



ARTICLE

Freshwater Ecology

Dietary composition and fatty acid content of giant salmonflies (*Pteronarcys californica*) in two Rocky Mountain rivers

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Abstract

Many aquatic invertebrates are declining or facing extinction from stressors that compromise physiology, resource consumption, reproduction, and phenology. However, the influence of these common stressors specifically on consumer–resource interactions for aquatic invertebrate consumers is only beginning to be understood. We conducted a field study to investigate *Pteronarcys californica* (i.e., the “giant salmonfly”), a large-bodied insect that is ecologically and culturally significant to rivers throughout the western United States. We sampled gut contents and polyunsaturated fatty acid composition of salmonflies to compare resource consumption across river (Madison or Gallatin, Montana), sex (male or female), and habitat (rock or woody debris). We found that allochthonous detritus comprised the majority of salmonfly diets in the Gallatin and Madison Rivers, making up 68% of the gut contents on average, followed by amorphous detritus, diatoms, and filamentous algae. Diets showed little variation across river, sex, or length. Minor differences in diets were detected by habitat type, with a higher proportion of diatoms in the diets of salmonflies collected from rocky habitat compared to woody debris. Fatty acid composition generally supported the results of gut content analysis but highlighted the importance of primary producers. The presence of eicosapentaenoic acid (20:5n-3) and alpha linolenic acid (18:3n-3) indicated consumption of diatoms and filamentous green algae, respectively. Our research underscores the importance of a healthy riparian zone that provides allochthonous detritus for invertebrate nutrition as well as the role of algae as an important source of fatty acids.

KEYWORDS

aquatic-terrestrial linkages, cross-boundary subsidy, feeding, invertebrate, Plecoptera, stonefly

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INTRODUCTION

Freshwater ecosystems are experiencing high levels of biodiversity loss due to pervasive anthropogenic climate warming and land-use change (Dudgeon et al., 2006; Sala et al., 2000). Freshwater invertebrates, in particular, are susceptible to myriad environmental stressors (i.e., warming temperatures, sedimentation, nutrient pollution, and altered flow regimes), and many taxa are consequently facing extinction or reduced abundance (Collier et al., 2016). Invertebrate physiology, resource consumption, reproduction, and phenology all respond to environmental stressors (Shah et al., 2020), but relatively little is known about emerging responses of consumer–resource interactions to these stressors. Although patterns of resource preference and dietary analysis have been central components of freshwater conceptual frameworks, including the river continuum concept, ecological stoichiometry, and food web analysis (Benke & Wallace, 1980; Cross et al., 2005; Rosi-Marshall, Vallis, et al., 2016; Vannote et al., 1980), limited information regarding diet composition of key taxa has made specific conservation actions that target invertebrates and the food resources that they require under global change challenging (Amarasekare, 2015; Cross et al., 2011).

Pteronarcys californica (i.e., the “giant salmonfly”) is a large-bodied insect that is ecologically and culturally significant to rivers throughout the western United States. Their large body size makes them an important prey item for aquatic consumers, including trout, and their highly synchronous emergence provides an important seasonal food resource for terrestrial consumers, including birds and spiders (Rockwell & Newell, 2009; Walters et al., 2018). Additionally, recreational fishing during salmonfly emergence is an economic boost for local communities. However, salmonflies are declining in abundance in many western rivers and their range is contracting due to rising water temperatures and sedimentation (Anderson et al., 2019; Birrell et al., 2019; Kowalski & Richer, 2020). Salmonflies are already extirpated from certain river systems in Montana, Utah, and Colorado (Birrell et al., 2019; Elder & Gaufin, 1973; Stagliano, 2010). Efforts to conserve and restore populations of salmonflies in western rivers will require comprehensive knowledge of habitat requirements, life history patterns, and feeding preferences.

Understanding salmonfly diets may have implications for restoration and conservation efforts targeting these insects. Salmonflies are often classified as shredders and presumed to consume allochthonous detritus. However, the few studies that have quantified salmonfly diets by gut content analysis indicate substantial variation in allochthony by location and season, with sometimes heavy reliance on autochthonous production (Blackadar et al., 2020; Rosi-Marshall, Vallis, et al., 2016). In addition

to traditional methods of gut content analysis, fatty acid (polyunsaturated fatty acid [PUFA]) composition of macroinvertebrate tissues can provide information on origins and quality of macroinvertebrate diets (Lau et al., 2009; Torres-Ruiz et al., 2007). Omega-3 long-chain polyunsaturated fatty acids (n-3 LCPUFAs) are a critical diet component for growth and reproduction in both aquatic and terrestrial consumers (Olsen, 1999; Twining et al., 2016). Eicosapentaenoic acid (EPA; 20:5n-3), an n-3 LCPUFA, is found in aquatic primary producers, but not terrestrial primary producers, whereas its n-3 PUFA precursor alpha linolenic acid (ALA; 18:3n-3) is found in both aquatic and terrestrial primary producers (Hixson et al., 2015; Twining et al., 2016). Aquatic invertebrates can accumulate EPA through consumption of diatoms whereas dietary sources of other PUFAs like ALA include filamentous algae, terrestrial detritus, and bryophytes (Guo et al., 2016; Torres-Ruiz et al., 2007; Twining et al., 2017; Whorley et al., 2019). Investigating the fatty acid composition of salmonflies can thus provide additional insight into their feeding ecology as well as their importance as a prey item for terrestrial consumers that gain n-3 LCPUFAs through consumption of adult aquatic insects (e.g., Twining et al., 2018, 2019).

Our objective was to quantify the diets and whole-body PUFA composition of giant salmonflies, which are ecologically, culturally, and economically important to rivers in the western United States. We used gut content analysis and fatty acid composition to elucidate dietary composition and to compare different rivers, habitats, and sexes. We hypothesized that different rivers (Gallatin or Madison) and habitats (rocky or woody) might supply different food resources because of variation in watershed size, land cover, damming status, and resource material itself (Wellard Kelly et al., 2013). We also hypothesized that diet might be different between the sexes because females consistently reach larger body sizes, perhaps due to differences in either resource amount or type consumed (Anderson et al., 2019), and females may have different diet requirements to support reproductive tissues. Understanding feeding habits of salmonflies could have important implications for the conservation of this ecologically significant insect and help guide future river restoration efforts.

METHODS

Study area

This study was conducted in the Madison and Gallatin Rivers, located in Southwest Montana (Figure 1). The Madison and Gallatin Rivers originate in Yellowstone

National Park, Wyoming, and flow north for 295 and 193 river kilometers, respectively. Both rivers join the Jefferson River near Three Forks, Montana, to form the Missouri River.

Study sites on the Madison River were located between Hebgen and Ennis Reservoirs, which were created by dams constructed in 1914 and 1906, respectively. In this area, the Madison River flows through a broad valley, with riparian vegetation dominated by willow and grasses. The Madison watershed (6620 km²) is 39% woodlands and 48% grass and shrublands (Anderson et al., 2019). Near our study sites, mean July water temperature and discharge were 14.7°C and 31.99 m³/s, respectively, in 2020 (USGS gage 06038800). Because the Gallatin River does not have a present-day USGS water temperature sensor, we evaluated historical temperature at the same location as the USGS gage from NorWeST (Isaak et al., 2017) to compare water temperature between rivers. NorWeST reported mean August water temperature of 16.4°C from 1993 to 2011.

Study sites on the Gallatin River were located between the towns of Big Sky and Gallatin Gateway. The Gallatin River in the study area flows through both valley and canyon topography. The Gallatin River is free flowing, with riparian vegetation dominated by willow and conifer forest. The Gallatin watershed (4784 km²) is 70% woodlands and 18% grass and shrublands (Anderson

et al., 2019). Mean July discharge was 27.6 m³/s in 2020 (USGS gage 06043120). Because the Gallatin River does not have a present-day USGS water temperature sensor, we collected historical temperature from NorWeST (Isaak et al., 2017) at the same location as the USGS gage. NorWeST reported mean August water temperature of 10.7°C from 1993 to 2011.

Salmonfly collection

Salmonflies were collected from three sites each on the Madison and Gallatin Rivers on 9–10 July 2020. Sites were chosen to span the length of each river known to support relatively dense salmonfly populations and were spaced at minimum approximately 12.5 river kilometers apart. We collected salmonflies in the evening between the hours of 5:00 PM and 9:00 PM to increase the likelihood of sampling a full gut and to maintain a consistent sampling window across all sites. At each site, we collected three male and three female late instars from two habitat substrate types: rock surfaces and woody debris ($n = 12$ individuals per site; 36 per river). Individuals were collected directly from substrates using forceps. Salmonflies were immediately preserved in Kahle's solution in individual vials and transported to the laboratory.

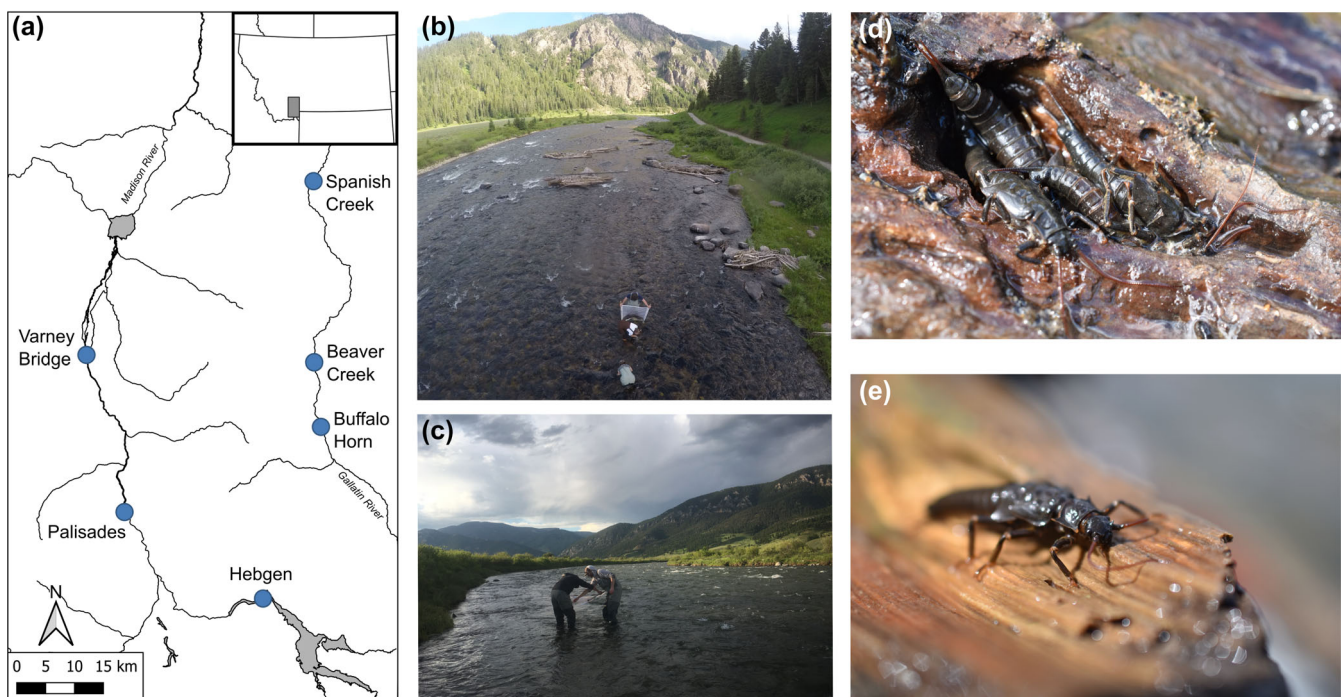


FIGURE 1 (a) Sampling locations on the Madison and Gallatin Rivers in Southwest Montana (inset); collecting salmonflies on the (b) Madison River and (c) Gallatin River; (d, e) larval salmonflies have been observed to congregate on woody debris. Salmonfly photo credits: M. MacDonald; location: Madison River, Montana

Gut content analysis

Gut contents were identified and analyzed in the laboratory following the methods described by Rosi-Marshall, Wellard Kelly, et al. (2016). We measured salmonfly body length (mean = 34.86; SE = 0.46 mm) and dissected each individual to remove the gut tract. Gut contents from the

upper portion of the gut tract were emptied into water, the gut wall was removed, and contents were placed in a beaker. We placed each beaker in a water bath sonicator for 30 s to disperse clumps. Gut contents were then filtered onto Metrical membrane filters (25-mm diameter, 0.45- μ m pore size). Filters were dried at 60°C for 10 min and then mounted onto slides using standard immersion

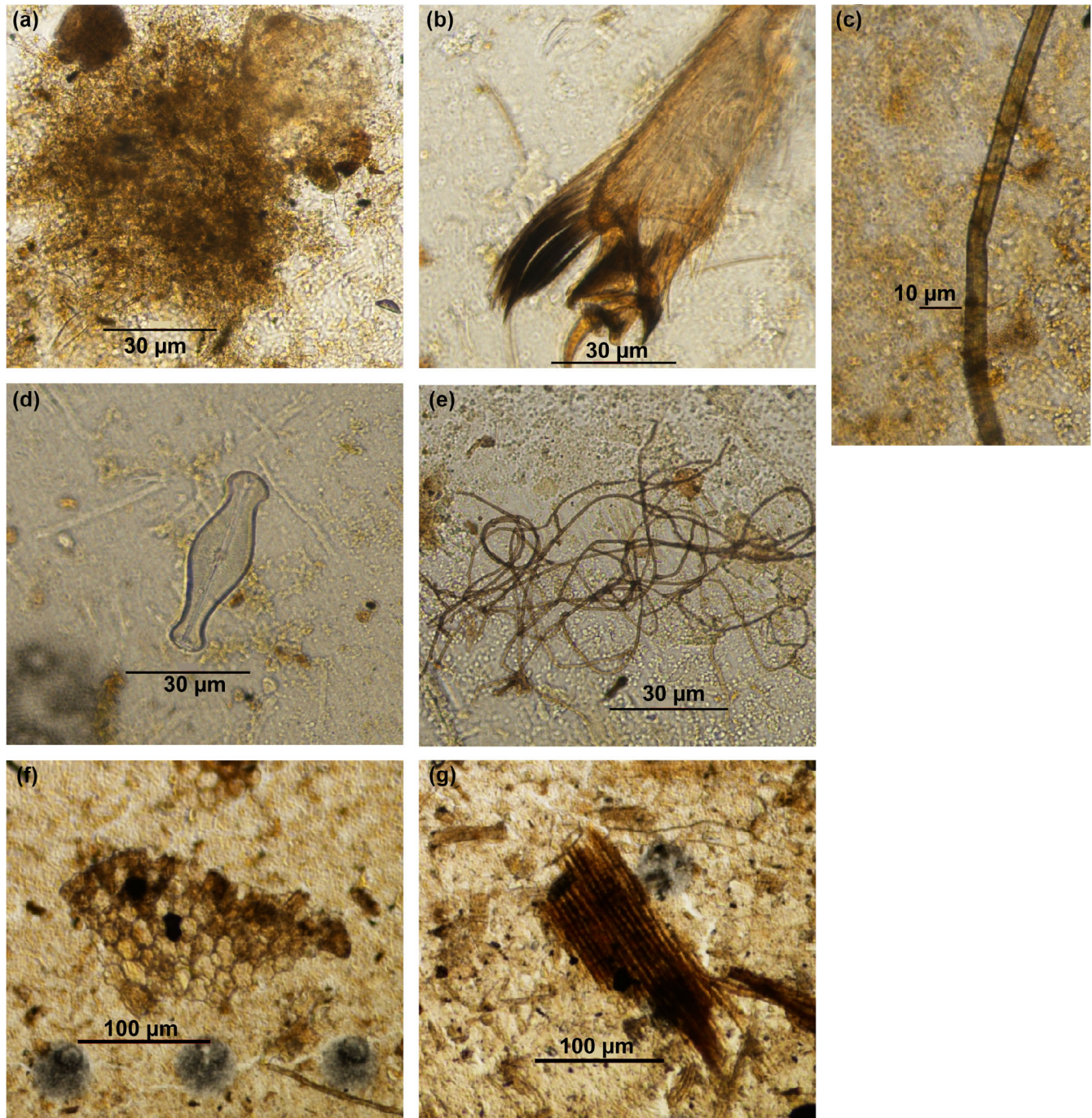


FIGURE 2 Representative images of diet items found in salmonfly guts, including (a) amorphous detritus, (b) animal material, (c) filamentous algae, (d) diatoms, (e) fungi, (f) leaf material, and (g) woody debris. Panels (a)–(e) were photographed at 200 \times magnification and panels (f) and (g) were photographed at 40 \times magnification. Scale bars are 30 μ m (a, b, d, and e); 10 μ m (c); or 100 μ m (f and g). Photo credits: M. Briggs and S. Swart

oil. We photographed particles at 100 \times to 400 \times magnification depending on the size of item and the resolution required to accurately identify the item type. We identified and measured the area of up to 150 particles per slide using NIS-Elements imaging software (Nikon Instruments Inc., Melville, New York, USA) and estimated the proportion of area comprised of each diet item type as our response variable. Diet item types identified included wood and leaf debris combined, amorphous detritus, animal material, fungi, diatoms, and filamentous algae (Figure 2).

Fatty acid analysis

Late-instar larval salmonflies were collected from two sites on the Madison River, Palisades and Varney Bridge, on 27 November 2019. We collected three females and three males from each site for fatty acid composition analyses. Salmonflies were frozen, shipped overnight, and stored at -80°C upon arrival at the processing facility. We freeze-dried and then weighed salmonflies prior to extracting fatty acid methyl esters (FAMES) for composition analyses. Freeze-dried salmonflies were deposited in 7 ml dichloromethane: methanol (2:1, v:v), crushed using a glass rod, vigorously sonicated, and stored over night at -20°C . Total lipids were extracted three times from tissues with dichloromethane:methanol (2:1, v:v). Pooled cell-free lipid extracts were evaporated to dryness under N_2 -atmosphere and transesterified with methanolic HCl (3 mol/L, 60°C , 15 min, Sigma-Aldrich 33050-U). FAMES were extracted three times with isohexane (2 ml). Pooled FAME-containing fractions were evaporated to dryness

under N_2 and resuspended in 50 μl of isohexane. FAMES were analyzed by gas chromatography (GC) using a HP 6890 gas chromatograph (Agilent Technologies) equipped with a flame ionization detector (FID) and a DB-225 (J&W Scientific, 30 m \times 0.25 mm inner diameter [id] \times 0.25 μm film) capillary column. Configuration details are given elsewhere (Martin-Creuzburg et al., 2010, 2017). FAMES were quantified by comparison to internal standards (C17:0, C23:0 ME) of known concentrations (adapted to dry mass in the sample), using multipoint calibration curves generated using FAME standards (Sigma). FAMES were identified by their retention times and their mass spectra, which were recorded with a quadrupole gas chromatograph-mass spectrometer (GC-MS; Agilent Technologies, 5975C inert MSD) equipped with a DB-225MS fused-silica capillary column (J&W Scientific, 30 m \times 0.25 mm id \times 0.25 μm film); gas chromatographic settings as for FID. Mass spectra were recorded between 50 and 600 m/z in the electron ionization mode. The limit of quantitation was 10 ng of fatty acid.

Data analysis

We used both multivariate and univariate approaches to analyze diet proportions for differences by sex, habitat, and river. We used nonmetric multidimensional scaling based on Bray-Curtis dissimilarities to visualize how salmonfly gut contents differed by sex, habitat, and river. To test whether the multivariate position of diets differed by these variables, we used a mixed effects permutational multivariate analysis of variance (PERMANOVA) including sex, habitat, and river as fixed effects and site as a random effect. Testing for differences in multivariate position provides information on the composition of the diet items. We used a permutational analyses of multivariate dispersions to test whether multivariate dispersion of diets differed across the same three covariates. Testing for differences in multivariate dispersion, or spread, provides information about how variable the diet items were (Anderson, 2006). Multivariate analysis was conducted using the vegan package (Oksanen et al., 2018).

We used a univariate approach to determine if the proportions either of wood and leaf material or of diatoms, two of the most abundant diet groups, differed by length, sex, habitat, and river. We constructed linear mixed effects models with either proportion of wood and leaf or proportion of diatoms as the response variable. We included site as a random effect and each combination of length, river, habitat, and sex as fixed effects. We chose not to include any interaction terms as we had no a priori reason to do so. We constructed a total of 16 models for each response variable, ranging from a

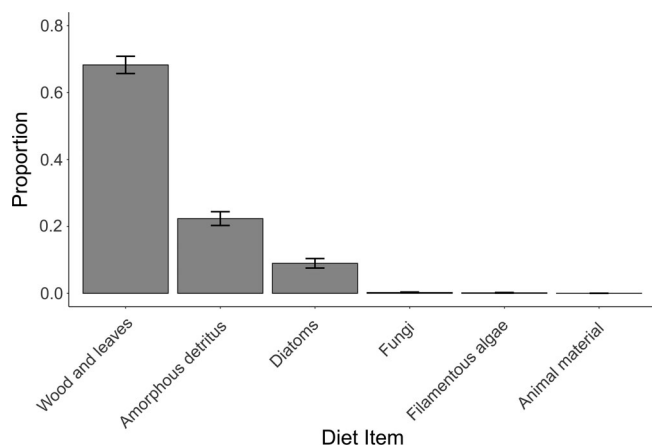


FIGURE 3 Mean proportion by area of diet items found in the guts of all salmonflies collected ($n = 72$) from sampling locations on the Madison and Gallatin rivers in Southwest Montana 9–10 July 2020. Error bars indicate SE

model including all four fixed effects to a null model which included only the random effect. We used Akaike's information criterion adjusted for small sample sizes (AIC_c) for model selection, and we selected the models with the lowest AIC_c scores for each response variable as our final models. Linear mixed effects models were fit using the lme4 package (Bates et al., 2015), and we used the MuMIn package for AIC_c model selection (Barton 2015). We used two-sided Student's t tests to determine if

the percent composition of fatty acids differed by site. All statistical analysis was completed in R version 4.0.4 (R Core Team, 2019).

RESULTS

Wood and leaf debris combined made up the majority of salmonfly gut contents, comprising a mean of 68.2%

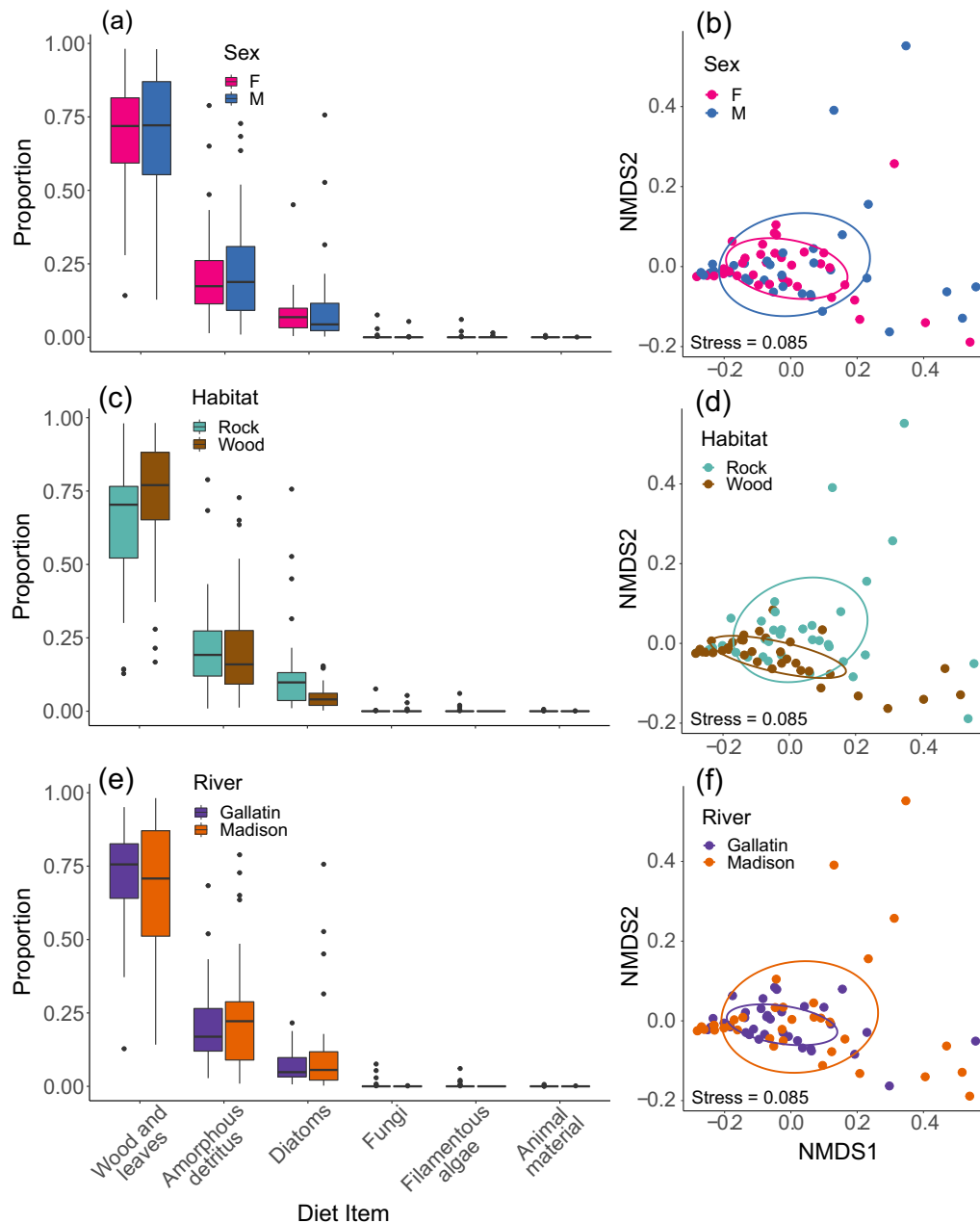


FIGURE 4 Standard box plots depicting proportional area of diet items in guts of salmonflies across (a) sex, (c) habitat type, and (e) river. Nonmetric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities of salmonfly diet items grouped by (b) sex (F = female; M = male), (d) habitat type, and (f) river. Ellipses show standard deviation. Diets were consistent across sex, habitat type, and river, with wood and leaves comprising the majority of gut contents, followed by amorphous detritus and diatoms. For all panels, site ($n = 3$ per river) is treated as a random effect. Salmonflies were collected from locations on the Madison and Gallatin rivers in Southwest Montana 9–10 July 2020

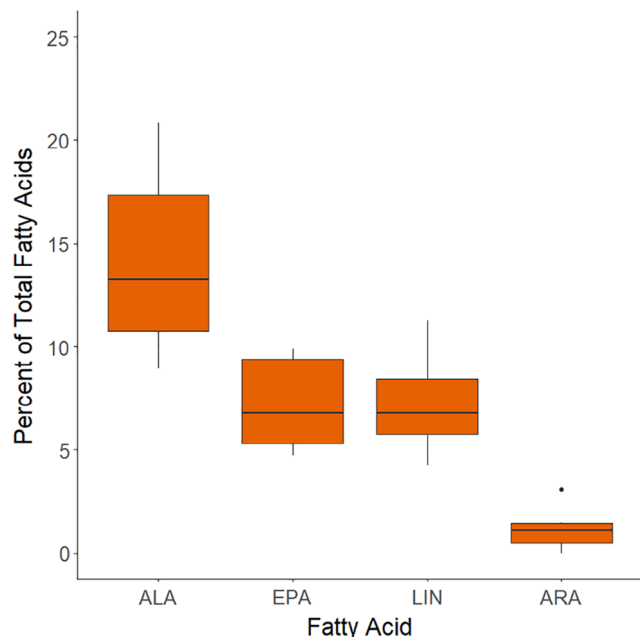


FIGURE 5 Standard box plot depicting percent composition (percent of total fatty acids) of two omega-3 polyunsaturated fatty acids, ALA (α -linoleic acid; 18:3n-3) and EPA (eicosapentaenoic acid; 20:5n-3), and two omega-6 polyunsaturated fatty acids, LIN (linoleic acid; 18:2n-6) and ARA (arachidonic acid; 20:4n-6), in salmonflies ($n = 12$) in the Madison River, Montana, collected 9–10 July 2020. Omega-3 polyunsaturated fatty acids in salmonflies support the gut content results showing that salmonflies consume diatoms and filamentous algae. Filamentous green algae are likely the source of ALA, diatoms are likely the source of EPA, and terrestrial detritus is likely the source of LIN, the precursor to ARA

(SE = 2.6 of the diet by area, Figure 3). The next most common diet categories included amorphous detritus (mean = 22.3%, SE = 2.1) and diatoms (mean = 9.0%, SE = 1.4). Filamentous algae, fungus, and animal material each comprised <1.0% of gut contents, on average.

Results of a PERMANOVA indicated that the multivariate position of diets, which represents diet compositions, differed by habitat ($p = 0.037$) but not by sex or river (Figure 4). Results of a PERMDISP indicated that multivariate dispersion of diets differed by river ($p = 0.018$) but not by sex or habitat, with higher dispersion among salmonfly diets from the Madison River than for those from the Gallatin River (Figure 4f). This result indicates that salmonfly individuals were more variable in their diet composition in the Madison River compared to the Gallatin River. Based on AIC_c model selection, the best model for the proportion of wood and leaves in salmonfly diets was the null model, which only included site as a random effect (Appendix S1: Table S1). The best model for the proportion of diatoms in salmonfly diets included habitat type as a fixed effect ($t = -3.267$, $p = 0.002$) and site as a random effect (Appendix S1: Table S2). Results of this

model indicated that diatoms comprise an additional 8.3% of diet items in the guts of salmonflies collected from rocky habitat when compared to salmonflies collected from woody debris.

Salmonflies collected from the Madison River contained EPA, ALA, LIN, and ARA, common and expected types of fatty acids in aquatic insect tissues. We expected LIN > EPA > ALA > ARA based on the areal proportions of diet items from the gut content analysis. However, we found that ALA > EPA > LIN > ARA (Figure 5). ALA, an n-3 precursor to EPA that is likely acquired through the consumption of filamentous green algae, comprised an average of 14.0% of total fatty acids. EPA, a n-3 LCPUFA that can be acquired from the consumption of aquatic primary producers such as diatoms, comprised an average of 7.22% of total fatty acids in salmonflies. LIN, a precursor to ARA that is generally acquired from terrestrial detritus, made up an average of 7.15% of total fatty acids, while ARA made up an average of 1.05% of total fatty acids.

DISCUSSION

A comprehensive understanding of the influence of land-use change, pollution, flow modification, and other stressors on energy flows through food webs requires knowledge of feeding habits. Establishing diet patterns of freshwater insects may help evaluate how stressors affect resource quantity, resource quality, and the resulting ecosystem services associated with high biodiversity and densities of insects that support food webs and angling economies. We found that allochthonous detritus (i.e., particulate wood and leaves) comprised the majority of salmonfly diets in the Gallatin and Madison Rivers, followed by amorphous detritus, diatoms, and filamentous algae. Diets showed little variation across river, sex, or length. Minor differences in diets were detected by habitat type, with more diatoms in guts of salmonflies sampled from rocky habitat compared to woody debris. Fatty acid composition generally supported the results of gut content analysis. The presence of EPA and ALA in salmonfly bodies is consistent with consumption of diatoms and filamentous algae, respectively. The percent composition of LIN was lower than expected for the high amount of terrestrial wood and leaf resource found in gut content analysis of the salmonfly diets.

Terrestrial detritus is a commonly consumed food item for shredders and detritivores, which likely gain much of their nutrition from fungi and bacteria growing on wood and leaves in streams (Eggert & Wallace, 2007). Thus, our finding that salmonflies consume primarily allochthonous material aligns with expectations for diets

of shredding invertebrates (Cummins et al., 1989). However, our findings contrast with previous studies of salmonfly diets which found a much greater dependence on autochthonous material, including diatoms and animal parts (Blackadar et al., 2020; Freilich, 1991; Rosi-Marshall, Vallis, et al., 2016). Both seasonal and site-specific factors may contribute to the differences between our findings and previous studies. For example, both the Madison and Gallatin Rivers achieve stream order 7, while sites in previous studies were in 4–6th order streams (Blackadar et al., 2020; Rosi-Marshall, Vallis, et al., 2016). Leaf litter standing stocks are likely relatively low during mid-summer, when we sampled in these rivers. However, biofilms on slowly decomposing woody debris can provide a year-round source of nutrition for invertebrates when leaf litter availability is low (Eggert & Wallace, 2007). Although we did not separately distinguish aquatic macrophyte leaf material from terrestrial leaf material in this study, macrophytes at our study sites are anecdotally rare. Future work might investigate salmonfly use of aquatic macrophyte beds as both food and habitat, especially during warm summer months (Angradi, 1993). Seasonal flow patterns, competition with other invertebrates, or availability of particular diet items in the environment may regulate salmonfly resource use, leading to a higher dependence on terrestrial detritus than documented in previous studies on salmonfly diets. In particular, the dependence on terrestrial detritus in rivers such as the Madison, which is an open-canopy water body flowing through a wide, agricultural valley, deserves further exploration. The absolute inputs of terrestrial, detrital particles to rivers such as the Madison might be small relative to algal production, but the interstitial benthic habitat may sufficiently retain allochthonous materials from the large upstream drainage area, as well as from riparian and upland soils. Future targeted work could establish the mechanisms responsible for driving variation in salmonfly reliance on allochthonous inputs across additional rivers.

Autochthonous resources like diatoms and green algae typically contain higher proportions of n-3 LCPUFA than terrestrial sources and therefore represent a high-quality food for macroinvertebrate consumers (Cashman et al., 2016; Guo et al., 2016; Torres-Ruiz et al., 2007). While gut content analysis demonstrated that salmonflies in these rivers primarily consume terrestrial detritus, EPA and ALA suggest a reliance on autochthonous food resources as well. Although we expected high proportions of LIN given the large consumption of terrestrial diet items, we found relatively small proportions of these LCPUFAs. The presence of EPA in salmonfly tissues suggests that they have the potential to serve as an important direct dietary source of n-3

LCPUFA for terrestrial consumers, such as birds and spiders, which consume aquatic n-3 LCPUFA sources during large emergence events (Twining et al., 2018, 2019). Together, gut contents and fatty acids provide a more complete understanding of salmonfly feeding ecology and their role in aquatic and terrestrial food webs.

We detected small differences in diets of salmonflies collected on rocky habitat compared to woody debris, with more diatoms consumed by salmonflies sampled from rocks. Perhaps salmonflies using rocks are consuming more diatoms, which is a high-quality food source for invertebrates (Guo et al., 2021; Marcarelli et al., 2011). The underside of rocks could also provide a refuge from potential predation. However, future work could examine the extent of habitat fidelity (Freilich, 1991) to evaluate whether the minor differences in diet composition we detected relate to limited movement and habitat fidelity of salmonflies across different substrate types.

Salmonflies in the Madison and Gallatin Rivers depend on wood and leaf debris as a primary food source. A healthy and intact riparian area that provides wood and leaf detritus to streams is vital for salmonflies in Southwestern Montana and is likely important throughout the range of this ecologically significant insect. Yet, riparian zones face many threats, including clear-cutting, lack of floodplain connection, invasive species establishment, and intense grazing pressure (Goodwin et al., 1997; Poff et al., 2011). In addition, reliance on high-quality autochthonous primary producers such as diatoms that are also sensitive to anthropogenic river alteration may further limit availability of preferred food resources (Larras et al., 2017). Salmonflies are declining throughout the western United States and this trend is likely to continue as the climate warms (Anderson et al., 2019; Kowalski & Richer, 2020; Walters et al., 2018). Maintaining habitat that meets the need of salmonflies will be key to conserving and restoring salmonfly populations in reaches where they are at risk of extirpation. Our research underscores the importance of a healthy riparian zone that provides allochthonous detritus for invertebrate nutrition as well as invertebrate habitat (Benke & Wallace, 2003; Fenoy et al., 2021). Understanding invertebrate diets and feeding ecology can guide future efforts to restore and conserve imperiled freshwater biodiversity.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

Data (Briggs et al., 2021) are available from Dryad: <https://doi.org/10.5061/dryad.6djh9w122>.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

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