



Feeding ecology of three freshwater mussel species (Family: Unionidae) in a North American lentic system

Kaelyn J. Fogelman · James A. Stoeckel ·
Jonathan M. Miller · Brian S. Helms

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Abstract Freshwater mussels are typically considered to be primarily filter-feeders with an ability to pedal feed, however there is limited information regarding interspecific differences in food resources and feeding modes. The objective of this study was to investigate interspecific variation in food resource usage among mussels in a lentic system associated with a reservoir drawdown. We quantified carbon-13 and nitrogen-15 isotopic signatures for three mussel species, including a Federally Threatened species, and their potential food resources in Gantt Lake, Alabama, USA. For all species, carbon-13 derived from limnetic, benthic fine particulate organic matter (FPOM) contributed on average 99% to the mussel diet. Paradoxically, mussels collected from the littoral zone relied primarily on food resources from the limnetic zone, although this spatial disconnect may have been an artifact of the sampling regime. Carbon-13 associated with littoral FPOM, limnetic and littoral suspended particulate organic matter (SPOM) and coarse particulate organic matter (CPOM)

contributed <1%. *Elliptio pullata* (Lea, Proceedings of the Academy of Natural Science 8: 262, 1856) collected live but emersed for 8 weeks were enriched in nitrogen-15, providing evidence of catabolism during emersion. Results suggest that benthic sources can be a dominant food resource for unionids and that stranded unionids rely on internal energy stores to survive emersion.

Keywords Unionidae · Stable isotopes · Feeding ecology · Catabolism · Lake management · Benthic food web

Introduction

Freshwater mussels (Unionidae) can consume a wide range of food resources including live algae, bacteria, and particulate organic material. Individual constituents of mussel diets have been identified through several methodological approaches as algae, rotifers, protozoans, detritus, bacteria, and dissolved organic carbon (Strayer, 2008; Vaughn et al., 2008; Haag, 2012). In both river and lake habitats, diatoms (Bacillariophyceae) and green algae (Chlorophyta) can be found in high concentrations in the mantle cavity and gut of mussels, suggesting preferential feeding on these dietary items (Nichols & Garling, 2000). Despite the positive selection for planktonic algae, bacteria are often of equal or greater importance to mussel diets in both riverine and lake systems

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K. J. Fogelman (✉) · J. A. Stoeckel
School of Fisheries, Aquaculture, and Aquatic Sciences,
Auburn University, Auburn, AL 36849, USA
e-mail: kjf0021@auburn.edu

J. M. Miller · B. S. Helms
Department of Biological and Environmental Sciences,
Troy University, Troy, AL 36082, USA

(Nichols & Garling, 2000; Christian et al., 2004; Newton et al., 2013). Bacteria are found in seston but also dominate microbial communities associated with benthic detrital pathways (Krumins et al., 2013; Boyd, 2019).

Although typically described as suspension feeders, unionids are capable of exploiting benthic-derived food resources (Raikow & Hamilton, 2001; Nichols et al., 2005) and some studies have shown that both juvenile and adult unionids derive more than 50% of their diet from benthic sources (Yeager et al., 1994; Gatenby et al., 1996, 1997; Fogelman et al., 2022). The positive relationship observed between juvenile and adult *Elliptio complanata* (Lightfoot, 1786) growth and sediment organic content in lentic systems (Cyr, 2020) suggests that mussels are able to utilize benthic food sources when suspended sources are limiting. The mouth and foot of a mussel are located anteriorly while the incurrent siphon is located posteriorly. During filter feeding, particles are moved from the posterior incurrent siphon anteriorly across the gills where cilia direct filtered products to the mouth while during pedal feeding particles could move from the foot, located ventrally, to the dorsally located mouth. Ultimately, the mouth is located closer to the foot than to the entry of the incurrent siphon. Sympatric mussels are capable of partitioning food resources, likely via differences in gill morphology (Galbraith et al., 2009). Furthermore, mussels in lentic environments may experience different feeding opportunities and exhibit different resource acquisition patterns as compared to mussels in lotic environments. Algae, organic matter, and nutrient fluctuation, particularly associated with resuspension events, are particularly instrumental in ecosystem dynamics in lentic systems (Bloesch 1995; Wetzel, 2001) and are likely important phenomena for freshwater mussels. Thus, evaluating the diets of multiple mussel species while considering a combination of potential food items could elucidate mechanisms driving rarity, allowing for sympatry, or other distributional phenomena.

It is difficult to evaluate the diets of freshwater mussels. Particles uptaken through filtration may be rejected as pseudofeces prior to ingestion, and particles ingested may pass through the digestive track without being assimilated (Vaughn et al., 2008; Haag, 2012). Carbon-13 and nitrogen-15 stable isotopes and stoichiometric ratios can provide useful insight into

aspects of primary food sources, trophic position, food quality, and stress of unionid consumers (Nichols & Garling, 2000; Raikow & Hamilton, 2001; Christian et al., 2004; Newton et al., 2013; Weber et al., 2017; Fogelman et al., 2022). Typically dietary constituents are inferred via carbon-13 (^{13}C) stable isotope signatures ($^{13}\text{C}/^{12}\text{C}$, or $\delta^{13}\text{C}$) and trophic position is inferred via nitrogen-15 (^{15}N) isotopes ($^{15}\text{N}/^{14}\text{N}$, or $\delta^{15}\text{N}$; DeNiro & Epstein, 1978, 1981).

In addition to elucidating trophic position, nitrogen-15 values can be used to detect food limitation or stress in consumers. Nitrogen-15 isotopic-enrichment above one trophic level (i.e., $>3.4\text{‰}$) can be caused by “self” isotopic discrimination due to catabolism of tissues that leads to metabolic retention of $\delta^{15}\text{N}$ in the organism (Cherel et al., 2005). This enrichment has been shown in unionids, oysters, and fish and is indicative of nutrient and environmental stress (Bowes et al., 2014; Patterson & Carmichael, 2018). There is also evidence that $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ in bivalves can change with shell size, indicating varying trophic niches or ontogenetic changes in food resources at different size/age ranges (Tai Tue et al., 2012; Yasuno et al., 2014; Fogelman et al., 2022), but there is limited consensus on the mechanisms behind these relationships. Additionally, consumer and food source C:N ratios can provide insight on food quality and origins (Sterner & Elser, 2002; Cross et al., 2005; Trochine et al., 2019). Food resources with low C:N ratios are of higher quality than resources with high C:N ratios (Trochine et al., 2019) and elevated C:N ratios in consumers can be an indicator of environmental stress or bioenergetic limitation (Frost et al., 2004; Sterner & Elser, 2002). Food source stoichiometry can also indicate origins of dietary items as C:N ratios can differ between allochthonous and autochthonous origins and/or between benthic and suspended resource pools (Cross et al., 2005).

With 65% of North American unionid mussels facing imperilement (Haag & Williams, 2014), it is important to evaluate drivers of their decline. Freshwater systems are experiencing altered flow regimes, including an increase in the frequency and duration of droughts (IPCC, 2021). These low-flow and drought events can cause shifts in mussel community assemblages, driven by the mortality of specialist or drought intolerant species (Gagnon et al., 2004; Gough et al., 2012; DuBose et al., 2019). When mussels experience drought conditions, they follow one of three

strategies to cope with emersion: drought intolerant species track receding water, semi-tolerant species track the water and then burrow, and tolerant species burrow (Gough et al., 2012). A behavioral mechanism unionids exhibit during stress events, including emersion, is increased valve activity (Hasler et al., 2017; Curley et al., 2021). Increased frequency and duration of valve closures has increased energetic costs for bivalves as it limits their ability to uptake oxygen and reduces feeding rates (Schick et al., 1986; Robson et al., 2010). To further understand the effects of low-flow and drought events on unionid mussels, it is important to consider the physiological effects of emersion as it relates to reduced feeding and anaerobic respiration.

In this study, we compare the extent to which adults of three mussel species assimilate suspended versus benthic food resources in a lentic system, and whether usage patterns vary across species and sizes. Our specific objectives were to: (1) determine whether primary diet components vary across species, (2) compare the relative importance of benthic and suspended carbon-13 sources to gain insight into feeding mechanisms (deposit versus suspension feeding), (3) evaluate relationships between isotopic signatures and body sizes, and (4) compare isotopic signatures between recently-immersed (≤ 48 h) and emersed (8 weeks) individuals.

Methods

Study site and species

Gantt Lake is a reservoir impounded in 1922 on the Conecuh River in Southeast Alabama, USA (Smillie, 1927). Gantt Lake has a surface area of 11.12 km² and the substrate associated with mussel habitat is a mixture of sand and clay and organic detritus (Cook & Moss, 2007; Miller et al., 2021). The mean depth of the lake is 1.4 m with a maximum depth of 10.7 m, a storage capacity of 0.018 km³ and a hydraulic retention time of 7 days (US EPA, 1976; Cook & Moss, 2007). Trophic state of the lake fluctuates from oligotrophic to eutrophic throughout the year, but generally it can be considered a mesotrophic system with an average Secchi depth of approximately 1 m (US EPA, 1976; ADEM, 2017). Growing season suspended chlorophyll *a* ranges from 4.3 to 8.2 $\mu\text{g/l}$ (ADEM,

2017). The lake weakly stratifies in the late spring but is largely isothermal through the summer and fall seasons (ADEM, 2017).

Three mussel species were sampled from Gantt Lake for isotope analysis during a planned drawdown for maintenance on the reservoir's hydroelectric dam. *Fusconaia escambia* Clench & Turner, 1956 is a Federally Threatened species (USFWS, 2010) restricted to the Conecuh/Escambia and Yellow River drainages in Alabama and Florida. *Utterbackiana hartfieldorum* (Williams et al., 2009) is a species with a similar distribution in the southeastern United States, including Mississippi, Alabama, Florida, and Georgia. In Alabama, *U. hartfieldorum* is listed as Critically Imperiled but has an Apparently Secure national status (NatureServe, 2021). *Elliptio pullata* (Lea, 1856) is a common species found in the southeast United States, including Alabama, Florida, and Georgia.

The drawdown of Gantt Reservoir began on September 20, 2019, and took 16-days to reach full drawdown. This occurred at a rate of about 0.15 m per day during the first 2 days, then increased to 0.35 m per day for 9 days, and 0.7 m per day for the next 4 days, and 0.91 m per day on the last day. Full drawdown took the reservoir to the original river elevation (pre-impoundment) or 8.53 m below normal water level within the reservoir (i.e., river level). The reservoir was refilled to normal pool level on December 23, 2019, with the drawdown lasting approximately 13 weeks. The reservoir elevation remained at river level for about 10 weeks; reservoir refill lasted approximately 17 days.

Mussel sampling

All three species were sampled from Gantt Lake in September 2019 during the planned drawdown event. Twelve individuals per species were collected from exposed substrates within 1–2 day of emersion at approximately 1–2 m pre-drawdown depth (Miller et al., 2019). Length (posterior to anterior margin of shell; mm) was measured for each individual. To obtain tissues for stable isotope analyses, mussels were opened using a pair of flat-tipped, reverse-action pliers and one to two sublethal tissue samples were taken from the foot tissue (nasal biopsy tool #453,733, Karl Storz, Tuttlingen, Germany). Foot tissue was sampled as it has the largest tissue availability to sample sublethally, and it has a long-term

tissue turnover rate (~1 year, Raikow & Hamilton, 2001). To avoid tissue degradation, all mussel samples were stored on ice immediately after collection (Perkins et al., 2018). *Elliptio pullata* and *U. hartfordorum* samples were transported to the laboratory on ice then frozen at $-20\text{ }^{\circ}\text{C}$ before being preserved and shells were deposited in the Troy University Zoological Museum. *Fusconaia escambia* were allowed to recover on site and then translocated as a condition of a Biological Opinion (BO# 2016-F-0576) between the Federal Energy Regulatory Commission, Power-South Energy Cooperative (federal licensee) and U.S. Fish and Wildlife Service (USFWS).

Tissue samples were dried at $80\text{ }^{\circ}\text{C}$ to a constant mass, ground using a mortar and pestle, weighed (nearest 10^{-5} g) and placed in a 4 mm \times 5 mm tin capsule (Costech Analytical, Valencia, CA, USA). In November 2019, 12 additional live *Elliptio pullata* were recovered (Miller et al., 2021) that had been emersed but partially buried in exposed lakebed substrates for the previous 8 weeks since the initial Gantt Lake drawdown event. All 8 week-emersed *E. pullata* were processed for stable isotopes in the same manner as initial, recently-immersed mussels. For the purposes of this study, we refer to the mussels initially collected within 48 h of stranding as “recently-immersed” as $\leq 48\text{ h}$ is likely not enough time to substantially change isotopic signatures from the immersed state (Hawkins, 1985; Raikow & Hamilton, 2001; Gustafson et al., 2007). We refer to those collected 8 weeks after stranding as “emersed.”

Potential food resource sampling

Hypothesized food sources [suspended particulate organic matter (SPOM), fine particulate organic matter (FPOM), and coarse particulate organic matter (CPOM; benthic detritus)] were sampled 1 week prior to the beginning of lake drawdown and sampling of mussels. Considering the lake is generally isothermal during this time of year (ADEM, 2017), and the fact there were no major weather events during this timeframe, we made the assumption that SPOM, FPOM, and CPOM did not appreciably change over this period of time. Water samples for SPOM were collected from the limnetic and littoral zones one week before the drawdown. Samples taken from the littoral zone were collected $< 15\text{ m}$ from shore and samples taken from the limnetic

zone were taken nearer the channel approximately 80 m from shore. Seven to eight 1 l water column samples were collected for littoral samples ($< 1\text{ m}$ subsurface). Seven to eight limnetic samples (approximately 9 m depth) were collected with a 2.2 l Van Dorn horizontal water sampler (Wildco® Alpha). All water samples were pre-filtered onsite through 55 μm mesh to remove larger particles (Vaughn et al., 2008). Prefiltered water was then transported back to the laboratory on ice and stored at $-80\text{ }^{\circ}\text{C}$. Samples were then thawed and vacuum-filtered through a pre-combusted ($450\text{ }^{\circ}\text{C}$ for 4 h) 47 mm Whatman® glass fiber (GF/F) filter (nominal pore size = $0.7\text{ }\mu\text{m}$) to isolate suspended solids between 0.7 and 55 μm , reflecting the size fraction typically consumed by mussels (Post, 2002; Strayer, 2008; Vaughn et al., 2008). Filters were dried at $80\text{ }^{\circ}\text{C}$ to a constant mass and fumigated in 3 N H_3PO_4 for 8 h to remove carbonates (Harris et al., 2001).

Five, shallow, surface-sediment samples were collected from the littoral zone ($\leq 15\text{ m}$ from shore, $< 1\text{ m}$ subsurface) for littoral FPOM (detritus, algae, bacteria, and fungi mixed with sand) using a turkey baster ($\sim 25\text{ ml/sample}$) and five deep surface-sediment samples were collected for limnetic FPOM at approximately 10 m using a 3.5 l Ekman grab sampler (Wildco®). Samples were pre-filtered (55 μm) and vacuum filtered (0.7 μm) in the same manner as the water samples. After vacuum filtration, FPOM samples were dried to a constant mass at $80\text{ }^{\circ}\text{C}$. The dried sediment was then removed from the filter, fumigated in 3 N H_3PO_4 for 8 h to remove carbonates (Harris et al., 2001), ground to a fine powder using a mortar and pestle, weighed (nearest 10^{-5} g), and encapsulated in 4 mm \times 6 mm tin capsules.

Approximately 85 g of submerged CPOM (mixed leaf packs and amorphous organic detritus) was collected from the littoral zone and transported in the same manner as SPOM and FPOM samples. CPOM was then fumigated, ground, and encapsulated in same manner as the FPOM samples. All prepared isotopic samples of mussel tissues and food resources were shipped to Washington State University Stable Isotope Core Laboratory for $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ analysis.

Isotope ratios are reported in parts per thousand (‰) relative to standards [Vienna Pee Dee Belemnite (VPDB) for carbon and atmospheric N for nitrogen], defined in delta notation as:

$$\delta^{13}\text{C} \text{ or } \delta^{15}\text{N} = (R_{\text{sample}}/R_{\text{standard}} - 1) \times 10^3$$

where $R = {}^{13}\text{C} / {}^{12}\text{C}$ or ${}^{15}\text{N} / {}^{14}\text{N}$, respectively (DeNiro & Epstein, 1978, 1981).

Statistical analyses

To account for variation in lipid content between consumers and food resources, all $\delta^{13}\text{C}$ values were mathematically-adjusted using the linear relationships between $\delta^{13}\text{C}$ and C:N for mussels or % carbon for food resources (Post et al., 2007). All mussel $\delta^{13}\text{C}$ values were adjusted for lipid influence using C:N ratios as follows:

$$\delta^{13}\text{C}_{\text{adjusted}} = \delta^{13}\text{C}_{\text{untreated}} - 3.32 + 0.99 \times \text{C} : \text{N}$$

All food resource $\delta^{13}\text{C}$ values were corrected for lipid influence using % carbon as follows:

$$\delta^{13}\text{C}_{\text{adjusted}} = \delta^{13}\text{C}_{\text{untreated}} - 3.02 + 0.09 \times \% \text{ carbon}$$

Adjusted $\delta^{13}\text{C}$ values were used for all statistical analyses. Raw isotopic data are publicly available on Auburn University's scholarly repository, AUora, at <https://aurora.auburn.edu/> and directly from the corresponding author.

We used a combination of linear models and supportive Bayesian mixing models to address our objectives. First, to determine whether isotopic values or C:N ratios varied across species (objective 1) or across recently-immersed and 8 weeks-emersed individuals (objective 4) we used a one-way analysis of variance (ANOVA) to test the effects of species on mussel $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Second, to identify possible relationships between isotopic values and consumer size (objective 3), we used linear regression analysis. When data did not meet assumptions of variance or normality, rank transformations were performed on response variables and Kruskal–Wallis H-tests were reported in lieu of F-tests. When species effect was significant, a Dunn's test was conducted for the post hoc analysis. Statistical significance was set at $P < 0.05$. All analyses of variance tests were performed using SigmaPlot Version 13.0 (SigmaPlot, 2014).

To determine the relative contribution of each potential food source to mussel diets (objective 3), we used a Bayesian tracer mixing model framework (MixSIAR; R Core Team, 2020; Stock & Semmens,

2016). MixSIAR accounts for hierarchical structure associated with food chains, uncertainty in the consumer tissue mixture, and food source variability (Semmens et al., 2009; Stock et al., 2018). This analysis was used to determine whether primary diet components varied across species (objective 1) and to provide insight into dietary carbon-13 sources (objective 2). The MixSIAR model used two tracers ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$) and five food sources (CPOM, littoral FPOM, limnetic FPOM, littoral SPOM, and limnetic SPOM). To evaluate whether our isotope mixing model was valid, we used a Monte Carlo simulation of the resource mixing region to evaluate the probability that our consumers stable isotope contributions were satisfied by the resource polygon (Smith et al., 2013). The point-in-polygon method utilizes iterations to generate mixing polygons with the distribution of the dietary food sources using a user-chosen discrimination factor (Smith et al., 2013). The proportion of the polygon that has a solution is given as a frequentist probability that the proposed mixing model data can accurately calculate source contributions to explain a consumer's isotopic value, and a 95% mixing region is used as a basis for consumer exclusion (Smith et al., 2013). Carbon discrimination used in the mixing model was $+0.4 \text{‰} \pm 1.3 \text{ SD}$ and N was $+3.4 \text{‰} \pm 1 \text{ SD}$ (Post, 2002). After using the point-in-polygon method to evaluate the validity of our mixing model, we excluded consumers that fell outside of the 95% mixing region (i.e., had $< 5\%$ chance of having their isotopic value explained by the source contributions in the MixSIAR analysis (Fig. 1; Smith et al., 2013).

Two models, a null model and a model using species as a variable, were initially tested using an uninformative prior and compared using deviance information criterion (DIC) scores (Table 1; Phillips et al., 2014; Stock & Semmens, 2016; Stock et al., 2018). Model convergence comparisons were made by assessing Gelman-Rubin diagnostics and Geweke diagnostics (Phillips et al., 2014; Stock & Semmens, 2016; Stock et al., 2018). Two priors for food source contribution were compared for use in the model: an uninformative prior and a literature-based prior attributing 41% contributions from both limnetic and littoral SPOM, 8% from both limnetic and littoral FPOM, and 51% from CPOM (Table 1; Fogelman et al., 2022). We used an informative prior from a lotic system because existing stable isotope studies

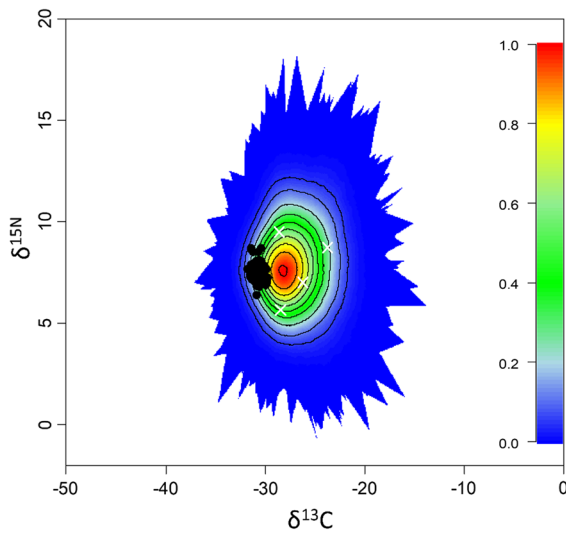


Fig. 1 Simulated mixing regions for *Elliptio pullata*, *Utterbackiana hartfieldorum*, and *Fusconaia escambia* (black dots) and the average source values (white crosses). Consumers within 95% of the mixing region (outermost, dark blue, contour) are included, and consumers with <5% probability of satisfying the mixing model were excluded. Consumers outside the 95% mixing region ($n=0$) are not used in the MixSIAR model as they need an alternative model to explain their isotopic composition, although all in the current study fit the model with >20% probability. Contours represent probabilities within the mixing region at the 5% outermost contour and every 10% interval

identifying benthic food sources as significant contributors to the mussel diet have been conducted in lotic, rather than lentic, systems (Raikow & Hamilton, 2001; Weber et al., 2017; Fogelman et al., 2022).

Results

Mussel isotopic composition and carbon:nitrogen ratios

There were no significant differences across recently-immersed species in $\delta^{15}\text{N}$ values, but there were significant differences in $\delta^{13}\text{C}$ and C:N ratios between *F. escambia* and recently-immersed *E. pullata*. *Fusconaia escambia* was more depleted in ^{13}C than recently-immersed *E. pullata* and *F. escambia* exhibited a significantly lower C:N ratio relative to recently-immersed *E. pullata*. *Utterbackiana hartfieldorum* exhibited intermediate $\delta^{13}\text{C}$ and C:N ratios and was not significantly different from either of the other species (Table 2). Eight week emerged *E. pullata* were enriched in ^{15}N relative to recently-immersed *E. pullata* but did not differ in $\delta^{15}\text{N}$ from the other two recently-immersed species (Table 2).

Recently immersed *E. pullata* ranged in length from 54.78 to 88.66 mm ($n=15$) and 8-week emerged individuals sampled ranged in length from 56.50 to 92.50 mm ($n=12$). The length of *U. hartfieldorum* sampled ranged from 50.94 to 95.09 mm ($n=12$) and *F. escambia* length ranged from 45.34 to 79.43 ($n=13$). Shell length had a significant negative relationship with $\delta^{13}\text{C}$ ($F_{1,10}=70.65$, $P<0.001$, $R^2=0.783$) and a significant positive relationship with $\delta^{15}\text{N}$ ($F_{1,10}=24.76$, $P<0.001$, $R^2=0.683$) for *U. hartfieldorum* (Fig. 2). There was no relationship between shell length and isotopic values for any other species.

Table 1 MixSIAR model parameter estimates used for model selection criteria

Model ID	Model	N	Gelman rubin diagnostic		Geweke diagnostic		DIC score
			<i>n</i>	%	<i>n</i>	%	
1	Null Model, Uninformative Prior	60	58	97	3	5.00	5.55
2	Species, Uninformative Prior	96	56	58	3	3.13	39
3	Species, Informative Prior	96	96	100	3.33	3.47	− 6.74*

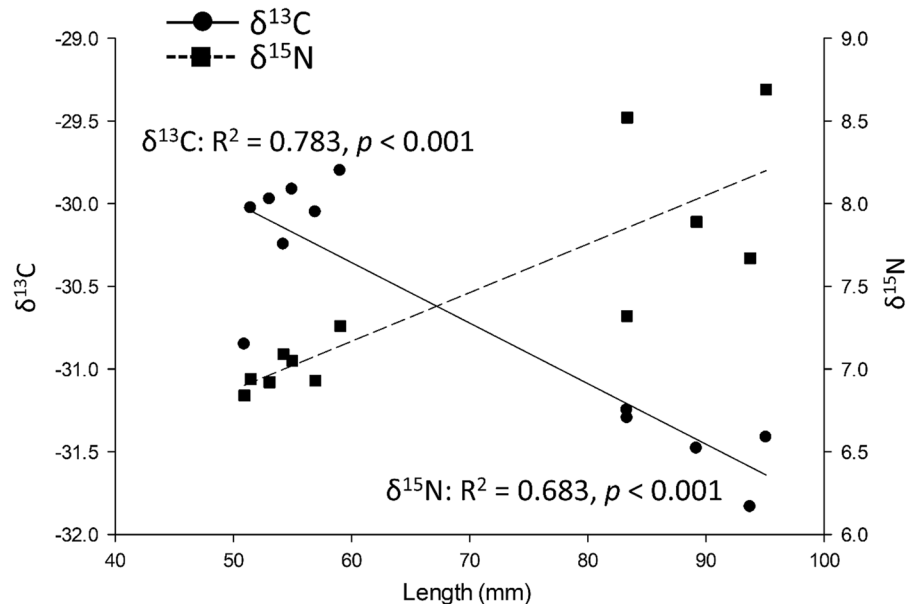
Optimum Gelman-Rubin diagnostics have the greatest proportion of variables below the R cutoff < 1.05 (~100%) and optimum Geweke diagnostics should have all parameters < $5\% \pm 1.96$. For model criteria, N indicates the number of model variables and *n* indicates the number of model parameters below the Gelman-Rubin diagnostic of 1.05. Gelman-Rubin and Geweke diagnostics indicate whether a model has converged. Lowest deviance information criterion (DIC) score indicates the best fitting model. The best DIC score, and thus best-fitting model used in this study, is denoted with an*

Table 2 One-way analysis of variance (ANOVA) results for freshwater mussel $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N with species as an effect

Parameter	Mean ($\%$) \pm SE				Test statistic	P-value
	<i>U. hartfieldorum</i> (recently immersed) Mean \pm SE	<i>F. escambia</i> (recently immersed) Mean \pm SE/SESE	<i>E. pullata</i> (recently immersed) Mean \pm SE	<i>E. pullata</i> (8-week emersed) Mean \pm SE		
$\delta^{13}\text{C}$	$-30.68 \pm 0.22^{\text{ab}}$	$-31.07 \pm 0.12^{\text{a}}$	$-30.53 \pm 0.09^{\text{b}}$	$-30.54 \pm 0.10^{\text{ab}}$	$H_3 = 8.63$	0.035
$\delta^{15}\text{N}$	$+7.43 \pm 0.18^{\text{ab}}$	$+7.80 \pm 0.16^{\text{ab}}$	$+7.33 \pm 0.10^{\text{b}}$	$+7.80 \pm 0.08^{\text{a}}$	$H_3 = 11.24$	0.01
C:N	$+3.94 \pm 0.04^{\text{ab}}$	$+3.87 \pm 0.04^{\text{b}}$	$+4.33 \pm 0.11^{\text{a}}$	$+4.04 \pm 0.06^{\text{ab}}$	$H_3 = 15.64$	0.001

Mean estimates and standard error (SE) are presented for species effect analyzed for $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, and C:N. Statistical differences in mean $\delta^{13}\text{C}$, $\delta^{15}\text{N}$ or C:N ratio are denoted with different letters (Dunn's Test) at $P < 0.05$ if (bold)

Fig. 2 Scatter plot of the relationship between shell length (mm) and $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ isotopic values in *Utterbackiana hartfieldorum*



Food source contributions

The best fitting model analyzed species as a fixed effect with a literature-based informative prior. This informative prior had a lower DIC score than the null model or a model using an uninformative prior (Table 1). Based on results of the point-in-polygon analysis, no consumer values were removed for any of the species (Fig. 1). For all species, limnetic FPOM was the dominant carbon-13 source with littoral FPOM, limnetic and littoral SPOM, and CPOM comprising relatively minor contributions. FPOM was enriched in ^{13}C and depleted in ^{15}N compared to all mussel species and exhibited approximately one trophic level of isotopic discrimination from consumers (Fig. 3). Limnetic FPOM dietary contributions to

recently-immersed *Elliptio pullata* were $98.7\% \pm 1.8$ SD. For 8-w emersed *E. pullata* limnetic FPOM contributions were $99.4\% \pm 0.7$ SD. Limnetic FPOM contributions for *U. hartfieldorum* and *F. escambia* were 99.0 ± 1.3 SD and 99.1 ± 1.3 SD, respectively. For all species, whether recently-immersed or 8-weeks emersed, littoral FPOM, limnetic and littoral SPOM, and CPOM contributions were $< 1\%$ (Table 3).

Discussion

We quantified food resources in three mussel species via stable isotopes in Gantt Lake, Alabama and found that mussels primarily relied on benthic, as opposed to suspended, organic food resources. The primary

Fig. 3 ^{13}C and $\delta^{15}\text{N}$ isotope biplots for immersed and emersed *Elliptio pullata*, *Utterbackiana hartfieldorum*, and *Fusconaia escambia* and food sources [coarse particulate organic matter from benthic detritus (CPOM), fine particulate organic matter from benthic sediments (FPOM), suspended particulate organic matter (SPOM)]. Each point is an average and error bars represent the associated standard deviation

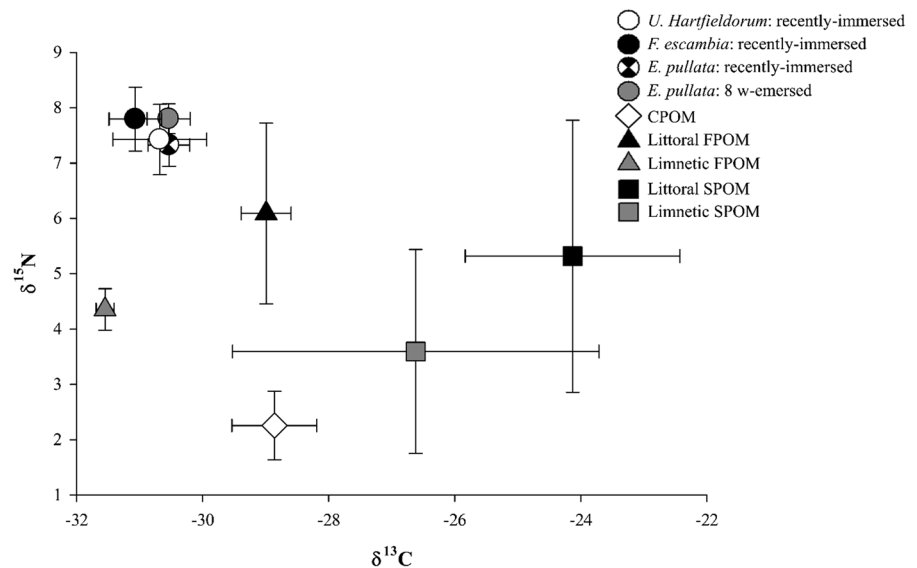


Table 3 Mean estimated dietary source contributions (%) for recently-immersed *Utterbackiana hartfieldorum*, *Fusconaia escambia*, *Elliptio pullata*, and 8-week emersed *E. pullata* with standard deviation (SD)

Food source	<i>U. hartfieldorum</i> (recently immersed) Mean \pm SD	<i>F. escambia</i> (recently immersed) Mean \pm SD	<i>E. pullata</i> (recently immersed) Mean \pm SD	<i>E. pullata</i> (8-week emersed) Mean \pm SD
CPOM	0.7 \pm 1.2	0.7 \pm 1.2	0.8 \pm 1.4	0.4 \pm 0.6
Littoral FPOM	0.1 \pm 0.3	0 \pm 0.2	0.1 \pm 0.7	0 \pm 0.2
Limnetic FPOM	99.0 \pm 1.3	99.1 \pm 1.3	98.7 \pm 1.8	99.4 \pm 0.7
Littoral SPOM	0.1 \pm 0.2	0.1 \pm 0.2	0.1 \pm 0.4	0.1 \pm 0.1
Limnetic SPOM	0.2 \pm 0.4	0.1 \pm 0.3	0.2 \pm 0.6	0.1 \pm 0.3

This table reflects mean estimate values of potential $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ contributions to mussel tissue composition determined using Bayesian mixing models in MixSIAR

carbon-13 source for *F. escambia*, *U. hartfieldorum*, and *E. pullata* was consistently dominated by FPOM associated with limnetic benthic (i.e., profundal) sediments. Littoral fine particulate organic matter, coarse particulate organic matter, and suspended particulate organic material contributed less than 1% to dietary carbon-13. This suggests that all three species had access to organic material associated with littoral benthic sediments.

Despite mussels being predominately classified as filter feeders, many recent studies using stable isotopes have shown that mussels can exploit benthic materials as food sources (Nichols & Garling, 2000; Raikow & Hamilton, 2001; Christian et al., 2004; Nichols et al., 2005; Weber et al., 2017; Fogelman et al., 2022). Detrital material can serve as the primary food source in smaller lotic systems (Fogelman

et al., 2022) while larger (especially lentic) systems may provide adequate suspended food resources in addition to detrital sources. Mussels are capable of digesting detrital material (Christian et al., 2004), and allochthonous material has been shown to contribute 1/3–1/2 of mussel biomass and dietary carbon-13 in riverine systems (Weber et al., 2017; Fogelman et al., 2022). Freshwater sediments can trap and store organic and inorganic particles that can form the base of aquatic food webs (Krumins et al., 2013) and there is a positive relationship between mussel growth rate and sediment organic content (Cyr, 2020). The isotopic values of mussel species in this study overlapped with organic particles associated with limnetic benthic FPOM as the dominant food source. This pattern is consistent with prior stable isotope studies on unionids where sympatric individuals have similar

isotopic values irrespective of taxonomy (Weber et al., 2017; Fogelman et al., 2022). This broad dietary overlap could be attributed to the effects of site-specific drivers (e.g., nutrient cycling, dominant carbon sources) on feeding (Peipoch et al., 2012).

Freshwater mussels can deposit-feed to access benthic food resources such as FPOM and CPOM, and it has been suggested that they preferentially assimilate the microbial communities associated with benthic organic matter (Nichols and Garling, 2000; Christian et al., 2004; Nichols et al., 2005). In aquatic systems, “green” pathways have carbon entering the system as algae living on the sediment surface and, “brown” pathways have the majority of carbon entering the system through decomposition of organic materials (e.g., bacteria; Krumins et al., 2013). In shallow freshwater lakes there is a direct coupling between sediment food webs and primary producers such as benthic algae (Krumins et al., 2013). Mussels in Gantt Lake may be relying on both “green” and “brown” pathways when using FPOM as a primary food source. The relative contribution of algae or bacteria from FPOM was not quantified in this study and warrants further investigation. Additionally, food resource sampling in this study was constrained by the timing of lake drawdown, thus we did not capture potential temporal variability in “green” and “brown” pathways that may be contributing to the mussel diet. Mussel, CPOM, and FPOM exhibited little variation in isotopic signatures among replicate samples, and may be expected to show minimal seasonal variation as the tissue turnover rate in mussel foot tissue is approximately 1 year (Raikow & Hamilton, 2001). When mussel foot tissue has been examined seasonally its isotopic values have shown minimal temporal variation (Fogelman et al., 2022). However, there was notably higher variation in SPOM signatures than in mussel foot tissue signatures in this study, which would be expected to fluctuate seasonally and thus could be contributing a higher proportion to mussel diet than we report here.

It was surprising that the dominant food source of these mussels was benthic organic matter sampled from the limnetic zone, while most individuals inhabited littoral zones. While mussels were not collected where the predominate food source was sampled, the isotopic composition of this deepwater benthic sediment most closely represents the isotopic composition of the mussel tissue. Although

not directly quantified, limnetic sediments were gray to black in color and uniformly fine in texture (i.e., hypoxic/anoxic “muck”), whereas littoral sediments were brown in color and positioned within a sand matrix. It is likely that accumulated benthic organic matter in the littoral sediments is comprised of material associated with vegetation, algae, and associated consumers, whereas limnetic sediments are comprised of previously suspended lentic algal biomass and transported lotic organic matter that has settled. Littoral and limnetic samples came from representative sites and not across the entire lake, so the true spatial extent of limnetic vs. littoral sediment “types” is not fully known. Limnetic sediments can be found closer to shore than where we sampled FPOM (~ 10 m depth) and these sediments border the fringes of general mussel occurrences throughout the lake environment. Thus it is possible some individual mussels, particularly in deep habitats, have direct benthic access to this putative food source.

Deep-water sediments can routinely become resuspended (Evans, 1994; Bloesch, 1995), and such episodic events can have strong effects on subsequent ephemeral algal proliferation and overall ecological conditions (Dzialowski et al., 2008). The mechanisms for sediment suspension are numerous, including wind and rain from periodic events, recreational use, seasonal fluctuations in river discharge, seasonal lake turnover and drawdown (Anthony and Downing, 2003; Schallenberg and Burns, 2004; Dzialowski et al., 2008; Effler and Matthews, 2004). All of these mechanisms are potentially exacerbated by the daily water level fluctuations associated with hydroelectric power generation. Gantt Lake is situated in an environment that experiences frequent summer storms (Chagnon, 2001) and is a popular recreational boating area. Conecuh River discharge upstream of Gantt Lake can fluctuate from over 85 m³/s to less than 2.8 m³/s over a year and the lake weakly stratifies but is isothermal for much of the year, facilitating mixing (ADEM, 2017; USGS, 2022). Such resuspension events may play a pivotal role in providing littoral mussels access to limnetic food resources, either through filtration of resuspended materials or pedal feeding of settled material. Although the precise mechanisms remain unclear, episodic resuspension events may provide deep sediment organic matter availability (or the nutrients thereof) to littoral

mussels and be particularly important in their growth and development.

Eight-week emerged *E. pullata* had a significantly higher nitrogen-15 value than *E. pullata* individuals that were collected immediately after drawdown. The enrichment of $\delta^{15}\text{N}$ of emerged versus recently-immersed *E. pullata* could be caused by nutritional and environmental stress. “Self” isotopic discrimination can occur in consumers due to self-catabolism of tissues and subsequent metabolic retention of $\delta^{15}\text{N}$ (Cherel & Hobson, 2007; Bowes et al., 2014). Because consumers are of a higher trophic level than their basal food resources, self catabolizing and using their own tissue stores results in an increased trophic signature. Such a physiological process has been used to explain high trophic discrimination factors in bivalves (Patterson & Carmichael, 2018; Fogelman et al., 2022). It is likely that during 8 weeks of emersion, *E. pullata* was experiencing significant nutritional and environmental stress and utilizing its own tissue stores for nutrition due either to the absence of an ability to deposit or suspension feed or risks associated with this behaviour in dessicated environments.

Increased catabolism can be evidenced in both increased nitrogen-15 values and elemental C:N ratios, however the hypothesis of increased catabolism by emerged mussels was not supported by C:N ratios. Increased C:N ratios are indicative of increased N excretion and depletion of energy stores (Sterner & Elser, 2002), but C:N ratios of emerged *E. pullata* were not significantly different than those of recently-immersed *E. pullata* in our study, even after 8 weeks out of water and presumably no access to external food sources. This suggests that ^{15}N -enrichment is a more sensitive indicator of catabolism in stranded mussels than C:N ratios, and/or that they recycle needed nutrients when stranded and require only a small amount of internal energy stores for basic maintenance. An alternative to self-catabolism is that the change in nitrogen-15 isotopic values were a result of pedal feeding on another fraction of sediment organic matter while emerged (Brendelberger & Klauke, 2009) but this would increase the risk of dessication as it would require the valves to be open. While it may be possible that mussels were pedal feeding while emerged, the mechanisms of food uptake are not understood as pedal feeding is generally known for juveniles and it is not known if pedal feeding can occur out of water.

We detected a negative relationship between shell size and ^{13}C -depletion and a positive relationship between shell size and ^{15}N -enrichment for *U. hartfieldorum*, but not the other species sampled in this study. Previous studies examining relationships between unionid shell size and isotopic values are rare, but have generally shown both ^{13}C - and ^{15}N -enrichment with increasing shell length. The reason for this discrepancy among studies is unknown but it suggests that in some systems such as Gantt Lake, *U. hartfieldorum* may preferentially assimilate more ^{13}C -depleted and ^{15}N -enriched fractions of organic pools in benthic sediments as they grow. *Utterbackiana hartfieldorum* sampled in this study fell into two size/age classes (50–60 mm and 80–100 mm) although age was not determined in this study. The pattern in length versus isotopic enrichment/depletion may be due to diet shifts associated with observed ontogenetic niche shifts in habitat use between young (shallow habitats) and older (deep habitats) individuals in Gantt and Point A Lakes (Miller, pers. obs.). Further research is necessary to discern the physiological mechanisms and relationships behind isotopic incorporation, preferential food particle selection, and variation among mussel size/age.

A central question when developing conservation plans for mussels is whether all species in a community have similar environmental needs or whether they need to be managed differently. In this study, the answer was unequivocal with regard to food resources. All species showed evidence of using FPOM as a major food resource—indicating that management of benthic food resource quality would benefit multiple species. Specifically, control of sediment transport from upstream sources would be beneficial, as low-quality transported inorganic sediment could potentially smother high quality organic sediments. It would also be beneficial to evaluate how mussels are accessing limnetic FPOM in Gantt Lake to ensure that this pathway remains unaltered in the system, especially since the largest known population of *F. escambia* exists here. Determining whether dam withdrawals, in tandem with rain events, play a role in resuspension of FPOM for mussel availability would allow for implementation of best management practices associated with mussel dependence.

These findings suggest that food resources are not limiting and are able to support multiple species

as we have no evidence of food source partitioning across sympatric species in this system. Additionally, we found evidence that *E. pullata* were undergoing catabolism during 8 weeks of emersion. Further investigations into the relationships between energy stores and survival of stranded mussels may be extremely useful for our understanding of how mussel survival is affected by dewatering events as such extremes are becoming more common in natural environments with climate change. As freshwater mussel populations continue to decline in North America (Haag, 2014), it is critical to understand their feeding ecology and the relative importance and quality of the benthos as a provider of food resources. Continued investigation of nutritional pathways to mussels across a range of systems and environmental conditions will allow for more effective management and conservation efforts for these imperiled species.

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Author contributions KF: analysis, data curation, writing (original), writing (review and editing). JS: sample collection, writing (original), writing (review and editing), supervision. JM: conceptualization, methodology, data and sample collection, writing (review and editing). BH: conceptualization, methodology, data and sample collection, analysis, data curation, writing (original), writing (review and editing), supervision.

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Data availability The data that support the findings of this study are available from the corresponding author, K. Fogelman, upon reasonable request.

Declarations

Conflict of interest The authors declare that there are no competing interest.

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