapid water current and a water depth of less than 10 cm (Table 1) may preclude substantial primary production in the water column. It was assumed, therefore, that truly planktonic algae were unavailable in the streams studied. After processing, the samples of organic matter were frozen and freeze-dried for 24 h (Delta 1–24 LSC, Christ GmbH, Osterode am Harz, Germany).

Samples of semi-adult FPM with lengths ranging between 28 and 38 mm from rearing cages were collected concurrently or a few days after samples of organic matter were taken. Four individuals were collected at random from S1 at the end of August, and at the end of November, four randomly collected individuals were sampled from S1, S2, and S3, respectively. Samples of juvenile FPM were collected by removing individuals from Buddensiek cages at the end of November 2017. Sampling FPM from the streams was permitted by the authority for nature conservation of the federal state of Saxony. However, a specific licence was not necessary as the work was restricted to captive-bred and caged mussels only.

Semi-adult FPM were placed on ice until later preparation. After 3–5 h, semi-adult FPM were dissected in the laboratory into four types of tissue: shell muscle, foot muscle, gills, and mantle tissue. Semi-adult tissue and juveniles were freeze-dried.

2.3  Stable isotope analysis

To prepare samples for stable isotope analysis, mussel tissue and food resources were ground with an electronic ball mill and weighed into tin capsules. Shells of juvenile FPM were removed by acid treatment. Samples from each stream were split, and 100 individuals were treated with 1.2 N HCl and subsequently dried for 12 h at 65°C. As acid treatment may interfere with nitrogen isotopes, 100 untreated individuals were analysed to serve as a control. The subsequent statistical and mixing model analysis was conducted with acid-treated δ¹³C but untreated δ¹⁵N.

Concentrations of δ¹³C and δ¹⁵N were measured using a Thermo-Finnigan Flash 2000 elemental analyser connected to a Delta V Advantage mass spectrometer (Thermo Electron Corporation, Waltham, USA). Stable isotope data were expressed as the relative difference between ratios of samples and standards (PeeDee Belemnite for δ¹³C, atmospheric N₂ for δ¹⁵N):

\[ \delta R(\%o) = \left( \frac{R_{\text{SAMPLE}}}{R_{\text{STANDARD}}} - 1 \right) \times 10^3 \]

(1)

where \( R = 13C/12C \) or \( 15N/14N \).

Analytical precision (SD) from multiple runs was less than 0.2‰ for δ¹³C and δ¹⁵N.

2.4  Statistical analysis

Daily growth of FPM was calculated from the increase in the length, width, and thickness (semi-adults only) of individual mussels during
88 days during summer. Growth rates of semi-adult FPM were compared among streams using one-way ANOVA and Tukey’s HSD post-hoc test in R (R Core Team, 2013). A conventional linear model and mixed-effects models were used to identify the major causes of variation. Starting with a single predictor, model complexity was increased in a step-wise manner to examine the added value of additional predictors. All models fitted were compared using Akaike information criterion (AIC). Among a set of related models, the one with the lowest AIC represents the optimum trade-off between goodness-of-fit and model complexity. Standard linear and mixed-effects models were fitted with R using the methods ‘lm’ from package ‘base’ and ‘lmer’ from package ‘lme4’ (Bates et al., 2015). In addition, one-way ANOVA and Tukey’s HSD post-hoc test in R were used to determine whether the stable isotope composition of various BOM sources differed. Tissue turnover of juvenile FPM was calculated as the difference between initial and final isotope values. Differences were not tested for significance owing to the low replication (N = 2 per stream).

2.4.1 Mixing models

Before mixing model analysis, outliers in the stable isotope data of FPM were identified by inspecting stable isotope biplots (Figure 1). Juvenile and semi-adult FPM from S1 were outside the mixing polygon delimited by the resources after accounting for trophic discrimination (Figure 1); hence, mixing model analysis had to be restricted to S2 and S3. Bayesian mixing models as implemented in the MixSIAR package (Stock & Semmens, 2013) were applied to estimate the contribution of different food resources to juvenile and semi-adult FPM separately for each stream.

Dietary proportions of semi-adult FPM are driven by individual variation, and variation induced by differences in stable isotope composition between tissue types (Gustafson et al., 2007). The latter is associated with different biochemical compositions, e.g. lipid-rich tissues are often enriched in δ13C (DeNiro & Epstein, 1977). By contrast, differences in metabolic activity also contribute to variation in tissue stable isotope composition (Auerswald et al., 2010). Both potential sources of variation were accounted for, and models were run with a hierarchical setup with tissue type nested in individuals (Semmens et al., 2009). The contribution of individual and tissue type to explaining the dietary variability of semi-adult FPM was assessed by comparing the estimated posterior density for the standard deviation of factors ‘individual’ and ‘tissue type’.

Models for juvenile FPM were run with ‘individual’ as the only source of variation. Trophic discrimination was considered by using factors and uncertainties specific for aquatic invertebrates: 0.1 ± 2.2‰ for δ13C and 2.6 ± 2.0‰ for δ15N (Brauns et al., 2018). Mixing models were run with concentration dependence (Phillips & Koch, 2002) and without residual error terms (Parnell et al., 2013). The convergence of each model run was verified using diagnostic plots and tests provided by the software. Mixing models resulted in
3,000 posterior estimates of the proportional contribution of each resource to the diet of FPM. Posterior estimates of terrestrial leaves and terrestrial herbaceous vegetation were pooled into the resource category ‘t-POM’, those of BOM from stream bottom and cages into ‘BOM’, and those of moss and submerged macrophytes into ‘submerged macrophytes’ by taking the sum of the individual contributions. The resulting 3,000 posterior estimates of the proportional contribution of each resource category to FPM diet was used to calculate the mean and 95% credible intervals.

3 | RESULTS

3.1 | Sources of BOM

BOM from the stream bottom was significantly depleted in $\delta^{13}$C and $\delta^{15}$N compared with BOM from Buddensiek cages in S2, but differences were relatively small (Table 2). Stable isotopes of BOM from the stream bottom did not differ significantly from BOM extracted from the cages in which juvenile and semi-adult FPM were kept.

3.2 | Juveniles

Stable isotope values of juvenile FPM from the breeding station were substantially depleted in $\delta^{13}$C compared with individuals exposed in the streams (Table 3, Figure 1). After 148 days of exposure in the streams, stable isotopes of juvenile FPM fell within the range of semi-adult FPM, indicating that tissue turnover was completed (Figure 1). The differences between initial and final tissue isotope values ranged from 4.09‰ to 1.27‰ for $\delta^{13}$C and from 1.89‰ to −0.95‰ for $\delta^{15}$N (Table 3).

Mixing model analysis showed that juvenile FPM preferentially assimilated organic matter of terrestrial origin in S2. Concomitantly, there was no clear preference for a given resource at S3 (Figure 2).

3.3 | Semi-adults

Growth rates based on shell length and height did not significantly differ between streams (Table 4). Shell thickness was significantly lower in S3 compared with the other two streams.

The stream of origin primarily drove the stable isotope composition of semi-adult FPM (Table 5). The tissue type explained a relevant fraction of the residual variance, and the model’s $R^2$ generally approached values close to 0.9. A further improvement in the explanatory power of models was obtained when random intercepts were introduced to allow the baseline values of $\delta^{13}$C and $\delta^{15}$N to vary between individuals (Table 5). The resource use of semi-adult FPM in S2 and S3 did not substantially differ from that of juvenile FPM (Figure 2). Diets of semi-adult FPM were dominated by t-POM (49%) and BOM (34%) at S2, whereas biofilm, BOM, submerged macrophytes, and t-POM contributed equally to the diet of semi-adults at S3.

Comparing components of variation in dietary contributions showed that variability attributed to tissue types was greater than the variability attributed to individuals in both streams (Figure 3).

### Table 2

| Benthic organic matter | Buddensiek cages | Box | Instream |
|------------------------|------------------|-----|----------|
| $\delta^{13}$C         | −28.79 ± 0.04$^a$| −29.01 ± 0.11$^b$| −29.11 ± 0.06$^b$|
| $\delta^{15}$N         | 4.97 ± 0.08$^a$  | 4.50 ± 0.15$^b$  | 3.97 ± 0.53$^b$  |

Note: Significant differences between benthic organic matter sources ($P < 0.05$, 1-way ANOVA, Tukey’s HSD) are indicated by different superscript letters. Buddensiek cages are plates with small holes for young FPM.

### Table 3

|              | $\delta^{13}$C |         | $\delta^{15}$N |         |
|--------------|----------------|---------|----------------|---------|
|              | Untreated      | Acid    | Untreated      | Acid    |
| Rearing station | −22.47 ± 1.63  | −35.37 ± 0.19 | 3.11 ± 0.08  | 4.10 ± 0.54  |
| S1           | −18.56 ± 0.81  | −31.28 ± 0.11 | 4.71 ± 0.07  | 5.23 ± 0.01  |
| S2           | −19.07 ± 0.03  | −29.12 ± 0.05 | 6.60 ± 0.38  | 7.06 ± 0.08  |
| S3           | −17.81 ± 0.23  | −29.94 ± 0.83 | 5.66 ± 0.85  | NA       |

Note: Shell carbonates were removed with acid treatment, and untreated values are given for comparison.
**DISCUSSION**

### 4.1 Juvenile FPM

Juvenile FPM were isotopically similar to semi-adult FPM after 148 days irrespective of the stream studied, indicating complete tissue turnover within this period. The results of this study do not allow the estimation of half-life rates or quantifying the exact time-point of isotopic equilibrium, given the constraints in obtaining a sufficient number of individuals. We are unaware of published studies on stable isotope tissue turnover of FPM. The available literature on other freshwater unionids is too sparse for a robust generalization of the results obtained. Tissue turnover of *Elliptio complanata* took approximately 9 months in Piedmont streams of North Carolina (USA) (Gustafson et al., 2007). Estimated turnover rates for *Pleurobema sintoxia* and *Fusconaia flava* of $\delta^{15}N$ in different tissue types ranged from 78 to 85 days in Michigan streams (Raikow & Hamilton, 2001). To what extent these turnover rates apply to juvenile FPM remains unknown; thus, further studies are needed to evaluate the dynamics of isotopic incorporation as it is a crucial prerequisite for quantifying the trophic ecology of FPM using stable isotopes (Vander Zanden et al., 2015).

### 4.2 Semi-adult FPM

Tissue type was an important predictor of isotopic variability and contributed substantially to the dietary variability of semi-adult FPM. This finding is in line with a previous study that showed statistically significant differences in isotope composition between mantle and foot tissue of freshwater unionids (Lafrançois et al., 2018). Thus, this study, together with previous findings, indicates that tissue types have to be considered in stable isotope studies of FPM, and mixing models should be run with all available tissue types. The role of tissue type in explaining dietary variability also calls for establishing specific trophic discrimination factors for FPM tissue type. We assumed that

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**TABLE 4** Mean (±1 SD) daily growth rates of shell length, height, and thickness ($\mu$m d$^{-1}$) for semi-adult freshwater pearl mussels

| Stream | Length | Height | Thickness | n  |
|--------|--------|--------|-----------|----|
| S1     | 54 ± 11$^a$ | 25 ± 6$^a$ | 16 ± 8$^a$ | 7  |
| S2     | 46 ± 27$^a$ | 21 ± 12$^a$ | 12 ± 8$^{ab}$ | 4  |
| S3     | 45 ± 18$^a$ | 16 ± 11$^a$ | 3 ± 2$^b$   | 4  |

Note: Different superscript letters indicate significant differences in growth rates among the three streams ($P < 0.05$, 1-way ANOVA, Tukey’s HSD), n = number of individuals measured.

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**FIGURE 2** The proportional contribution of resources to the diet of juvenile and semi-adult freshwater pearl mussels in stream S2 and S3. Shown are means and 95% credibility intervals for each resource category. BOM = benthic organic matter; sub. macrophytes = submerged macrophytes; t-POM = terrestrial particulate organic matter.
discrimination factors between resource and individual tissues are within the range of those for resource and whole-body, and have thus corrected tissue isotopic signatures with values from a meta-analysis of whole-body values (Brauns et al., 2018). This conjecture was based on the rather large SD of whole-body discrimination factors. The extent to which both types of discrimination factors are similar remains unknown and requires controlled feeding experiments. However, epilithic biofilm isotopes most probably mirror the isotopic signal from biofilm associated with sediments accessible to FPM. Autochthonous microalgae usually have a much better nutritional value than terrestrial organic matter and are often the predominant food of bivalves in fresh waters (Jantz & Neumann, 1998). In contrast, stable isotope analyses indicated that autochthonous algal primary production seems to be of minor importance for FPM in the streams studied.

Particulate resources such as BOM were important dietary components for FPM in both streams, and the high reliance on terrestrial resources in S2 was remarkable (Figure 2). We assume that t-POM resources became accessible to semi-adult FPM after decomposition into finer particles and the resuspension of such particles from the stream bottom. We furthermore assume that particulate resources were assimilated via filtration, as pedal feeding is inefficient for individuals >2.2 mm (Schartum et al., 2017). This assumption is corroborated by findings from Geist, Auerswald & Boom (2005), showing that fine suspended particles are an essential food resource for FPM.

The results on the role of terrestrial resources are conservative, given that the estimates rely only on t-POM. BOM, which contributed up to 73% to FPM diet, can contain significant amounts of organic matter of terrestrial origin. BOM can also serve as a substrate for autochthonous biofilm production. Chlorophyll-a values from BOM measured in the streams ranged between 70 and 150 μg L⁻¹ (data not shown) and indicate that the role of biofilm as a resource for FPM may be underestimated. It is impossible at present to differentiate the sources of BOM based on bulk stable isotope analyses. Improved techniques such as compound-specific stable isotope analyses are needed to quantify the exact degree of the assimilation of terrestrial resources for FPM. Unless such data are available, mixing models demonstrated that FPM strongly relies on terrestrially-derived resources and shows that FPM can be an important consumer coupling terrestrial and freshwater ecosystems. These results support earlier studies that demonstrated POM from terrestrial vegetation to be essential for mussels (Hruska, 1999).

**TABLE 5** Performance of different statistical models to explain the observed values of δ¹³C and δ¹⁵N in semi-adult freshwater pearl mussels

| Predictors                      | Fitting method | SSR  | R²   | AIC    |
|--------------------------------|----------------|------|------|--------|
| δ¹³C Stream                     | lm             | 7.6  | 0.83 | 54.2   |
| δ¹³C Tissue                     | lm             | 40.4 | 0.09 | 156.6  |
| δ¹³C Stream + tissue            | lm             | 5.0  | 0.89 | 34.5   |
| δ¹³C Stream + (1|individual) | lmer  | 1.0  | n.c. | −1.3   |
| δ¹⁵N Stream                     | lm             | 8.6  | 0.76 | 61.6   |
| δ¹⁵N Tissue                     | lm             | 30.5 | 0.14 | 139.6  |
| δ¹⁵N Stream + tissue            | lm             | 4.7  | 0.87 | 30.8   |
| δ¹⁵N Stream + (1|individual) | lmer  | 1.0  | n.c. | 0.9    |

Note: All predictors were categorical. The term ‘1|individual’ indicates that the intercept was allowed to vary between individuals.

Abbreviations: AIC, Akaike information criterion; n.c., not computed; SSR, sum of squared residuals.

**FIGURE 3** Variation in the diet of semi-adult freshwater pearl mussels attributed to individual variation, and variation between tissue types. The standard deviation of the estimated posterior density is shown for the factors ‘individual’ and ‘tissue type’ (see methods for explanation) as means and 95% credibility intervals.
Successful conservation strategies for FPM require a holistic understanding of its ecological demands and life history, population dynamics, and habitat requirements. Water quality, quantity, flow velocity, and substrate properties are known to be important drivers of population dynamics (Geist & Auerswald, 2007; Geist, 2010), whereas food requirements of populations at different life stages have received almost no attention. This study indicates that the availability of particulate resources, especially those from the riparian area, are essential drivers of both juvenile and semi-adult FPM. This provides empirical support based on modern stable isotope analyses for earlier assumptions that detritus from wet grass meadows are beneficial food resources for juvenile FPM (Hruska, 1999). Terrestrial POM may represent the primary food source, especially in streams with low nutrient levels and large canopy cover. The high dependence on allochthonous inputs in natural FPM habitats demonstrated in this study highlights the need to preserve intact riparian and associated areas to manage threatened FPM populations. For example, an integrated catchment management plan focusing on the remediation and protection of intact riparian areas, including wetlands with their specific vegetation, may be a fruitful avenue for the successful conservation of endangered FPM populations. This includes regulating various activities, including, but not limited to, grazing of livestock, the addition of fertilizers, the use of pesticides or herbicides, ploughing, or land drainage. Furthermore, the protection and fostering of diverse (wet) meadows by supporting extensive grazing and organic farming could be critical elements in a successful conservation plan. Whether these management strategies have to facilitate the settlement and growth of specific terrestrial plants, e.g. species from wet meadows, needs to be further investigated. Metabarcoding approaches, in conjunction with the analyses of food particles ingested by FPM, can help to address this question. Such data may improve the understanding of the specific food requirements of different FPM age-classes in the field, which may also contribute to refining recent monitoring approaches for FPM (Boon et al., 2019) by adding parameters that describe the availability of resources that FPM require. For example, mapping the structural integrity of the riparian zone, including the presence of specific plants, may be a way to determine or monitor the status of a given stream reach to serve as a refugium for FPM. Such an amendment of monitoring standards requires data from other streams across Europe.

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CONFLICT OF INTEREST
The authors have no conflict of interest to declare.
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