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# WINTER ECOLOGY OF A WILDERNESS RIVER: EFFECTS OF ICE DISTURBANCE AND SUBSIDIES OF UNGULATE CARCASSES ON AQUATIC FOOD WEBS

by

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To the Graduate Faculty:

The members of the committee appointed to examine the thesis of Ryan J. Blackadar find it satisfactory and recommend that it be accepted.

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# WINTER ECOLOGY OF A WILDERNESS RIVER: EFFECTS OF ICE DISTURBANCE AND SUBSIDIES OF UNGULATE CARCASSES ON AQUATIC FOOD WEBS

Thesis Abstract – Idaho State University (2013)

Knowledge of the winter ecology of rivers is deficient for aquatic ecosystems globally and disturbance by ice is often not considered in natural disturbance regimes of rivers. I investigated how disturbance by river-ice affects organic-matter dynamics and feeding ecology of aquatic insects in Big Creek, a 6th - order tributary of the Middle Fork Salmon River in central Idaho, USA. Also, I used opportunistic sampling to investigate how elk (*Cervus elaphus*) carcasses from gray wolf (*Canis lupus*) kills, which often occur in rivers or on ice during winter, may serve as resource subsidies to river organisms. I compared samples of biofilm, transported organic matter, and gut-contents of aquatic insects before and after ice break-up events. In addition, I compared dissolved nutrients, biofilm, and diet analyses (gut-contents and stable isotopes) of aquatic insects collected from an elk carcass to samples collected at similar sites without a carcass. Scour associated with 2 ice break-up events reduced the quantity of biofilm by 39% but increased the quality by 41%. Several aquatic insect taxa consumed greater proportions of diatom frustules following ice break-up, although some taxa consistently consumed large proportions of high-quality food resources. Diet analyses indicated that aquatic insects collected from an elk carcass consumed biofilm rather than directly consuming carcass material. This study indicates ice regimes in rivers affect organic-matter dynamics and feeding ecology of aquatic insects. Additionally, this study provides insight to a unique subsidy to river ecosystems and indicates a potential pathway in which predation by wolves may be altering river processes. More research on the winter ecology of aquatic ecosystems is needed to predict potential effects of climate change.

# PREFACE

Disturbance is a critical factor in aquatic communities and ecosystems; however, disturbances by ice often are not considered in the natural disturbance regime of rivers. Furthermore, knowledge of the winter ecology of aquatic ecosystems is deficient globally. Recent evidence indicates that winter temperatures may be most affected by climate change. Increased temperatures in winter associated will likely alter the spatial extent, duration, and disturbances of river ice. The response of river ecosystems to such shifts in ice, however, is uncertain because of the paucity of studies exploring the winter ecology of rivers. Based upon studies of physical processes of river ice, I predict that shifts in river ice and associated disturbances would have dramatic consequences for the quantity and quality of basal resources available to aquatic consumers.

The objective of this study was to quantify how river ice affects organic-matter dynamics and the feeding ecology of aquatic insects. I also assessed the response of dissolved nutrients, biofilm, and aquatic insects to a unique subsidy, elk carcasses originating from wolf kills, initially predicted to occur in winter. This thesis is separated into two chapters in manuscript form. The first chapter describes the ice regime in winter of a wilderness river. Chapter 1 also describes how disturbance from ice affects biofilm attached to rocks and organic matter transported in the water column. Further, Chapter 1 presents results of gut-content analysis of aquatic insects and how the diets shift following disturbance by ice. The second chapter describes how elk carcasses originating from wolf kills may be resource subsidies to organisms in aquatic ecosystems when delivered to rivers. Chapter 2 explains how basal resources and use of these resources by aquatic insects may be affected by the addition of an ungulate carcass to rivers.

# CHAPTER 1

## River ice disturbance: effects on organic-matter dynamics and feeding ecology of aquatic insects

Ryan J. Blackadar, Colden V. Baxter, and John M. Davis

## Abstract

Disturbance is a critical factor shaping the structure and function of aquatic communities and ecosystems. Disturbances from ice, however, often are not considered within the ecological disturbance-regime of rivers. We investigated effects of a river-ice regime on organic-matter dynamics and the feeding ecology of aquatic insects during winter. We collected monthly samples of organic matter (attached biofilm and organic matter in transport) and aquatic insects from Big Creek, a 6th - order tributary of the Middle Fork Salmon River in central Idaho, USA, during winter 2010 - 2011. We documented patterns in river ice via direct observation and use of digital-interval cameras. Our results indicate that river ice affects both the quantity and quality of organic matter available to consumers. Specifically, scour from December and February ice break-up events reduced biofilm biomass (ash-free dry mass and chlorophyll-*a*) by one-half and one-third, respectively, whereas quality (chlorophyll-*a* : ash-free dry mass) increased. Diets of scrapers, *Rithrogena* (Heptageniidae) and *Bibiocephala* (Blephariceridae), collector-gatherer, *Baetis* (Baetidae), and collector-filterer, *Simulium* (Simulidae) appeared to follow the patterns of organic matter in response to disturbance by river ice. These taxa consumed greater proportions of diatom frustules following ice break-up events. In contrast, other taxa, such as collector-gatherer, non-Tanypodinae

(Chironomidae), and the collector-filterer, *Arctopsyche* (Hydropsychidae), did not follow patterns observed in organic matter. Rather, these insects consistently consumed high proportions of diatom frustules and insect material, which are high-quality food resources. The shredder, *Pteronarcys* (Pteronarcyidae), exhibited the greatest variability in its diet, but followed patterns observed in algal resources. Our study indicates that ice regimes in rivers affect organic-matter dynamics and the feeding ecology of aquatic insects**.** Understanding the ecological consequences of river-ice is important in the context of a changing climate. Furthermore, predicting the net effects of global climate change is hindered because of the lack of aquatic research conducted during winter.

**Key words**

river ice, organic matter, aquatic invertebrates, gut-content analysis, disturbance, algae

## Introduction

Disturbance is a critical factor shaping the structure and function of aquatic communities and ecosystems (Resh et al. 1988). Flooding, drought, landslides, fire, and human alterations to the hydrologic-flow regime represent disturbances to aquatic ecosystems that affect river productivity and the survival of aquatic organisms (Poff et al. 1997, Lake 2003, Minshall 2003, Cover et al. 2010, Poff and Zimmerman 2010). Also, those disturbances affect the quantity and quality of organic matter available to consumers within aquatic foodwebs. For instance, floods transport accumulated organic matter to downstream regions and link the mainstem channel to floodplain habitat, where exchange of organic matter and organisms may occur (Junk et al. 1989, Poff et al. 1997, Biggs & Smith 2002). In addition, elevated flows mobilize sediments and riverbed particles, which scour the substrate and expose areas of riverbed for colonization by biofilm communities (Fisher et al. 1982, Power & Stewart 1987). New growth of biofilm communities is considered high quality, because diatom frustules in biofilm contain large proportions of lipids and fatty acids compared to accumulations of diatom stalks composed of muco-polysaccharides (Fisher et al. 1982, Spaulding & Elwell 2007). Droughts also are critical periods for organic-matter availability (Lake 2003), reducing the transport of fine particulate organic matter, and leading to accumulations of detritus and sediments (Cuffney & Wallace 1989, Boulton & Lake 1990). In contrast the ice regimes of rivers have received less attention, and in particular, studies are needed that investigate the ecological effects of ice dynamics (Scrimgeour et al. 1994, Prowse 2001a,b, Beltaos 2007).

Periods of river-ice formation and break-up are critical physical disturbances affecting river ecosystems at high latitudes and altitudes (Scrimgeour et al. 1994, Prowse 2001a, b). Ice may form at the water surface, thereby stabilizing water temperatures relative to atmospheric temperatures (Gard 1963), reducing water velocity (Turcotte et al. 2011), and concentrations of dissolved oxygen under ice (Prowse 2001a). Turbulent flows, in contrast, prevent formation of ice at the water surface and promote the formation of small ice crystals in the water column (frazil ice; Prowse 2001a, Hirayama et al. 2002). Frazil ice affects the viscosity of flowing waters and may scour the riverbed (Reimnitz et al. 1990, Prowse 2001a). In addition, frazil ice may attach to river substrates to form anchor ice, which can influence hydraulic conditions by confining the channel, shielding riverbed materials from entrainment, and disturbing river substrates (Prowse 2001a, Turcotte et al. 2011). Although many types of river ice affect physical processes in rivers, ice break-up events may do so disproportionately. Ice break-up events result from the degradation of river ice in large ice flows, and are characterized by dramatic increases in discharge, water temperature, suspended materials, and intense scouring of the river channel and riparian area (Scrimgeour et al. 1994, Prowse & Culp 2003, Beltaos 2007, Turcotte et al. 2011, Engstrom et al. 2011). In general, ice break-up events are assumed to occur in spring with warming temperatures and thawing of rivers, however, timing and frequency of ice break-up events are likely to be affected by climate change. Previous studies have focused on physical effects of river ice (hydraulic and geomorphic), however, less is known regarding the ecological effects of disturbance from river ice.

By affecting physical processes, ice may influence the quantity and quality of organic matter in river ecosystems. For instance, surface ice can decrease light available to primary producers (Steinhardt & Wurtsbaugh 2003), and anchor ice displaces macrophytes (Prowse 2001a). In addition, scour from ice break-up events may stimulate primary producers by providing a clean substrate for new growth (Scrimgeor et al. 1994). Ice break-up events also scour riparian areas, promote seedling development (Rood et al. 2007), and deliver riparian organic matter to river channels (Scrimgeour et al. 1994). Disturbance from river ice likely affects quantity and quality of organic matter, however, few studies have specifically addressed the effects of river ice on aquatic food webs.

River ice may alter the structure of food webs by affecting the distribution of organisms and availability of food resources. Ice formation and break-up can alter the distribution of fishes and may also have consequences for their populations by freezing their eggs (Brown et al. 2001, Cunjak et al. 1998, Huusko et al. 2007). Furthermore, ice can alter food webs by displacing aquatic macroinvertebrates, effects that may be exacerbated by ice out events (Bradt et al. 1999). Ice may also affect food webs by changing the availability of organic matter through scour. For instance, disturbance by floods reduced biofilm biomass and subsequently increased gross primary production (Fisher et al. 1982, Power and Stewart 1987). Additionally, floods in the Colorado River, USA, increased the quality of food resources available to aquatic insects, which also increased the production of rainbow trout (*Oncorhynchus mykiss*) (Cross et al. 2011, Wellard-Kelly et al. 2013). Scour from ice may play a similar role in structuring foodwebs of rivers; however, no study has directly investigated the feeding ecology of aquatic insects in response to a river-ice regime.

The objective of this research was to investigate how river ice affects organic-matter dynamics and the feeding ecology of aquatic insects. We hypothesized that disturbance by ice alters food-web dynamics in rivers. We predicted that ice break-up events scour the riverbed thereby reducing the quantity of biofilm, but increasing the quality of biofilm. Further, we predicted that scour from ice break-up events mobilize riverbed materials, subsequently increasing transported organic matter. Because scour from ice may increase the quality of organic matter, we predicted that aquatic insects would consume a greater proportion of high-quality food resources (i.e., diatoms) following ice break-up events. Our broader aim was to investigate winter ecology in river ecosystems, which is globally understudied. Winter temperatures in the Pacific Northwest of North America are predicted to be most affected by climate change (Mote et al. 2003); therefore, river ice regimes are likely to be altered. Results from our research will improve understanding of how climate-induced shifts in ice-regimes may affect aquatic ecosystems.

## Methods

### *Study site*

Our research was based at Taylor Wilderness Research Station (TWRS, 45.1019° and 114.8517°W), within the Frank Church 'River of No Return' Wilderness Area in central Idaho, USA. This area is roadless and accessible only by trail or small aircraft. We studied effects of a river ice-regime in this wilderness watershed to measure responses of an intact aquatic ecosystem that exhibits a natural ice regime. Study locations were located in the mainstem of Big Creek, a 6th order river (drainage area: 1444 $km^2$), which flows into the Middle Fork of the Salmon River (Fig. 1). Big Creek has an intact natural flow regime as well as intact diverse suite of native macroinvertebrates that encompass a range of feeding modes that occur in montane streams and rivers within this region and other similar regions of the world. Big Creek flows through steep, mountainous terrain with severe changes in elevation from the valley bottoms near the station (1200 m) to high elevation peaks (3150 m). Forested slopes consist primarily of Douglas fir (*Pseudotsuga menziesii*) and Ponderosa pine (*Pinus ponderosa*), but xeric grass communities and sagebrush (*Artemisia*) are abundant on drier slopes. Annual precipitation ranges from 38 to 50 cm in valleys and from 76 to 100 cm at higher elevations (Robinson et al. 2005). Most precipitation occurs as snow, resulting in peak stream flows from May to June. Baseflow conditions occur from late summer through winter. We selected study locations along a 3 km segment of Big Creek encompassing pool and riffle habitats that were representative of the geomorphic character of the river. Selection of study locations was restricted to areas immediately upstream and downstream of the research station to allow for sample collection and processing.

*River ice monitoring*

We used a combination of techniques to describe patterns in river ice. Monthly sampling trips (December 2010 to March 2011) allowed for direct observation of river-ice characteristics. In addition, managers of TWRS (A. J. Brumble and T. Morrison) recorded digital photographs of Big Creek at known locations prior to an initial sampling trip, and between monthly trips made by authors. Also, we used digital-interval cameras (Moultrie, Farmington, NY, USA) to monitor patterns in river ice. Two cameras, one upstream and one downstream of the field station (Fig. 1), were programmed to record 3 photos hourly. We collected photographs monitoring river-ice regime from 11 December 2010 through 9 February 2011. Lastly, we used a gauge in the mainstem of Big Creek located at TWRS that continuously recorded stage-height of Big Creek to monitor river ice. We identified ice break-up events by loss of surface ice and change in color of water in digital photographs. Additionally, we identified ice break-up events by rise in the stage height of Big Creek, measured by gauge at TWRS.

*Biofilm*

We sampled biofilm (assemblage of living and dead autotrophs, heterotrophic microbes, and detritus on rocks) monthly from December 2010 to March 2011 at 36 study locations (18 thalweg and 18 edge) in the mainstem of Big Creek. Study locations were evenly distributed along the 3 km study segment. Three rocks were chosen haphazardly at each location, placed into plastics bags, and transported to the research station for processing. Rocks were scrubbed with a wire brush to remove all attached biofilm. The composite slurry from the combined 3 rocks was sub-sampled (20 - 50 mL), filtered onto pre-combusted (400 °C for 30 min) and pre-weighed glass-fiber filters (0.7

μm**)**, and frozen immediately. Samples were then transported back to Idaho State University for further analysis. The planar area of sampled rocks was traced onto paper. Rock tracings were cut, weighed, and converted to planar area with linear regression. This resulted in c. 36 composite samples (18 thalweg and 18 edge) for each of the 4 sampling periods.

Based on standard methods (APHA, 1998, Davis et al. 2013), filters from each location were analyzed for chlorophyll-*a* (chl-*a*, measure of algal pigments and index of living primary producers) and ash-free dry mass (AFDM, measure of living and senesced primary producers). Filters were extracted in methanol for c. 12 h, analyzed for chl-*a* with a spectrophotometer and corrected for phaeophytin. After extraction, the filters and methanol extract containing the chl-*a* component were recombined, dried at 60 °C for at least 48 h, weighed and ashed at 550 °C for c. 4 h. Samples were then reweighed to calculate AFDM. Additionally, the chl-*a* values were divided by the corresponding AFDM values to generate the ratio of chl-*a* to AFDM, an indicator of biofilm quality by contrasting the portion of living autotrophs of biofilm (chl-*a*) to the portion of living and senesced autotrophs of biofilm (AFDM).

### *Transported organic matter*

We sampled transported organic matter in Big Creek monthly from December 2010 to March 2011. During each month, transported organic matter was collected during 3 time periods (morning, mid-day, and night) for a 24 h period to encompass diel variability. Each month, coarse (>1 mm) and fine (<1 mm, ≥ 53 μm) transported organic matter were collected from the thalweg for 1 - 4 h. Coarse transported organic matter (CTOM) was collected with a hoop net (22.9 cm diameter, 1-mm mesh) with attached flowmeter (General Oceanics Inc., Miami, FL, USA) connected to a fencepost. Fine transported organic matter (FTOM) was collected mechanically by pumping river water through a hose. The intake was surrounded by a 1-mm mesh and was positioned in the thalweg at c. 0.6 total depth. The water was then pumped to the shore and into 53-µm mesh net. At the end of each sampling period, the CTOM and FTOM nets were removed and rinsed with filtered river water.  Both the CTOM and FTOM were sub-sampled (20 - 50 mL), filtered onto pre-combusted (400 °C for 30 min) and pre-weighed glass-fiber filters (0.7 µm**)**, and frozen immediately. The remaining volume of CTOM and FTOM was sieved (250 and 53 µm, respectively), transferred to plastic containers, and frozen immediately for later calculation of bulk AFDM. CTOM and FTOM filtered sub-samples were used to quantify chl-*a* concentrations as previously described. FTOM samples were analyzed for chl-*a* concentrations and not for AFDM. After chl-a analysis, the CTOM extract and filter were recombined and added to the remaining bulk AFDM for the calculated total AFDM. The total AFDM values of CTOM were used to calculate the ratio of chl-*a* to AFDM, as described previously.

### *Aquatic insect collection and laboratory processing*

We collected aquatic insects monthly (December 2010 to March 2011) from thalweg and edge habitats with kick-nets (250-µm mesh). Individuals were preserved in Kahle’s solution (Stehr 1987) and returned to the laboratory for gut-content analysis. Prior to gut removal, individuals were identified to genus or species, except for Chironomidae, which were identified as non-Tanypodinae (Merritt and Cummins 1996). Most insects were identified to genus and correspond to the Standard Taxonomic Effort Level (Adams et al. 2004). We examined diets of taxa representing 4 functional feeding groups (Cummins and Klug 1979): scrapers [*Rithrogena* (Ephemeroptera: Heptageniidae) 4 - 11 mm] and [*Bibiocephala* (Diptera: Blephariceridae) 7 – 13 mm], collector-gatherers [*Baetis* (Ephemeroptera: Baetidae) 3 – 6 mm] and [Non-Tanypodinae (Diptera: Chironomidae) 8 – 11 mm], collector-filterers [*Simulium* (Diptera: Simulidae) 4 – 7 mm] and [*Arctopsyche grandis* (Trichoptera: Hydropsychidae) 10 – 24 mm], and a shredder [*Pteronarcys* (Plecoptera: Pteronarcyidae) 8 – 48 mm]. These taxa are important members of the assemblage of insects in Big Creek, representing, for instance, 53% of total benthic abundance, and 25% of the total benthic biomass of aquatic invertebrates collected during summer monitoring (Baxter, unpublished).

Diets were evaluated with standard techniques for gut-content analysis (Benke and Wallace 1980, Rosi-Marshall and Wallace 2002). Gut contents were removed from the insect body, suspended in distilled water, sonicated 60 s, and filtered onto membrane filters (0.45 μm). Filters with gut-contents were dried in an oven at 60 °C, mounted on slides with immersion oil, and sealed with clear, nail polish. The number of individuals per slide varied from 1 - 4, based on the size and gut fullness of insects. Three to 7 slides per month were analyzed for each taxon.

We measured the fractional area of food particles to calculate the proportion of each particle type in the diets of aquatic insects. Food particles were identified as amorphous detritus, diatom frustule, diatom stalk, filamentous algae, fungi, leaf material, insect material, and wood with a compound microscope at 200 - 400 × (Leica DMRB, Buffalo Grove, IL, USA) mounted with a digital camera (SPOT CCD, Sterling Heights, Michigan, USA). We identified diatom frustules and diatom stalks separately because stalks were large particles that were easily distinguished from the frustules and because they differ in nutritional quality (i.e., diatoms composed of lipids and fatty acids, stalks composed of muco-polysaccharides). We measured the area of 100 particles on each slide along transects (1 – 5) using ImageJ (National Institutes of Health, Bethesda, Maryland, USA; http://imagej.nih.gov/ij/).

*Statistical Analyses*

Data on organic matter from each monthly sampling period were averaged. We estimated means and 95% confidence intervals for the chl-*a*, AFDM, and chl-*a* : AFDM of biofilm and seston using bootstrap analysis. Data were re-sampled with replacement 1000 times, and the mean and 2.5% and 97.5% quantiles were calculated from the bootstrap distribution. For all metrics, differences were considered significant if 95 % confidence intervals did not overlap. We examined differences in biofilm values (AFDM, chl-*a*, chl-*a* : AFDM) between habitats by plotting monthly means and 95% confidence intervals for each month. Because biofilm values were similar between habitats (i.e., 95% confidence intervals overlapped), values for biofilm were combined across habitats for additional analyses. We used sign analysis to investigate the direction of differences in biofilm metrics following ice break-up events, and associated $p$ - values $< 0.05$ were considered significant (Conover 1980).

Data on diets of aquatic insects from each monthly sampling period were averaged to calculate the proportion of all food-particle types in the diet of each taxon. We examined differences in diets of insects by comparing monthly mean proportions of dominant food resources (diatom frustule, amorphous detritus, leaf material, filamentous algae, etc.). We estimated means and 95% confidence intervals for the proportion of each food resource consumed using bootstrap analysis. For each taxon, data on the proportion of food resource consumed were re-sampled with replacement 1000 times, and the mean and 2.5% and 97.5% quantiles were calculated from the bootstrap analysis. Differences were considered significant if 95 % confidence intervals from the mean did not overlap.

We used correlation analysis to investigate relationships among diets of aquatic insects and days since initial ice break-up. We correlated the proportion diatom frustules observed in gut-contents for individuals with the days since initial ice break-up for each taxon. Pearson correlation coefficient ($r$) was used to measure the strength of the linear association between variables and associated $p$ - values $< 0.05$ were considered significant. We also used sign analysis to investigate the direction of differences in diets of aquatic insects following ice break-up events (Conover 1980). We evaluated the proportion diatom frustules in gut-contents of insects before and after ice break-up events, and associated $p$ - values $< 0.05$ were considered significant. We used a weight-of-evidence approach (sensu Bowyer et al. 2003, Monteith et al. 2013) wherein results from a single variable may not be sufficient to draw a firm conclusion, but when multiple variables are considered, a clear pattern may become evident. Statistical analyses were performed with the software package SAS (SAS version 9.2, SAS Institute Inc., Cary, NC, 2008), and Microsoft Excel 2008 (Microsoft Corporation, Redmond, WA).

## Results

### *River ice regime*

Ice formation within our study segment in Big Creek commenced in November 2010, documented by photographs at known locations (Table 1). We observed surface ice along margins of the river channel in edge habitats from November 2010 to February 2011; however, surface ice was not observed in thalweg habitats for all months. In contrast, we observed frazil and anchor ice in both edge and thalweg habitats from November 2010 to February 2011. In addition, we documented 2 ice break-up events, via digital-interval cameras (Fig. 2). The initial ice break-up event occurred on 27 December 2010 and a second ice break-up event, of lower magnitude than initial, occurred on 3 February 2011. Following ice break-up events, we observed a wall of ice (c. 0.3 – 1.6 m in height) deposited along river margins that altered the morphology of the river channel. Overall in winter 2010 - 2011, formation of river-ice commenced in November 2010 and ice was no longer present in the river by March 2011. The ice regime in Big Creek was dominated by 2 ice break-up events in December and February.

### *Biofilm*

Monthly mean AFDM of biofilm collected from rocks decreased following 2 ice break-up events in thalweg and edge habitats, from December through February (Fig. 3A). AFDM in edge habitats was greater than in thalweg habitats for all months; however, differences were not significant (Fig. 3A). Overall, biofilm collected from the edge of the river channel exhibited parallel trends in AFDM as biofilm collected the thalweg of river channel (Fig. 3A). When biofilm values from edge and thalweg habitat were combined, AFDM decreased by 38%, following the initial ice break-up event in December (Fig. 3B). Also, AFDM of biofilm decreased by 54% between January and February, following a second ice break-up event of lower magnitude. Differences in AFDM between January and February were significant (Fig. 3B). The greatest shift in this variable occurred between February and March (Fig. 3A, B). AFDM values in March were 207% greater than values in February (Fig. 3B). Overall, AFDM of biofilm in thalweg and edge habitats decreased following 2 ice break-up events ($n$ = 66, x = 16, $p$ <

0.00003).

Patterns in mean chl-*a* of biofilm collected from rocks in both habitats were similar to trends in AFDM of biofilm, following 2 ice break-up events (Fig. 3A - D). In contrast to AFDM, chl-*a* in thalweg habitats was greater than in edge habitats for all months, although not significantly so (Fig. 3C). Overall, biofilm collected in thalweg habitats exhibited parallel trends in chl-*a* as in edge habitats (Fig. 3C). When biofilm values from thalweg and edge habitats were combined, chl-*a* decreased by 23%, following the initial ice break-up event in December (Fig. 3D). In addition, chl-*a* of biofilm decreased by 41% after the second ice break-up event of lower magnitude (i.e., between January and February, Fig. 3D). Differences in chl-*a* between January and February were significant (Fig. 3E). Chl-*a* of biofilm increased 103% from February to March, reaching chl-*a* values similar to pre ice-break-up conditions (Fig. 3D). Overall, chl-*a* of biofilm in thalweg and edge habitats decreased following 2 ice break-up events ($n$ = 66, x = 20, $p$ = 0.0011).

Means of the monthly ratio of chl-*a* to AFDM of biofilm, an indicator of quality of biofilm, exhibited trends opposite to those of AFDM and chl-*a* (Fig. 3A - F). When chl-*a* and AFDM of biofilm were highest (December & March), the ratio of chl-*a* to AFDM was lowest, and vice versa (Fig. 3A – F). Similar to chl-*a*, the ratio of chl-*a* to AFDM in thalweg habitats was greater than in edge habitats for all months (Fig. 3E). Overall, biofilm collected from the edge of the river channel exhibited parallel trends in the ratio of chl-*a* to AFDM as biofilm collected the thalweg of river channel (Fig. 3E). When biofilm values from edge and thalweg habitat were combined, the ratio of chl-*a* to AFDM increased by 43%, following the initial ice break-up event in December (Fig. 3F).

Differences in chl-*a* : AFDM between December and January were significant (Fig. 3F). The ratio of chl-*a* to AFDM decreased 38% from February to March, reaching values similar to pre ice-break-up conditions (Fig. 3F). Overall, the ratio of chl-*a* to AFDM of biofilm in thalweg and edge habitats increased following two ice break-up events ($n = 66$, $x = 18$, $p < 0.00023$).

*Transported organic matter*

Metrics of transported organic matter were highly variable among sampling times (morning, mid-day, night) and months. The AFDM of CTOM was greatest in December and January (Fig. 4A). Following 2 ice break-up events, the AFDM of CTOM decreased significantly by 80% from January to February and remained low through March (Fig. 4A). The chl-*a* content in CTOM was highly variable in December (large 95% CI) and declined through February (Fig. 4B). Differences in chl-*a* in CTOM between December and January, and between January and February were significant (Fig. 4B). The ratio of chl-*a* to AFDM for CTOM also was highly variable in December and March (large 95% CI) (Fig. 4C). Following a reduction from December to January, the ratio of chl-*a* to AFDM for CTOM increased from January to March (Fig. 4C). Differences in the ratio of chl-*a* to AFDM for CTOM between January and February were significant (Fig. 4C). Overall, CTOM was highly variable; however, AFDM and chl-*a* decreased following 2 ice break-up events. The ratio of chl-*a* to AFDM increased following the second ice break-up event (Fig. 4C).

Chl-*a* in FTOM exhibited a similar pattern to coarse seston (Fig. 4B & D). Chl-*a* in FTOM seston was greatest in December and March (Fig. 4D). Following ice break-up events, chl-*a* in FTOM decreased from December through February, but then increased from February to March (Fig. 4D). Differences in chl-*a* in FTOM between December and January were significant (Fig. 4D). Overall, chl-*a* in FTOM decreased following 2 ice break-up events.

### *Aquatic insect gut-content analysis*

Overall, diets of 5 taxa of aquatic insects (*Rithrogena, Bibiocephala, Baetis, Simulium,* and *Pteronarcys*) appeared to follow the patterns of organic matter in response to disturbance by river ice. These taxa consumed greater proportions of diatom frustules following 2 ice break-up events ($n$ = 58, x = 19, $p$ = 0.0064). Differences in the proportion of diatom frustules in gut-contents of these taxa were greatest following the initial ice break-up event that occurred in December 2011 ($n$ = 29, x = 19, $p$ = 0.00011). In contrast, 2 taxa of aquatic insects (non-Tanypodinae Chironomidae and *Arctopsyche*) did not follow patterns observed in organic matter. Rather, these insects consistently consumed high proportions of diatom frustules and insect material, respectively, which are high-quality food resources.

Diets of the scraper mayfly, *Rithrogena,* consisted primarily of amorphous detritus (49%), diatom frustules (32%), leaf material (8%), insect material (7%), and diatom stalks (2%) (Fig. 5A). On average, the monthly proportion of diatom frustules in gut-contents, a high-quality food resource to aquatic insects, was significantly lower in December, prior to ice break-up events, than all other months (Fig. 6A). Following the initial ice break-up event, however, the proportion of diatom frustules increased significantly by 166% between December and January (Fig. 6A). In addition, the proportion of diatom frustules increased 13% after the second ice break-up event, between January and February, although not significantly (Fig. 6A). A significant positive relationship occurred between days since initial ice-out and the proportion of diatom frustules in gut-contents (*n* = 24, *r* = 0.48, *p* = 0.0171). In contrast to diatom frustules, the proportion of diatom stalks, a lower-quality food resource to aquatic insects compared to frustules, was greatest in December, prior to ice break-up events, and decreased through March (Fig. 5A). The proportion of diatom stalks decreased by 45% and 57% following the ice break-up events in December and February, respectively (Fig. 5A). Similar to the pattern of diatom stalks in gut-contents, the proportion of amorphous detritus was significantly greater in December, prior to ice break-up events, than all other months (Fig. 6B). Following the initial ice break-up event, the proportion of amorphous detritus decreased by 34% between December and January, and the difference was significant (Fig. 6B). Overall, diets of *Rithrogena* were composed of a greater proportion of diatoms frustules following ice break-up events, which also corresponded to a reduction in the proportions of diatom stalks and amorphous detritus in gut-contents.

Diets of the scraper midge, *Bibiocephala*, consisted primarily of diatom frustules (52%), amorphous detritus (36%), diatom stalks (10%), and leaf material (2%) (Fig. 5B). On average, the monthly proportion of diatom frustules in gut-contents was lowest in December, prior to ice break-up events (Fig. 6A). Following the initial ice break-up event, however, the proportion of diatom frustules increased by 35% between December and January, although not significant (Fig. 6A). The proportion of diatom frustules in gut-contents remained at >50% from January through March (Fig. 6A). Days since initial ice break-up was not correlated with the proportion of diatom frustules in gut-contents (*n* = 28, *r* = 0.26, *p* = 0.1773). Following the initial ice break-up event, the proportion of diatom stalks decreased by 37% between December and January, although not significant

(Fig. 5B). Proportions of diatom stalks, however, were consistently variable (large 95% CI) throughout our study. Additionally, the proportion of amorphous detritus was greatest in December, prior to ice break-up events, and decreased by 17% and 7% following ice break-up events in December and February, respectively (Fig. 6B). Overall, diets of *Bibiocephala* were composed of a greater proportion of diatom frustules following ice break-up events, which also corresponded to a reduction in the proportion of amorphous detritus.

Diets of the collector-gatherer mayfly, *Baetis*, consisted primarily of diatom frustules (49%), amorphous detritus (34%), diatom stalks (7%), leaf material (7%), and filamentous algae (2%) (Fig. 5C). On average, the monthly proportion of diatom frustules was lowest in December, prior to ice break-up events (Fig. 6A). Following the initial ice break-up event, however, the proportion of diatom frustules increased by 46% between December and January, and the difference was significant (Fig. 6A). The proportion of diatom frustules in gut-contents remained at ~50% from January through March (Fig. 6A). Days since initial ice break-up was not correlated with the proportion of diatom frustules in gut-contents ($n = 24$, $r = 0.31$, $p = 0.1415$). In contrast to diatom frustules, the proportion of diatom stalks was greatest in December, prior to ice break-up (Fig. 5C). Following ice break-up events in December and February, the proportion of diatom stalks decreased by 71% and 42%, respectively, although not significantly (Fig. 5C). Similarly to diatom stalks, the proportion of amorphous detritus was greatest in December, prior to ice break-up (Fig. 6B). The proportion of amorphous detritus decreased by 22% between December and January, following initial ice break-up, and the difference was significant (Fig. 6B). Additionally, the proportion of amorphous detritus was significantly lower in

March than in all other months (Fig. 6B). Overall, diets of *Baetis* were composed of greater proportions of diatom frustules following ice break-up events, which also corresponded to a reduction in the proportions of diatom stalks and amorphous detritus.

Diets of the collector-gatherer midge, non-Tanypodinae Chironomidae, consisted primarily of diatom frustules (51%), amorphous detritus (30%), leaf material (12%), diatom stalks (4%), and fungi (3%) (Fig. 5D). On average, the monthly proportion of diatom frustules was greatest in March while the proportion of amorphous detritus was lowest (Fig. 7). Between December and February, the proportion of diatom frustules ranged from 40 – 50 % and did not differ significantly following ice break-up events (Fig. 7A). A significant positive relationship occurred between days since initial ice-out and the proportion of diatom frustules in gut-contents ($n = 24$, $r = 0.54$, $p = 0.0061$). This positive correlation was driven by high proportions of diatom frustules in March. In contrast to diatom frustules, the proportion of diatom stalks was greatest in December and decreased following the ice break-up events (Fig. 5D). Diatom stalks accounted for 15% and 2% of gut-contents in December and January, respectively, however diatom stalks were not observed in February or March, following 2 ice break-up events (Fig. 5D). Overall, non-Tanypodinae Chironomidae consistently consumed high proportions of diatom frustules, but their diets did not shift significantly following ice break-up events.

Diets of the collector-filterer black fly, *Simulium*, consisted primarily of amorphous detritus (49%), diatom frustules (29%), leaf material (16%), fungi (5%), and diatom stalks (3%) (Fig. 5E). On average, the monthly proportion of diatom frustules was lowest in December and increased through March (Fig. 6A). Following ice break-up events in December and February, the proportion of diatom frustules increased by 67% and 32%, respectively, although not significant (Fig. 6A). A significant positive relationship occurred between days since initial ice-out and the proportion of diatom frustules in gut-contents ($n = 21$, $r = 0.70$, $p = 0.0004$). Following the initial ice break-up event, diatom stalks accounted for 12% of gut-contents in January, however, diatom stalks were not observed in any other months (Fig. 5E). In contrast to diatoms, the proportion of amorphous detritus in gut-contents was significantly greater in December than all other months and decreased through February (Fig. 6B). Following the initial ice break-up event, the proportion of amorphous detritus decreased by 36% between December and January, and the difference was significant (Fig. 6B). Overall, *Simulium* consumed a greater proportion of diatoms frustules following 2 ice break-up events, which also corresponded to a reduction in the proportion of amorphous detritus.

Diets of the collector-filterer caddisfly, *Arctopsyche*, were dominated by insect material. Overall, the dominant proportion of food particles in the diets of *Arctopsyche* consisted of insect material (72%), diatom frustules (12%), amorphous detritus (8%), and diatom stalks (7%) (Fig. 5F). On average, the monthly proportion of insect material in gut-contents ranged from 64 – 80% and did not differ significantly following ice break-up events (Fig. 5F). Diatom frustules consistently composed <14% of gut-contents for all months and did not differ following ice break-up events (Fig. 7A). Days since initial ice break-up was not correlated with the proportion of diatom frustules in gut-contents ($n = 24$, $r = -0.0051$, $p = 0.9812$). In addition, diatom stalks consistently composed <12% of gut-contents for all months and did not differ following ice break-up events (Fig. 5F). Prior to ice break-up events in December, the proportion of amorphous detritus was greatest (Fig. 7B). Following the initial ice break-up event, however, the proportion of amorphous detritus decreased by 48% between December and January, although not significantly (Fig. 7B). Overall, *Arctopsyche* consistently consumed large proportions of insect material and diatom frustules, both high-quality food resources, but their diets did not shift significantly following ice break-up events.

Diets of the shredder stonefly, *Pteronarcys*, consisted of the greatest variety in food particles of all taxa examined. Overall, the dominant proportion of food particles in the diets of *Pteronarcys* consisted of wood (24%), diatom frustules (21%), leaf material (19%) amorphous detritus (16%), diatom stalks (11%), and insect material (6%) (Fig. 5G). The proportion of wood in the gut-contents was lowest in January and increased significantly by 302% from January to February, following the second ice break-up event (Fig. 5G). Prior to ice break-up, the proportion of diatom frustules was lowest in December (Fig. 6A). The proportion of diatom frustules increased by 176% between December and January, following the initial ice break-up event (Fig. 6A). A significant positive relationship existed between days since initial ice-out and the proportion of diatom frustules in gut-content ($n$ = 28, $r$ = 0.42, $p$ = 0.0248). In contrast to diatom frustules, the proportion of diatom stalks decreased from December through February following 2 ice break-up events (Fig. 5G). The proportion of diatom stalks decreased by 531% between January and February following the second ice break-up event, and the difference was significant (Fig. 5G). Leaf material consistently composed 15 – 24% of gut-contents for all months and did not differ following ice break-up events (Fig. 5G). Similarly, amorphous detritus and insect material composed 14 – 19% and 3 – 9% of gut-contents for all months, respectively, and did not differ following ice break-up events (Fig. 5G). Overall, diets of *Pteronarcys* were composed of greater proportions of diatom frustules following ice break-up events, which also corresponded to a reduction in the proportion of diatom stalks.

## Discussion

Our study demonstrates river ice is a crucial disturbance affecting river ecosystems in temperate zones, specifically the organic matter dynamics and feeding ecology of aquatic insects. Disturbance by river ice affected both the quantity and quality of organic matter available to aquatic consumers. Biomass of biofilm on rocks (AFDM and chl-*a*) was greatest prior to ice break-up events. Scour from ice break-up events, however, reduced biomass of biofilm (AFDM and chl-*a*) while the quality of biofilm (chl-*a* : AFDM) increased. Diets of most aquatic insects we studied (*Rithrogena, Bibiocephala, Baetis*, *Simulium, Pteronarcys*) appeared to follow the patterns of organic matter in response to disturbance by river ice. These taxa consumed greater proportions of diatom frustules, a high-quality food resource (i.e., rich in lipids and fatty acids), following 2 ice break-up events. In contrast, 2 taxa (non-Tanypodinae Chironomidae and *Acrtopsyche*) consistently consumed large proportions of diatom frustules and insect material, respectively, which are high-quality food resources. Our results indicate the ecological effects of ice break-up events may be similar to those of flood disturbance; however, disturbance by ice is distinctive because it typically occurs during times of the year not characterized by flooding (Grimm & Fisher 1989, Junk et al. 1989, Cross et al. 2011). Other aspects of river ice regimes may mirror the effects of drought disturbance, such as reduced habitat space, reduced organic matter in transport, and accumulation of sediments (Lake 2003). We hypothesize that ice disturbance in rivers is unique, because ice regimes incorporate both flood and drought disturbances. We acknowledge that our observations were limited to one river system; therefore, we suggest additional ecological studies of river ice regimes be conducted in other regions to investigate the extent to which the patterns we observed can be generalized. Nonetheless, just as every river has a unique flow-regime, there are likely different ice regimes for different river systems and thus, ice regimes need to be more consistently described as a dimension of the disturbance regime of rivers.

### *Organic matter dynamics affected by ice regime*

We documented that disturbance by river ice regimes affect organic matter dynamics of rivers in temperate zones. Congruent with our initial prediction that ice break-up events would reduce biomass of biofilm, both AFDM and chl-*a* of biofilm decreased following the December and February ice break-up events. In contrast, the quality of biofilm (chl-*a* : AFDM) increased following the 2 ice break-up events. Additionally, we visually documented a reduction of thick, brown mats of diatoms covering the riverbed following the initial ice break-up. Two, large diatoms dominated the brown mats collected from rocks prior to ice break-up, *Cymbella janischii* and *Didymosphenia geminata*; both species grow attached to substrates by vertical, muco-polysaccharide stalks (Bahls 2007, Blanco and Ector 2009). These 2 taxa of diatoms, which are native to the western United States, may grow large amounts of stalks that may cover the riverbed and cause ‘nuisance blooms’ (Bahls 2007, Blanco and Ector 2009). Our interpretation of these results is that scour from ice-out events may export accumulated organic matter of lower quality (detritus, diatom stalks, etc.) and expose areas of riverbed for colonization by organisms (diatom frustules, algae, etc.) that provide high-quality organic matter to consumers like insects. Our findings are consistent with prior research of the response of biofilm communities to flood disturbance, indicating a reduction in quantity of organic matter, but an increase in the quality of organic matter (Power & Stewart 1987, Grimm & Fisher 1989). In addition, our results indicate river-ice regimes may influence the abundance and distribution of nuisance blooms of diatoms, which are becoming problematic globally (Bahls 2007, Blanco and Ector 2009).

In contrast to the response of biofilm, our observations of seston did not support our initial prediction that ice break-up events would increase the biomass of transported organic matter. Responses of seston appeared to differ between the first and second ice break-up events. For example, AFDM increased slightly but chl-*a* decreased by 70% following the initial ice break-up in December. In contrast, AFDM decreased 80% but chl-*a* decreased slightly following the second ice break-up in February. No difference in AFDM in transport between December and January was likely attributed to residual export of organic matter from scour associated with the initial ice break-up event. Chl-*a* in transport, however, decreased following both ice break-up events, and colonization of scoured substrates by autotrophs that are less likely to slough may explain this result. From direct observation, however, ice break-up events greatly elevated suspended sediments and coarse organic matter in transport (Blackadar, personal observation). Our results for seston also may have been influenced by difficulties associated with placing nets into the water column with ice in transport. In spite of this, disturbance by river ice regimes is an importance process in controlling the quantity and quality of organic matter resources available to aquatic consumers during winter.

### *Biofilm in winter vs. summer*

Seasonal differences in biofilm values were dramatic in Big Creek and organic matter available to aquatic consumers in winter was not stable. Results from our study indicate that biofilm is highly productive and variable during winter. Average biofilm values during our winter study (December 2010 – March 2011) were substantially higher compared with average biofilm values over 4 years of summer monitoring (July 2008 – 2011, Baxter unpublished). Specifically, AFDM of biofilm in winter was 232% greater than biofilm in summer (17.27 vs. 5.20 g $m^{-2}$, respectively). Large AFDM values of biofilm in winter may be partially explained by senesced summer growth and accumulation of detritus following peak flow in spring (May – June). In addition, chl-*a* of biofilm in winter was 1234% greater than biofilm in summer (142.86 vs. 10.71 mg $m^{-2}$, respectively). This result is counterintuitive because summer is generally thought of as the most productive time of year, because of higher inputs of photosynthetically active radiation and warmer water temperatures. Additionally, the ratio of chl-*a* to AFDM was 288% greater than summer values (9.25 vs. 2.39 mg $g^{-1}$). Collectively, our results indicate that winter is a highly productive season in Big Creek. During winter 2010 – 2011, we observed the greatest biomass of biofilm and also the greatest quality of biofilm. Research focused on gross primary-production in wilderness rivers during winter is needed because such data are scarce. Ecologists must view winter as a critical period for aquatic productivity, a season generally overlooked because of the challenges associated with conducting aquatic research.

### *Response of aquatic insects to ice disturbance*

Our results indicated that diets of the scrapers, *Rithrogena* and *Bibiocephala*, and a collector-gatherer, *Baetis*, followed the patterns of biofilm in response to disturbance by river ice. In December, prior to ice break-up events, diets of *Rithrogena, Bibiocephala*, and *Baetis* were composed of high proportions of amorphous detritus and low proportions of diatom frustules. In contrast, following the ice break-up events that increased the quality (chl-*a* : AFDM) of biofilm, the proportion of amorphous detritus decreased and the proportion of diatom frustules increased in the diets of *Rithrogena, Bibiocephala,* and *Baetis*. In addition, the proportion of diatom stalks in diets was greatest in December, prior to ice break-up disturbances, but decreased following the initial ice break-up event. The trends observed in the diets of *Rithrogena, Bibiocephala*, and *Baetis* indicate that as the quantity (AFDM and chl-*a*) of biofilm decreased, these taxa consumed a greater proportion of diatom frustules. Additionally, diatom frustules in diets increased as biofilm quality (chl-*a* : AFDM) increased. These taxa are known to reside in fast, flowing waters and in cobble/rock habitats (Poff et al. 2006). Previous research documented these taxa feed primarily upon biofilm, and specifically diatom frustules, detritus, and algae (Poff et al. 2006). Our results are consistent with prior studies on diets of these taxa; however, our research highlights the foraging ecology of aquatic insects may shift in response to disturbance by river ice. Diatoms, a high-quality food source, may be more available for consumption following ice break-up events that reduce overall biofilm biomass, but expose freshly scoured substrate to colonization by diatoms. Scouring floods are known to decrease benthic biomass and primary producers may colonize the fresh substrate quickly (Power & Stewart 1987, Grimm & Fisher 1989). In addition, floods may promote diversity of aquatic producers allowing the most palatable species to persist (Elger et al. 2004). Ice break-up events in winter likely play a similar role as other types of flood disturbance in altering availability of high-quality food sources to aquatic consumers.

Diets of the collector-filtering black fly, *Simulium*, followed patterns in seston in response to disturbance by river ice. Analogous to taxa with diets that followed patterns of biofilm, diets of *Simulium* were composed of a high proportion of amorphous detritus and a low proportion of diatom frustules in December prior to ice break-up. In contrast, following the ice break-up events, the proportion of amorphous detritus decreased and the proportion of diatom frustules increased. Scour from ice break-up events exported accumulated organic matter of low quality to downstream environments. Following multiple ice break-up events, seston had lower concentrations of organic matter. Organic matter available for transport may have proportionally higher concentrations of high-quality diatoms because of the reduction in accumulated organic matter and the rapid colonization by autotrophs following ice break-up.  Increased proportions of diatoms in transport may have increased the proportion of diatoms frustules in guts-contents of *Simulium* following ice break-up events. *Simulium* also resides in fast, flowing waters (Merritt and Cummins 1996), and are collector-filterers that feed primarily on fine seston and consume a variety of food resources including detritus, diatom frustules, algae, and leaf material (Poff et al. 2006). Previous research has documented that they may track the availability of organic matter resources in seston (Erman and Chouteau 1979; Hart and Latta 1986; Hart 1987, Wellard-Kelly et al. 2013). Our research supports those previous conclusions; seston composition likely changed post ice break-up, and changes were manifested in the diets of *Simulium*.

Diets of the shredder stonefly, *Pteronarcys*, included the greatest variety of food resources and were the only taxa that consumed wood. Patterns observed in *Pteronarcys* diets mimicked patterns observed in *Rithrogena*, *Bibiocephala*, *Baetis,* and *Simulium* for particular diet items. For example, following ice break-up events, the proportion of diatom frustules increased and the proportion of diatom stalks decreased. This was likely attributed to changes in the relative availability of these resources.  Further patterns were difficult to distinguish because *Pteronarcys* exhibited the highest degree of omnivory in our study. Because *Pteronarcys* consumed a variety of organic matter resources, they are important in river ecosystems for processing a wide range of organic matter.

Diets of the collector-gatherer midge, non-Tanypodinae Chironomidae, and the collector-filterer caddisfly, *Arctopsyche* did not appear to follow the patterns in organic matter as closely as the previously discussed taxa. Chironomids consistently consumed large proportions of diatom frustules throughout our study. The proportion of diatom frustules in diets of Chironomids was not affected by the ice break-up. Previous research has documented the ability of chironomids to select for algal resources of high quality (Berg 1995; Henriques-Oliveira et al. 2003). We hypothesize the mobility and feeding mode of Chironomids may allow them to select for diatom frustules disproportionately to their availability. In addition, diets of *Arctopsyche,* did not change in response to ice break-up. Like Chironomids, *Arctopsyche*, may be able to select for a food item of particularly high quality. For example, *Arctopsyche,* consumed primarily insect material and this was consistent throughout our study. Insect material is a high-quality food, and *Arctopsyche* may specialize in obtaining that food resource. Collectively, non-Tanypodinae Chironomidae and *Arctopsyche* consistently consumed large proportions of high-quality food resources throughout our study and diets of these taxa did not shift following ice break-up events.

We faced several limitations while conducting this study. Our study did not evaluate the compositional changes of organic matter (biofilm and transported), therefore, we could not directly investigate patterns in resource use of aquatic insects compared with their availability. Additionally, we did not analyze differences in consumption of food resources compared with their relative assimilation. We recognize that observed differences in organic matter may be affected by factors that we did not quantify, such as light, temperature, and nutrients. Furthermore, our study focused on the diets of late-instars of aquatic insects, however, diets may shift during larval development so our results should be viewed accordingly. Nonetheless, we documented distinct patterns in organic matter, feeding ecology of aquatic insects, and river-ice processes during winter. Future research aimed at the issues listed previously would provide additional insight to the responses of both organic matter and feeding patterns of aquatic insects to disturbance by river ice.

### *Climate change and river ice*

Most concerns regarding temperature alteration expected to accompany climate change have focused on increases in summer water temperatures; however, major changes of thermal regimes in winter could affect river ice. We documented that organic matter and diets of aquatic insects were affected by ice break-up events during winter. Increased temperatures in winter associated with climate change, however, may alter the timing of ice break-up events and this shift has important consequences for basal resources and consumers in river ecosystems. For example, snowmelt and run-off in western North America have shifted to occur 10 - 30 days earlier in spring, and this change may alter the quantity and quality of biofilm available to aquatic invertebrates (Stewart, Cayan & Dettinger, 2004, Davis et al. 2013). Additionally, shifts in winter temperatures may become so extreme that river ice and associated ice break-up events may be eliminated completely in some regions. We hypothesize that loss of river ice and associated disturbances from ice will have dramatic consequences for quantity and quality of basal resources available to aquatic consumers. Although increased atmospheric temperatures have been correlated with decreased spatial extent and duration of ice cover within a few eco-regions (Prowse and Beltaos 2002), understanding how climate-induced shifts may affect aquatic ecosystems is limited because of the paucity of studies exploring the winter ecology of rivers. A more complete understanding of the winter ecology of rivers is needed in order to predict the potential effects of climate change that are not incorporated into our current understanding of effects from climate change.

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# CHAPTER 1: FIGURES & TABLE

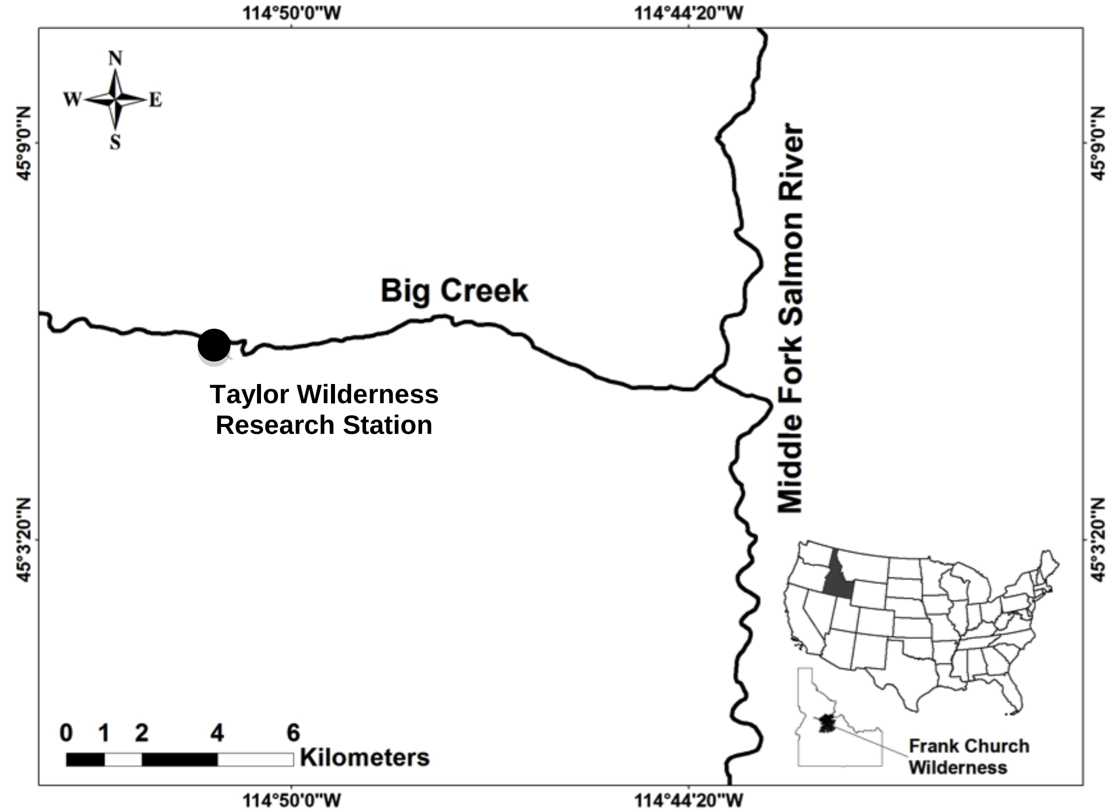

Figure 1. Map of Big Creek and Middle Fork Salmon River of central Idaho, USA. Location of Taylor Wilderness Research Station is noted on Big Creek by a closed circle. Inset shows the location of wilderness area in state of Idaho and USA.

Table 1. Ice regime of Big Creek, Idaho, USA, during winter 2010 – 2011.

| Date | Habitat | Ice type present (Yes or No) | | | |
|---|---|---|---|---|---|
| | | Surface | Frazil | Anchor | Ice break-up |
| Nov. 2010 | Edge | Yes | Yes | Yes | No |
| | Thalweg | No | Yes | Yes | No |
| Dec. 2010 | Edge | Yes | Yes | Yes | Yes |
| | Thalweg | No | Yes | Yes | Yes |
| | | | | | 27 Dec. 2010 |
| Jan. 2011 | Edge | Yes | Yes | Yes | No |
| | Thalweg | No | Yes | Yes | No |
| Feb. 2011 | Edge | Yes | Yes | Yes | Yes |
| | Thalweg | No | Yes | Yes | Yes |
| | | | | | 3 Feb. 2011 |
| Mar. 2011 | Edge | No | No | No | No |
| | Thalweg | No | No | No | No |

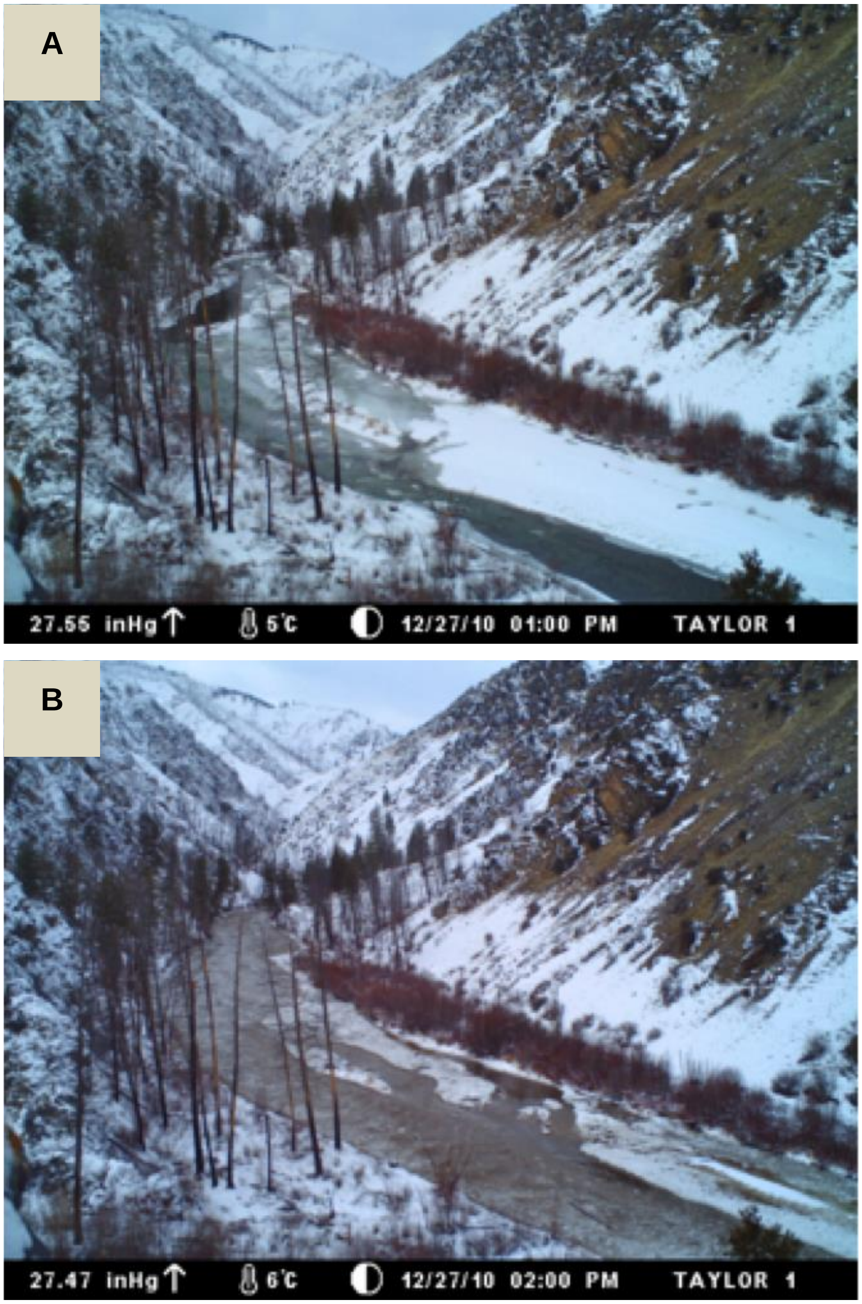

Figure 2. Photos from digital-interval camera documented conditions of Big Creek in Idaho, USA, immediately prior to initial ice break-up event (A) and one hour later during the December 27, 2010 ice break-up event (B). Note the increase in river discharge as indicated by the reduction of exposed gravel bar and the change in color of water.

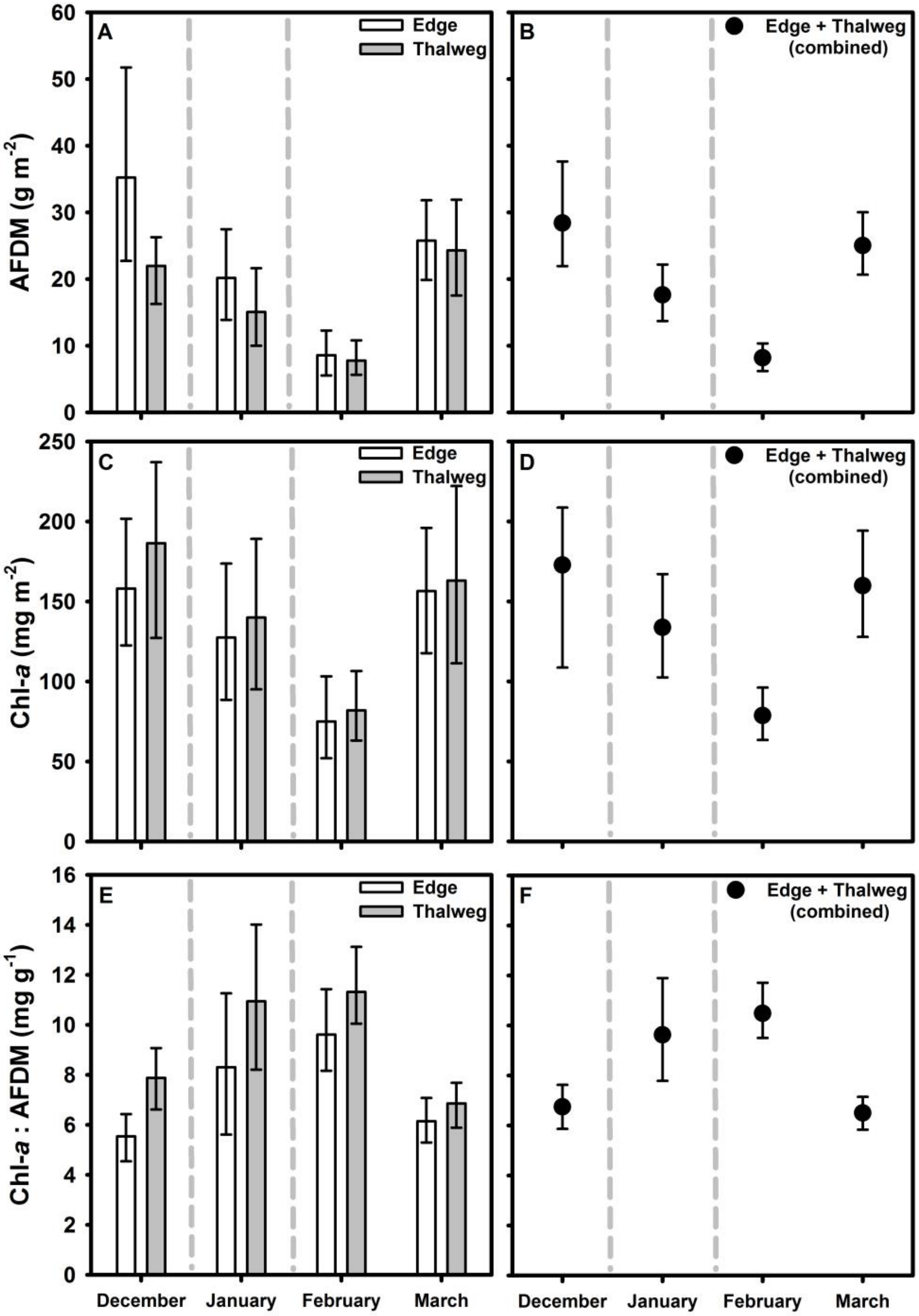

Figure 3. Biofilm monthly (Mean ± 95% CI) ash-free dry mass (A), chlorophyll-*a* (C), and ratio of chl-*a* to AFDM (E) in thalweg and edge habitats in Big Creek, Idaho, USA during winter 2010 - 2011. Monthly (Mean ± 95% CI) ash-free dry mass (B), and chlorophyll-*a* (D), and ratio of chl-*a* to AFDM (F) of biofilm in combined habitats (thalweg + edge). Dashed, vertical lines represent dates of ice break-up events.

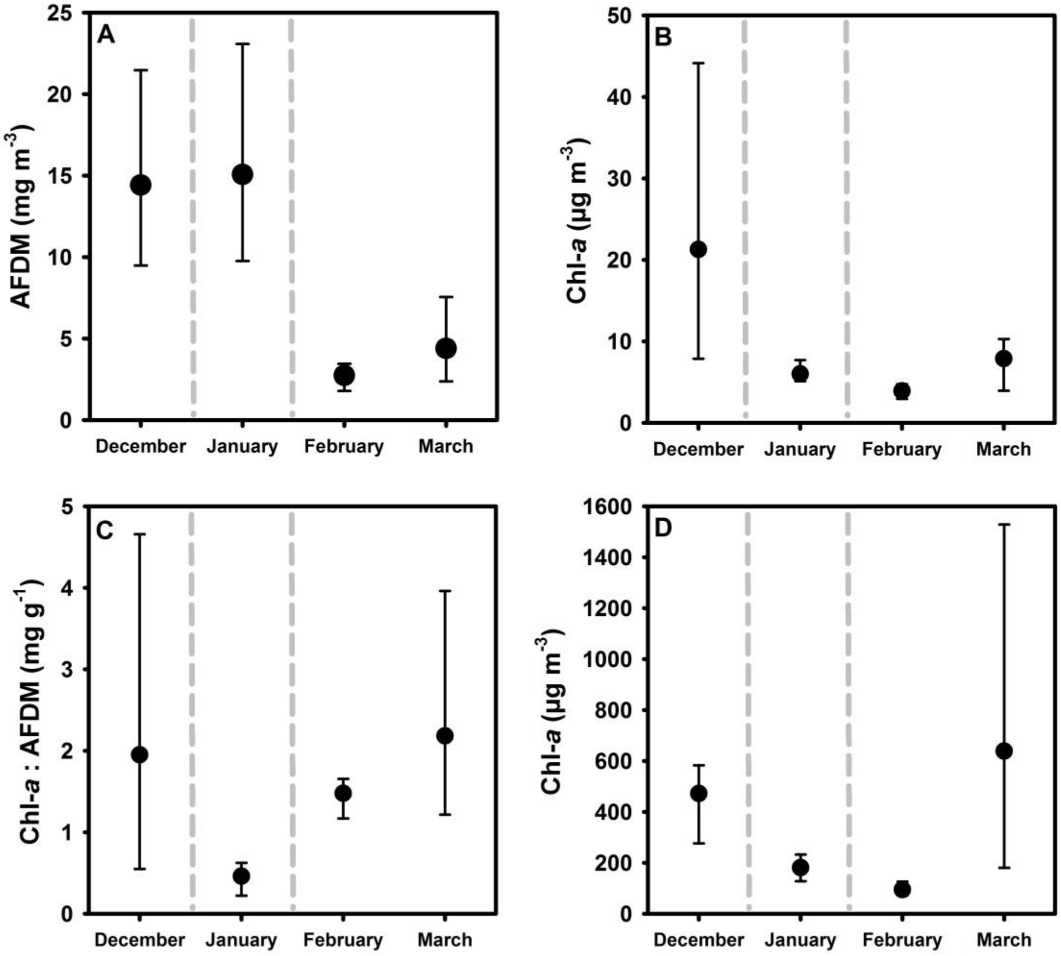

Figure 4. Transported organic matter monthly (Mean ± 95% CI) ash-free dry mass (A), chlorophyll-*a* (B), and the ratio of chl-*a* to AFDM (C) of coarse (>1mm) and chlorophyll-*a* (D) of fine (<1 mm, ≥ 53 µm) in Big Creek, Idaho, USA during winter 2010 - 2011. Dashed, vertical lines represent dates of ice break-up events.

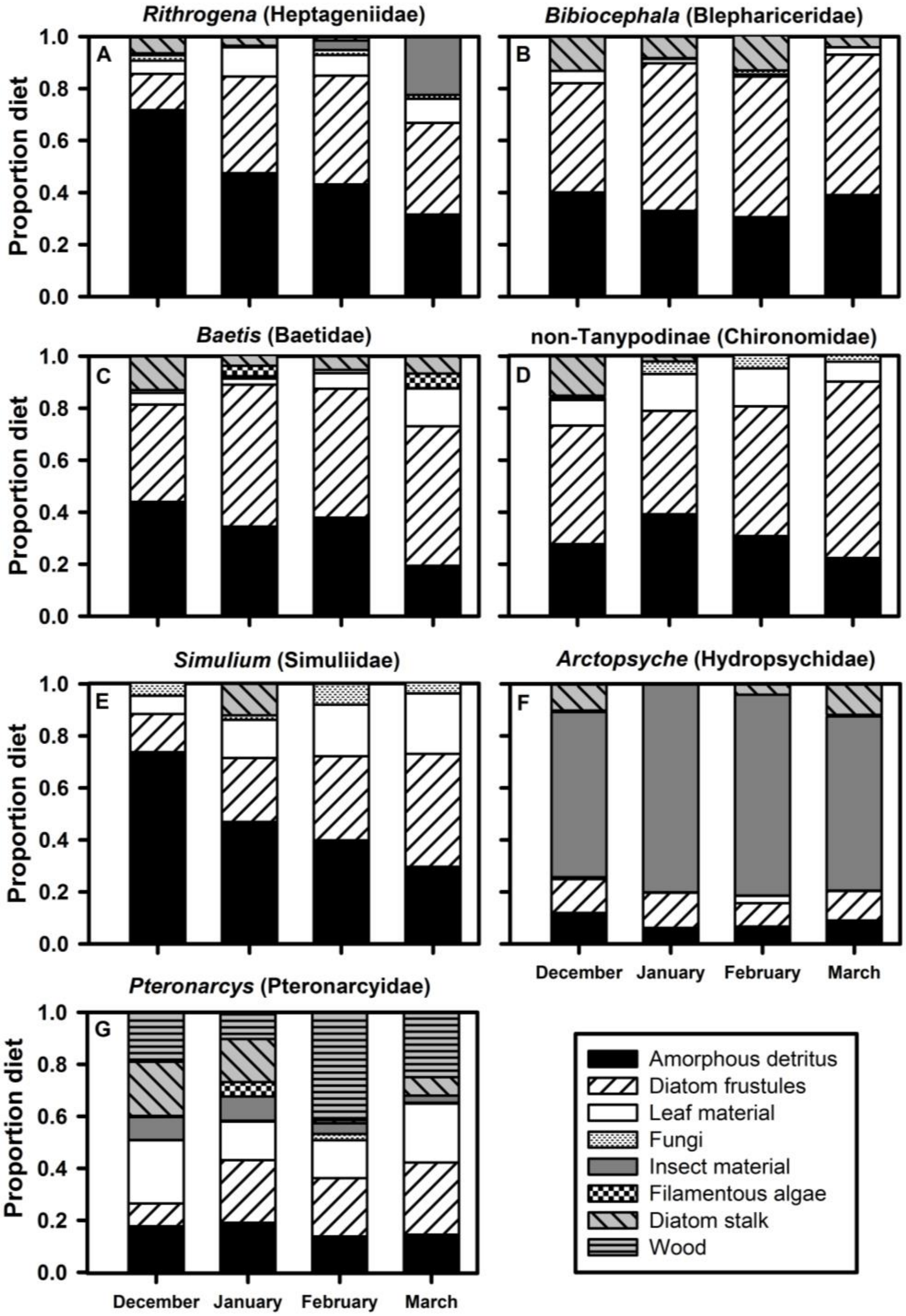

Figure 5. Proportion of different food items in the diets of aquatic insects collected from Big Creek in Idaho, USA. Individuals were collected between Dec. 2010 and Mar. 2011. Stacked bars represent the average ($n$ = 3 - 7) proportion of food-particle types in gut-contents of *Rithrogena* (A), *Bibiocephala* (B), *Baetis* (C), non-Tanypodinae Chironomidae (D), *Simulium* (E), *Arctopsyche* (F), and *Pteronarcys* (G).

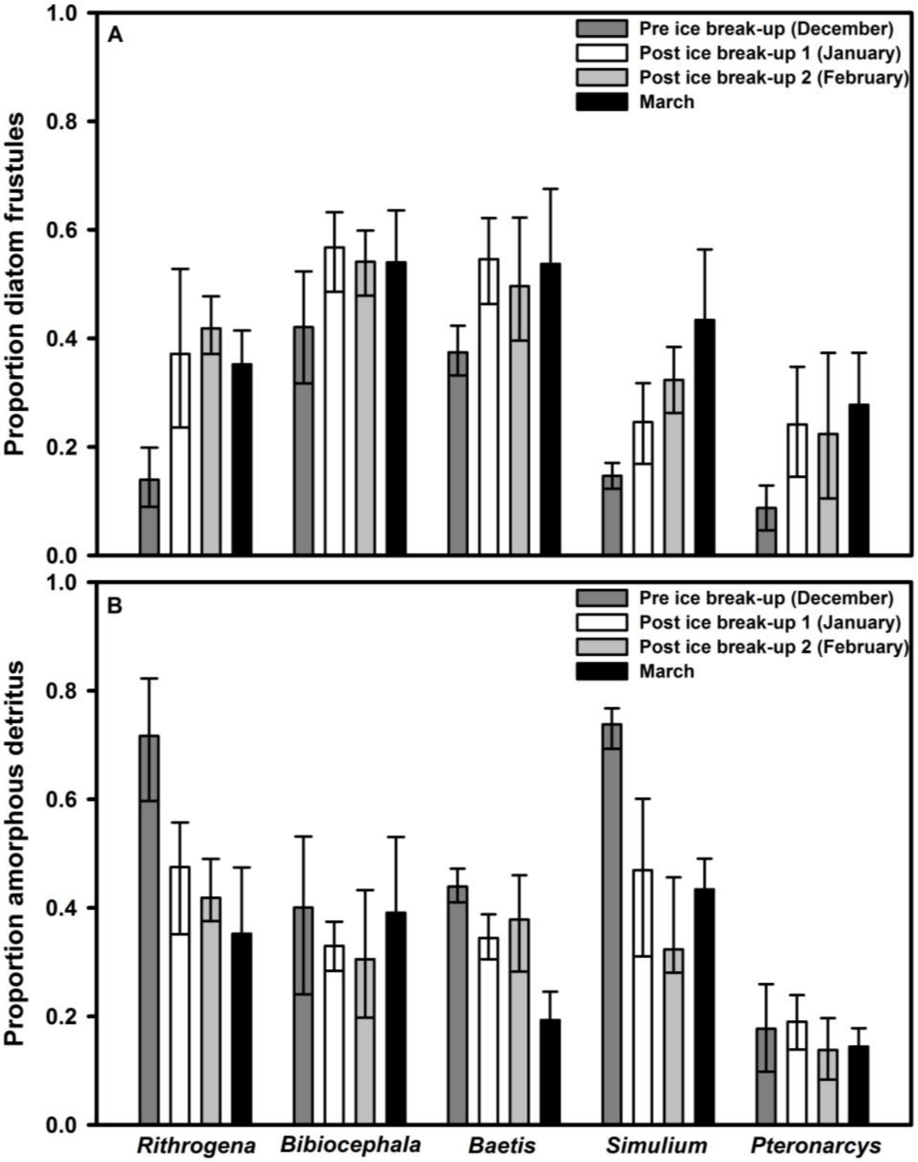

Figure 6. Monthly (Mean ± 95% CI) proportion of diatom frustules (A) and amorphous detritus (B) in gut-contents of aquatic insects collected from Big Creek in Idaho, USA. Proportions of diatom frustules increased and amorphous detritus decreased in diets of aquatic insects following ice break-up. Dark gray bars represent diets in December 2010 prior to ice break-up, white bars represent diets in January 2011 following initial ice break-up event, light gray bars represent diets in February 2011 following second ice break-up event, and black bars represent diets in March 2011.

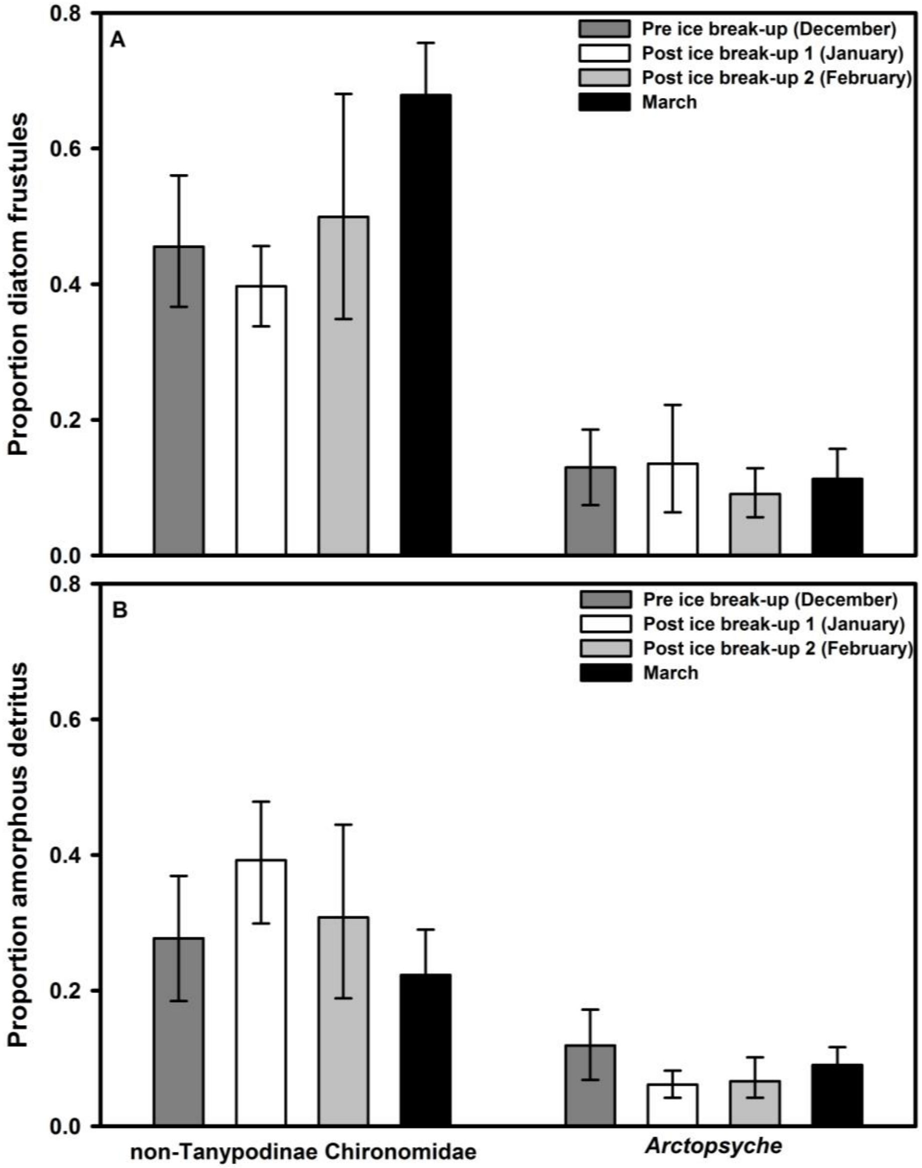

Figure 7. Monthly (Mean ± 95% CI) proportion of diatom frustules (A) and amorphous detritus (B) in gut-contents of aquatic insects collected from Big Creek in Idaho, USA. Proportions of diatom frustules and amorphous detritus were not affected by ice break-up events. Dark gray bars represent diets in December 2010 prior to ice break-up, white bars represent diets in January 2011 following initial ice break-up event, light gray bars represent diets in February 2011 following second ice break-up event, and black bars represent diets in March 2011.

# CHAPTER 2

## Of rivers, elk and wolves: effects of elk carcasses on nutrients, biofilm, and aquatic insects

Ryan J. Blackadar, Colden V. Baxter, and John M. Davis

## Abstract

Terrestrial and aquatic ecosystems are connected by the exchange of materials and organisms, referred to as resource subsidies. Vertebrate carcasses derived from terrestrial ecosystems, however, have not been previously considered as resource subsidies to aquatic habitats. We investigated effects of ungulate carcasses originating from gray wolf (*Canis lupus*) kills on aquatic food webs after the carcasses were deposited into Big Creek, a 6th - order tributary of the Middle Fork Salmon River in central Idaho, USA, in 2011. We collected samples of dissolved nutrients and biofilm to examine the effects of an elk (*Cervus elaphus*) carcass on aquatic producers. Additionally, we collected aquatic insects that had colonized a submerged elk carcass and from sites not receiving a carcass to determine the effects of a terrestrial resource subsidy on aquatic consumers. Dissolved nutrients and biofilm values, however, did not respond to submerged elk carcasses as initially predicted. Rather than direct consumption of elk carcass, results from gut-content analysis indicated aquatic insects that colonized a submerged elk carcass consumed a greater proportion of diatom frustules than aquatic insects at sites not receiving a carcass. Moreover, stable isotope values (δ13C and δ15N) of aquatic insects that colonized an elk carcass were more similar to biofilm values, whereas aquatic insects at sites without a carcass more closely resembled a mixture of biofilm and seston. Our results indicated that the input of ungulate carcasses to aquatic ecosystems provide fresh substrates for colonization by biofilm communities. In addition, ungulate carcasses in rivers may also provide nutrients to aquatic primary producers and consumers because aquatic insects that colonized a submerged elk carcass were enriched in stable isotope ratios of δ13C and δ15N. Additional studies are needed to determine if other aquatic organisms, such as aquatic macroinvertebrates, fishes, and river otters (*Lontra canadensis*), directly consume elk carcasses when deposited into river ecosystems. Future research should investigate other ecological consequences of terrestrial vertebrate carcasses as resource subsidies to aquatic ecosystems.

**Key words**

biofilm, nutrients, aquatic insects, gut-content analysis, stable isotopes, algae, wolves,

## Introduction

Ecologists have long recognized that streams and rivers are closely linked to adjacent, terrestrial ecosystems. Past research has demonstrated the connection of aquatic and terrestrial ecosystems by flows of nutrients, organisms, and energy, referred to as resource subsidies (Polis et al. 1997). Early research on resource subsidies focused on the flux of organic matter between ecosystems, particularly the input of terrestrial leaf litter to aquatic systems (Fisher and Likens 1973, Hynes 1975). More recent research has demonstrated that fluxes of aquatic and terrestrial invertebrates are critical food resources for organisms in both aquatic and terrestrial ecosystems (Baxter et al. 2005). For example, terrestrial invertebrates are food for predatory fishes when delivered to aquatic ecosystems; reciprocally, aquatic insects are prey to terrestrial consumers (i.e., birds, lizards, bats, etc.) when they emerge (Jackson and Fisher 1986, Nakano and Murakami

2001, Sabo and Power 2002). Furthermore, prior research has demonstrated that exchange of food resources between aquatic and terrestrial ecosystems, though quantitatively small (i.e., biomass), may represent subsidies to consumers in adjacent ecosystems of disproportionate quality (i.e., nutrients or energy, Baxter et al. 2005, Marcarelli et al. 2011).

The exchange of vertebrate carcasses across ecosystem boundaries may represent a resource subsidy of disproportionate quality. For instance, the death of whales (Cetacea) and deposition of their carcasses represents a rare subsidy from the upper ocean zones to the ocean floor, which supports a diverse range of organisms for decades (Smith and Baco 2003, Fujiara et al. 2007). Similarly, carcasses from marine vertebrates support terrestrial consumers (i.e., coyotes, *Canis latrans*) when they are washed ashore (Rose and Polis 1998). Though less rare than the previous example, salmon transport large amounts of marine-derived energy and nutrients to freshwater habitats and increase dissolved nutrients and biomass of biofilm communities (Wipfli et al. 1998, Cederholm et al. 1999). In addition, subsidies of salmon carcasses can stimulate aquatic insects directly through consumption (Winder et al. 2005), and indirectly by increasing the quality of their basal resources (Wipfli et al. 1998, Chaloner and Wipfli 2002). Because vertebrate carcasses can be important cross-ecosystem subsidies, their inputs to rivers may have similar positive effects on the recipient ecosystem.

Gray wolves frequently kill ungulates in and along streams and rivers, and the remains of these animals, though typically a small input in terms of biomass, may represent a high-quality resource subsidy to aquatic food webs. In terrestrial ecosystems, ungulate carcasses originating from wolf kills directly support a wide range of terrestrial scavengers (i.e. eagles, coyotes, beetles, etc.; Sikes 1998, Wilmers et al. 2003). Ungulate carcasses can increase the nutrient content and microbial biomass of soils underlying carcasses, as well as leaf nutrient content of plants, indicating a bottom-up pathway by which ungulate carcasses may stimulate higher trophic levels. (Bump et al. 2009a, Bump et al. 2009b). If ungulate carcasses are deposited into rivers, they may support aquatic insects directly by consumption of carcass material and indirectly by enhancing the nutritional quality of their food resources, but this has not been investigated. Because wolf populations in North America have increased recently (Smith et al. 2010), the possibility that wolf-kill carcasses might influence aquatic ecosystems deserves investigation.

The objective of this study was to investigate the potential of carcasses from terrestrial vertebrates, in this instance, North American elk (*Cervus elaphus*) to be a subsidy to organisms in river ecosystems. We hypothesized that elk carcasses deposited into rivers create ‘islands’ of high-quality food resources for aquatic organisms. We predicted that concentrations of dissolved nutrients and biomass of biofilm would be higher at sites receiving an ungulate carcass than at sites without a carcass. In addition, we predicted that aquatic insects would feed directly on the elk carcass and consume a greater proportion of algal resources than similar taxa at sites without a carcass. Our broader aim was to contribute understanding of a unique terrestrial subsidy to a wilderness river ecosystem.

## Methods

### *Study Site*

Our study was based at Taylor Wilderness Research Station (45.1019° and 114.8517°W), within the Frank Church 'River of No Return' Wilderness Area in central Idaho, USA. This area is roadless and accessible only by trail or small aircraft. We conducted opportunistic sampling of two ungulate carcasses in conjunction with the more detailed monitoring of the winter ecology in Big Creek (Blackadar, Ch. 1). We studied the potential effects of subsidies by ungulate carcasses in this wilderness watershed to measure responses of an intact aquatic ecosystem. Study locations were located in the mainstem of Big Creek, a 6th - order tributary to the Middle Fork of the Salmon River (Fig. 1). Big Creek has a natural flow regime and diverse suite of native macroinvertebrates that encompass the range of feeding modes for montane streams and rivers within this region and other similar regions of the world. Additionally, the watershed has a full complement of native ungulates including North American elk, mule deer (*Odocoileus hemionus*), and bighorn sheep (*Ovis canadensis*) in addition to native large carnivores including gray wolf, cougar (*Puma concolor*), coyote, and bobcat (*Lynx rufus*). Big Creek flows through steep, mountainous terrain with severe changes in elevation from the valley bottoms near the station (1200 m) to high elevation peaks (3150 m). Forested slopes consist primarily of Douglas Fir (*Pseudotsuga menziesii*) and Ponderosa Pine (*Pinus ponderosa*), but xeric grass communities and sagebrush (*Artemisia*) are abundant on drier slopes (Robinson et al. 2005). Annual precipitation ranges from 38 to 50 cm in valleys and from 76 to 100 cm at higher elevations (Robinson et al. 2005). Most precipitation occurs as snow, resulting in peak stream flows from May to June. Baseflow conditions occur from late summer through winter.

### *Surveys of ungulate carcasses*

We conducted monthly surveys (December 2010 to April 2011) of a 24 km segment of Big Creek surrounding Taylor Wilderness Research Station (TWRS), via fixed-wing aircraft and backpacking. During monthly fixed-wing aircraft flights used to access TWRS, we visually surveyed the river channel for evidence of ungulate carcasses. We also used a digital videocamera to record the flight over the river channel; video-recordings were examined for evidence of ungulate carcasses upon landing. In addition, we conducted monthly backpacking surveys of the river channel for evidence of ungulate carcasses. This combination of surveys along a 24 km segment of Big Creek surrounding TWRS, 12 km upstream and 12 km downstream, provided accurate detection of ungulate carcasses.

Upon discovery of an ungulate carcass in or along Big Creek, we performed a necropsy analysis on the located carcass (O'Gara 1978). We recorded the species, sex, age-class, percentage of carcass remaining, and body parts present. We evaluated the cause of death of ungulate by noting presence of tracks, predator feces, and hair. In addition, we evaluated the carcass for signs of subcutaneous hemorrhaging and recorded signs of a struggle at the site of the kill.

### *Dissolved nutrients*

We collected water samples for analysis of dissolved nutrients from 1 m upstream ($n$ = 3) and 1 m downstream ($n$ = 3) of an elk carcass 1 day after it had been deposited into Big Creek on 22 March 2011. We filtered river water (120 mL) through a membrane filter (0.45 µm, Millipore IRL Ltd, Tullagreen Ireland**)** into acid-washed bottles with no headspace.  Water samples were frozen immediately and kept frozen until analysis. Concentrations of ortho-phosphate ($PO_4$), ammonium ($NH_4$) and nitrate/nitrite ($NO_3$ /

$NO_2$) were analyzed with a SmartChem Water Analyzer (Mandel Scientific Company, Canada). The detection limit was 0.01 mg $L^{-1}$ for orthophosphate ($PO_4$) and all values (upstream and downstream) were below the detection limit for this constituent. One value of ammonium ($NH_4$) downstream of elk carcass was below the detection limit (0.02 mg $L^{-1}$) so we assigned a value of one-half the detection limit. No values of nitrate/nitrite ($NO_3$ / $NO_2$) were below the detection limit (0.01 mg $L^{-1}$).

*Biofilm*

We sampled biofilm (assemblage of living and dead autotrophs, heterotrophic microbes, and detritus on rocks) from 5 m downstream of an elk carcass (*n* = 4) 1 day after it was deposited into Big Creek on 22 March 2011. We also collected samples from sites with similar habitat (c. 1 – 2 m from river edge with analogous water depth, velocity, substrate) without an elk carcass (*n* = 20). Three rocks were chosen haphazardly at each location, placed into plastics bags, and transported to the research station for processing. Rocks were scrubbed with a wire brush to remove all attached biofilm. The composite slurry from the combined three rocks was sub-sampled (20 - 50 mL), filtered onto pre-combusted (400° C for 30 min.) and pre-weighed glass-fiber filters (0.7 μm, Millipore IRL Ltd, Tullagreen, Ireland**)**, and frozen immediately. Samples were then transported back to Idaho State University for further analysis. The planar area of sampled rocks was traced onto paper. Rock tracings were cut, weighed, and converted to planar area using linear regression. This resulted in 24 total samples (4 downstream samples from an elk carcass and 20 without a carcass) from March 2011. We included additional values from sites without a carcass because biofilm can be highly variable (Blackadar, Ch. 1).

Based on standard methods (APHA, 1998, Davis et al. 2013), filters from each location were analyzed for chlorophyll-*a* (chl-*a*, measure of algal pigments and index of living primary producers) and ash-free dry mass (AFDM, measure of living and senesced primary producers). Filters were extracted in methanol for c. 12 h, analyzed for chl-*a* with a spectrophotometer and corrected for phaeophytin. After extraction, the filters and methanol extract containing the chl-*a* component were recombined, dried at 60 °C for at least 48 h, weighed and ashed at 550 °C for c. 4 h. Samples were then reweighed to calculate AFDM. Additionally, the chl-*a* values were divided by the corresponding AFDM values to generate the ratio of chl-*a* to AFDM, an indicator of biofilm quality.

### *Aquatic insect collection and laboratory processing*

We collected aquatic insects for gut-content analysis directly from one elk carcass submerged in Big Creek on 2 May 2011. We removed the elk carcass from the river, which had remained in the river 43 days since the initial kill, and collected aquatic insects from haphazard locations throughout carcass. In addition, aquatic insects collected at sites without a carcass in March 2011 on prior sampling trip were used as reference samples (no-carcass treatment). We were restricted to compare the gut-contents of aquatic insects on an elk carcass in May 2011 and aquatic insects at sites without a carcass in March 2011 because of logistical constraints. Results from our study should be interpreted accordingly.

Individuals were preserved in Kahle’s solution (Stehr 1987) and returned to the laboratory for gut-content analysis. We examined diets of taxa representing three functional feeding groups (Cummins and Klug 1979): collector-gatherers [*Baetis* (Ephemeroptera: Baetidae) 3 – 6 mm] and [*Ephemerella* (Ephemeroptera:

Ephemerellidae) 5 – 7 mm], collector-filterers [*Hydropsyche* (Trichoptera: Hydropsychidae) 6 – 12 mm] and [*Brachycentrus* (Trichoptera: Brachycentridae) 3 – 10 mm], and a predator/engulfer [*Drunella spinifera* (Ephemeroptera: Ephemerellidae) 9 – 11 mm]. These taxa are important members of the insect assemblage at Big Creek, representing, for example, 18 % of total benthic abundance and 22 % of the total benthic biomass of aquatic invertebrates collected during summer monitoring (Baxter, unpublished).

Diets were evaluated using standard gut-content analysis (Benke and Wallace 1980, Rosi-Marshall and Wallace 2002). Gut contents were removed from the insect body, suspended in distilled water, sonicated 60 s, and filtered onto Millipore membrane filters (25 mm, 0.45 μm). Filters with gut contents were dried in a drying oven at 60° C, mounted on slides with immersion oil, and sealed with clear, nail polish. The number of individuals per slide varied from 1 - 2, based on the size and gut fullness of insects. Three to six slides per treatment (i.e. carcass vs. no carcass) were analyzed for each taxon.

We measured the fractional area of food particles to calculate the proportion of each resource type in the gut-contents of aquatic insects. Food particles were identified as amorphous detritus, diatom frustules, diatom stalk, filamentous algae, fungi, leaf material, insect material, and wood using a compound microscope at 200 - 400 × (Leica DMRB, Buffalo Grove, IL, USA) mounted with a digital camera at (SPOT CCD, Sterling Heights, Michigan, USA). Prior to taking photographs, we visually scanned the entire slide for evidence of carcass material in the gut-contents. Following the initial scan for carcass material, we measured the area of 100 particles on each slide along transects using ImageJ (National Institutes of Health, Bethesda, Maryland, USA; http://imagej.nih.gov/ij/).

*Isotope Analysis*

In addition to gut-content analysis of aquatic insects, we collected aquatic insects from the same elk carcass (May 2011) for isotope analysis. We also collected aquatic insects at sites without a carcass in March 2011 for reference samples (no-carcass treatment). Similarly to gut-content analysis, we were restricted to compare the isotope values of aquatic insects on an elk carcass in May 2011 and aquatic insects at sites without a carcass in March 2011 because of logistical constraints. Additionally, we collected potential food sources of aquatic insects for isotope analysis including elk muscle tissue (Mar. 2011), biofilm (Dec. 2010 – May 2011), seston (i.e. transported organic matter, Feb. – Mar. 2011), and leaves (Dec. 2010). All samples were frozen until analysis.

In the laboratory, samples were dried in a drying oven at 60 °C, except samples of elk muscle tissue were freeze-dried. After drying, we homogenized the samples with a mortar and pestle or grinding mill. All samples were analyzed with the Elemental Combustion System 4010 (Costech Analytical Technologies Inc., Santa Clarita, CA, USA) interfaced to a Delta V Advantage Mass Spectrometer through the ConFlo IV System (Thermo Scientific, Waltham, IL, USA). All samples were analyzed for isotope ratios of δ13 carbon (δ13C) and δ15 nitrogen (δ15N). Isotope ratios of δ13C are reported as per mil (‰) values relative to the VPDB scale; whereas δ15N values are reported as per mil (‰) values relative to air $N_2$. Additionally, all samples were analyzed for elemental composition of percent nitrogen and carbon. All isotope and elemental analyses were conducted at the Center for Archaeology, Materials and Applied Spectroscopy (CAMAS) Laboratory at Idaho State University, Pocatello, ID.

### *Statistical Analyses*

We examined difference in dissolved nutrients and biofilm data using PROC T-TEST in the software package SAS (SAS version 9.2, SAS Institute Inc., Cary, NC, 2008). We examined differences the gut-contents of aquatic insects by comparing mean proportions of food resources (amorphous detritus, diatom frustules, filamentous algae, etc.) between individuals collected from an elk carcass in May and individuals collected from sites without a carcass in March for each taxa. We estimated means and 95% confidence intervals for the proportion of each food resource consumed using bootstrap analysis. For each taxon, data on the proportion of food resource consumed were re-sampled with replacement 1000 times and the mean and 2.5% and 97.5% quantiles were calculated from the bootstrap analysis. Bootstrap analysis was conducted with Microsoft Excel 2008 (Microsoft Corporation, Redmond, WA). Low sample size precluded standard statistical analysis of isotope values of aquatic insects; therefore, we plotted the fractionation-corrected (+2.3 N and +0.3 C, McCutchan et al. 2003) isotope values of δ13 carbon and δ15 nitrogen (±1 SE) for potential food sources (elk tissue, biofilm, seston, and leaf). We also plotted the true isotope values of δ13 carbon and δ15 nitrogen (±1 SE) for aquatic insects collected from an elk carcass and from sites without an elk carcass to visually assess for differences between the two treatment types.

## Results

### *Ungulate carcasses deposited into river*

Contrary to expectations, we did not observe any ungulate carcasses deposited into Big Creek during winter 2010 – 2011. Rather, we located one ungulate carcass deposited into Big Creek, an adult cow elk on March 22 (Fig. 2). We conducted necropsy analysis to document the remaining portion of carcass and evaluate cause of death. On March 22, approximately 85 % of the elk carcass had been previously consumed. Small chunks of muscle tissue, ligaments / tendons, and hair was submerged in the river near the carcass. We observed the rumen and internal organs along the river edge, approximately 10 m from the river channel. We observed numerous wolf tracks, wolf beds with bones of elk carcass, and wolf feces (including those containing meat) near remains of elk. Furthermore, we observed subcutaneous hemorrhaging near the hind legs of the elk carcass, which is indicative of a wolf kill (O’Gara 1978). We concluded that the cow elk was likely killed by wolves approximately 10 m from the river channel, and then was dragged into Big Creek. We documented the elk carcass remained submerged in Big Creek for 43 days since the initial kill, via digital-interval camera, until we returned on 2 May 2011 to re-sample the carcass.

We located a second ungulate carcass deposited into Big Creek, another adult cow elk, on April 29. On initial observation, approximately 40 % of the elk carcass had been previously consumed. At this time the elk carcass was situated on the edge of the river and only partially submerged (50 %) in Big Creek. We returned to the second elk carcass 3 days later (May 1) and conducted a necropsy analysis. Most of the remaining carcass had been removed from the river and no longer was submerged in Big Creek (< 1 m from river channel). On May 1, approximately 95 % of the elk carcass had been previously consumed. One scapula, 2 - 3 rib bones, and portions of the rumen, however, were submerged in Big Creek. In addition, we observed numerous wolf tracks and wolf feces (including those containing meat) on trail near carcass. We did not observe subcutaneous hemorrhaging on the carcass because of the limited portion of carcass remains. We observed evidence of a struggle on the hill slope near carcass, indicating a chase may have originated upslope of carcass position. We concluded that the cow elk was likely chased from the hill slope and was likely killed by wolves on the edge of the river in downfall timber, approximately 5 m from the river.

### *Dissolved nutrients*

In contrast to our expectation, concentrations of ammonium ($NH_4$) tended to be lower downstream of the elk carcass than upstream in March 2011 (Fig 3A). This difference was not significant (*t*-test $t_4 = -2.49$, $p = 0.0673$) (Fig. 3A). Concentrations of nitrate / nitrite ($NO_3$ / $NO_2$) upstream of the elk carcass, however, did not differ from concentrations downstream of the carcass (*t*-test $t_4 = 0.91$, $p = 0.4155$) (Fig. 3B).

### *Biofilm*

Similar to results of dissolved nutrients, concentrations of chl-*a* on rocks downstream of the elk carcass did not differ from rocks collected at other edge locations in Big Creek (*t*-test $t_{22} = -1.17$, $p = 0.2529$) (Fig. 4A). The ratio of chl-*a* to AFDM also did not differ significantly on rocks downstream of the elk carcass compared to other rocks collected in edge locations during the same time period (*t*-test $t_{22} = -0.34$, $p = 0.7353$) (Fig. 4B).

### *Colonization of carcasses by aquatic insects*

We collected 98 individuals from the first submerged elk carcass on 2 May 2011, initially deposited into Big Creek on 22 March 2011. All gut-content and isotope analyses were conducted on aquatic insects collected from this carcass. Aquatic insects collected from this carcass encompassed 5 orders (Coleoptera, Diptera, Ephemeroptera, Plecoptera, and Trichoptera) and 12 families (Elmidae, Chironomidae, Simulidae, Athericidae, Ephemerellidae, Baetidae, Heptageniidae, Leptophlebiidae, Pteronarcyidae, Perlodidae, Brachycentridae, Hydropsychidae). Ephemerellidae (Ephemeroptera) were the most abundant aquatic insects on carcass, representing 41% of the total insects that were collected. Chironomidae (Diptera), Brachycentridae (Trichoptera), and Baetidae (Ephemeroptera) were the next most abundant taxa, representing 17%, 10%, and 9% of the total insects collected, respectively.

Additionally, we collected 69 individuals from the submerged remains (scapula and ribs) of the second elk carcass on May 1, initially deposited into Big Creek on April 29. Most of this carcass was removed from the river by scavengers; therefore, gut-content and isotope analyses of aquatic insects were not conducted. Aquatic insects collected from the submerged-carcass remains encompassed 5 orders (Coleoptera, Diptera, Ephemeroptera, Plecoptera, Trichoptera, and Trombidiformes) and 11 families (Elmidae, Chironomidae, Simulidae, Athericidae, Heptageniidae, Ephemerellidae, Baetidae, Leptophlebiidae, Capniidae, Brachycentridae, and Hydrachnoidae). Baetidae (Ephemeroptera) were the most abundant aquatic insects, representing 55% of the total insects that were collected. Ephemerellidae (Ephemeroptera) and Chironomidae (Diptera) were the next most abundant taxa, representing 14% and 9% of the total insects collected, respectively.

*Gut-content analysis*

Direct consumption of elk carcass material was not detected in diets of aquatic insects, via visual scan of gut-content slides (Fig. 5). Proportions of food types in *Baetis, Ephemerella,* and *Drunella spinifera* collected from a submerged elk carcass did not differ significantly (overlapping 95% CI) from individuals collected at similar sites without a carcass (Fig. 5). In contrast, *Hydropsyche* and *Brachycentrus* collected from an elk carcass had a greater proportion of diatom frustules in gut-contents than individuals at similar sites without a carcass (Fig. 6). Diets of *Hydropsyche* collected on an elk carcass were composed of more diatom frustules (54 vs. 15 %) and less insect material (15 vs. 61 %) than diets of *Hydropsyche* collected at sites without a carcass (Fig. 5). Differences in the proportion of diatom frustules in gut-contents of *Hydropsyche* collected from an elk carcass and sites without a carcass were significant (non-overlapping 95 % CI, Fig. 6A). The proportion of diatom frustules in diets of *Brachycentrus* collected from an elk carcass was higher than diets of *Brachycentrus* collected at sites without a carcass (22 vs. 14 %), although not significantly (Fig 6B).

### *Isotope analysis*

Overall, aquatic insects collected from one elk carcass in May were more enriched in δ13C (c. 2%) and δ15N (c. 0.5%) than the same taxa collected at sites without a carcass in March (Fig. 7). Isotope values of insects collected from an elk carcass were more similar to biofilm, while the same taxa at sites without a carcass resembled a mixture of biofilm and seston (Fig. 7). The mayflies, *Baetis* and *Drunella*, collected from an elk carcass were more enriched in δ13C (+ 3 – 6%) than the same taxa at sites without a carcass (Fig. 8). The caddisflies, *Hydropsyche* and *Brachycentrus*, and mayfly, *Ephemerella,* collected from an elk carcass were more enriched in δ15N (+ 1%) than same taxa at sites without a carcass (Fig. 8).

## Discussion

Our study indicated that ungulate carcasses originating from wolf-kills may be resource subsidies to organisms in aquatic ecosystems. We documented that 2 elk carcasses from wolf-kills were deposited into a wilderness river ecosystem. Contrary to our initial prediction, dissolved nutrients and biofilm communities did not increase downstream of a submerged elk carcass. Diets of aquatic insects may have responded to shifts in food resources driven by an elk carcass deposited into a river. Aquatic insects, *Hydropsyche* and *Brachycentrus*, collected directly from a submerged elk carcass consumed a greater proportion of diatom frustules than those collected from sites without a carcass. In addition, isotope values of aquatic insects collected from a submerged elk carcass were enriched in both δ13C and δ15N. Furthermore, isotope values of aquatic insects from an elk carcass were more similar to biofilm, whereas isotope values of aquatic insect at sites without a carcass tended to more closely resemble a mixture of biofilm and seston. Overall, aquatic insects collected from a submerged elk carcass in Big Creek consumed biofilm rather than feeding upon carcass material. We hypothesize that an elk carcass may have indirectly supported aquatic insects by enhancing the nutritional quality of biofilm, their primary food resource, and providing habitat for filter-feeding taxa, although further investigation is needed. Because of logistical constraints, we were restricted to compare diets (gut-contents and stable isotopes) of aquatic insects on an elk carcass in May 2011 and similar taxa without a carcass in March 2011. Results from our study should be interpreted accordingly. Our research provides insight to a unique subsidy that may link aquatic and wildlife ecology by new methods and suggest another pathway by which wolf predation may be altering river processes through changes in cross-boundary resource subsidies.

Although we observed only 2 ungulate carcasses deposited in Big Creek, prior researchers reported the number of submerged carcasses were much greater in other years. For example, previous managers of TWRS observed up to 8 ungulate carcasses per year were deposited into the mainstem and tributaries of Big Creek from 1999 – 2002 **(**J. Akenson and H. Akenson, personal communication**)**. In contrast, we located 2 ungulate carcasses that were deposited into the mainstem of Big Creek during spring 2011. The greater number of submerged carcasses recorded previously was likely a result of increased effort to locate carcasses, because researchers lived at TWRS full-time. In addition, climate conditions, which affect the distribution of ungulates by altering access to food resources (Turner et al. 1994) and predator – prey interactions (Huggard 1993, Post et al. 1999), were likely different between study periods. Perhaps we did not detect all submerged ungulate carcasses that were deposited into Big Creek. In general, our detection of submerged carcasses may have been inhibited by downstream transport of carcasses, lack of carcass evidence from terrestrial scavengers, and our monthly sampling regime. Regardless of the limited number of carcasses submerged in Big Creek during our study, our results indicate that basal resources and use of these resources by aquatic insects may be positively affected by this terrestrial subsidy.

Dissolved nutrients and biofilm communities did not increase downstream of a submerged elk carcass, however, sample size was limited. Concentrations of ammonium ($NH_4$) downstream of an elk carcass were lower compared to samples upstream of carcass, which may be explained by increased activity of microbes and autoptrophs (i.e., priming effect, Guenet et al. 2010). A submerged elk carcass likely contributes essential nutrients to organisms in the recipient habitat (Bump 2009a). Since aquatic autotrophs in the Salmon River are nutrient limited (Thomas et al. 2003, Marcarelli and Wurtsbaugh 2007), and uptake by aquatic organisms is likely rapid, detection of increased nutrients may be difficult. In addition, the elk was initially killed on the bank near Big Creek and much of the nutrients within the blood and internal organs may have been delivered to the terrestrial soils. Furthermore, vertebrate bones may leach nutrients slowly in aquatic ecosystems (Smith and Baco 2003, Fujiara et al. 2007). Additionally, biofilm is a highly variable (Blackadar, Ch. 1) and samples of biofilm were collected 1 day after the carcass was deposited into Big Creek, which is likely not adequate time to detect a response. Future studies should consider sampling a time-series of biofilm downstream of ungulate carcasses, as well as biofilm growing on ungulate carcasses to detect possible differences in species composition and nutrient content of biofilm (i.e., C : N, fatty acids, lipids, etc.).

Diets of aquatic insects from a submerged elk carcass shifted to a greater proportion of biofilm, potentially by three mechanisms. First, a submerged elk carcass may provide a fresh substrate for colonization by biofilm communities. Previous studies reported rapid colonization of fresh substrates by biofilm (Power & Stewart 1987, Szabo et al. 2008). In addition, other studies documented the quality of biofilm can be greater during early stages of colonization (Fisher et al. 1982). Furthermore, we observed that aquatic insects consumed a greater proportion of diatom frustules following disturbance, which altered the quantity and quality of biofilm growing on river substrates (Blackadar, Ch. 1). By creating fresh substrates, ungulate carcasses in rivers may similarly affect the quantity and quality of biofilm, as well as the use of this resource by aquatic insects. In addition to a substrate for biofilm, an elk carcass also may provide quality habitat for filter-feeding insects. The caddisflies, *Hydropsyche* and *Brachycentrus*, on an elk carcass consumed a greater proportion of diatom frustules compared to similar taxa at sites without a carcass. These insects, which filter food particles from the water column, may have used the elk carcass as a structure to obtain food resources. Previous research on salmon reported caddisflies used the remains of salmon carcasses to construct silken webs to collect food particles (Winder et al. 2005). Lastly, a submerged elk carcass also may provide essential nutrients to biofilm communities and organisms that fed on biofilm. For example, salmon carcasses have been documented to increase dissolved nutrients and stimulate biofilm in aquatic ecosystems (Wipfli et al. 1998, Chaloner and Wipfli 2002). Additionally, ungulate carcasses have been reported to increase nutrient content of soils and of terrestrial vegetation (Bump et al. 2009a,b). Because elk muscle tissue is high in nitrogen content (15%), we hypothesize that ungulate carcasses may play a similar role in aquatic ecosystems by stimulating biofilm production, thereby increasing consumption of biofilm by aquatic insects.

We did not document direct consumption of carcass material by aquatic insects, however, several factors in our study approach may have influenced our results. Because diet analyses of aquatic insects (gut-content and stable isotopes) were conducted only on individuals 43 days after the initial deposition in the river, aquatic insects may have directly consumed carcass material but were not detected in our analyses. For example, elk muscle tissue is a high-quality food resource (15% N and 51% C), therefore, aquatic insects may have assimilated the carcass material and would not be present in gut-

contents. Previous studies have reported aquatic insects rapidly colonize and possibly consume vertebrate carcasses (Keiper et al. 1997, Wipfli 1998, Barrios and Wolff 2010). Moreover, studies using stable isotopes (δ13C and δ15N) have reported aquatic insects incorporating salmon carcass nutrients and have suggested both direct and indirect consumption of carcass materials (Chaloner and Wipfli 2002). No study, however, has documented vertebrate carcass material in the gut-content of aquatic insects.
Evidence of direct consumption of vertebrate carcasses by aquatic insects is lacking.

Although our study has indicated ungulate carcasses originating from wolf-kills may positively affect aquatic insects, additional research is needed to fully quantify the response of aquatic ecosystems to this unique resource subsidy. Specifically, future research should aim to collect a time series of aquatic organisms to investigate the succession associated with the colonization of ungulate carcasses. In addition, studies of aquatic organisms from higher trophic levels, such as fishes and otters, should also be investigated to quantify the effects of ungulate carcasses on these organisms. Additional studies are needed to fully elucidate responses of aquatic ecosystems' to ungulate carcasses originating from wolf-kills.

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# CHAPTER 2: FIGURES

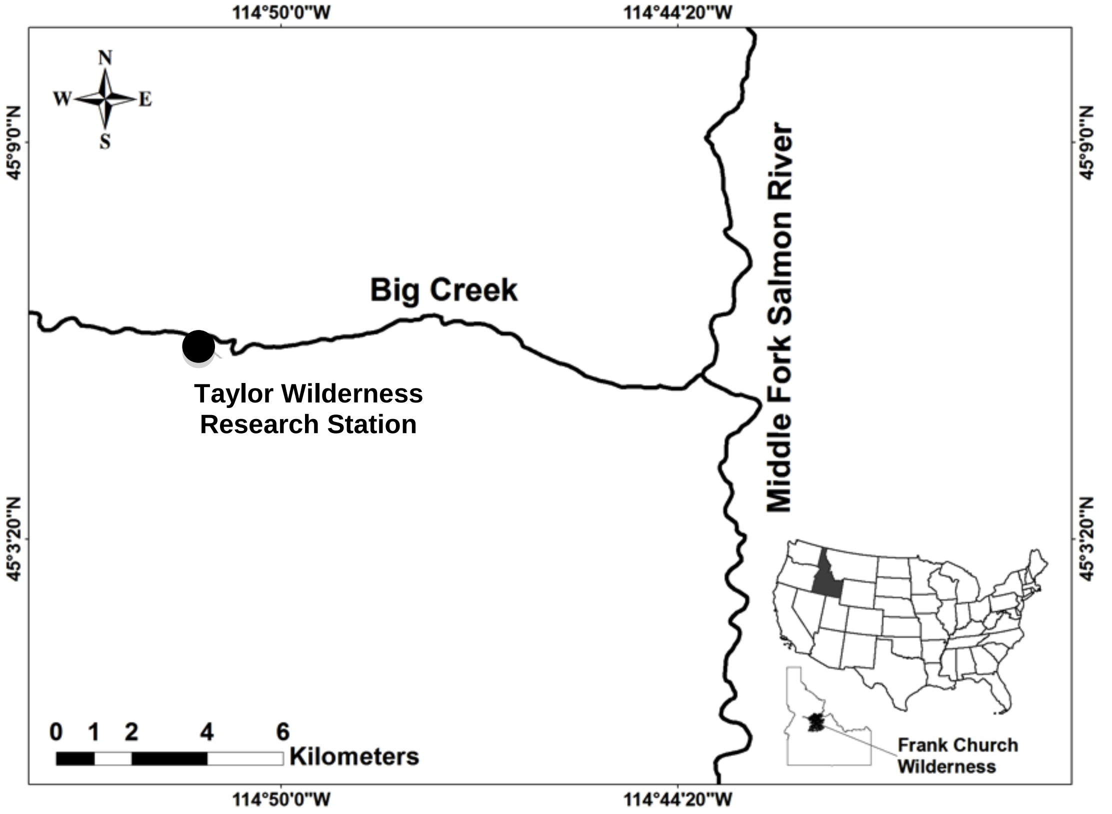

Figure 1. Map of Big Creek and Middle Fork Salmon River of central Idaho, USA. Location of Taylor Wilderness Research Station is noted on Big Creek by a closed circle. Inset shows the location of wilderness area in state of Idaho and USA.

Figure 2. Photo of author documenting an elk carcass (*Cervus elaphus*) deposited into Big Creek, Middle Fork Salmon River, USA. Photo was taken two days after the initial kill by wolves (*Canis lupus*).

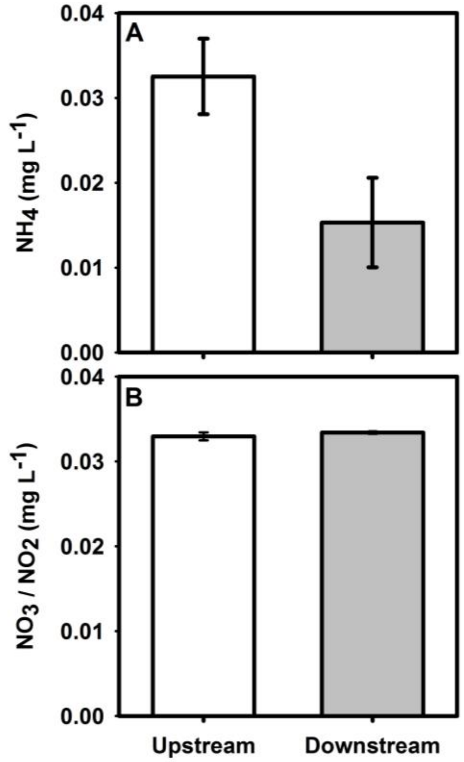

Figure 3. Concentrations of ammonium, $NH_4$, (mg $L^{-1}$) (A) and of nitrate / nitrite, $NO_3$ / $NO_2$, (mg $L^{-1}$) (B) in river water collected upstream (*n* = 3) and downstream (*n* = 3) of submerged elk carcass 1 day after it was deposited in Big Creek, Middle Fork Salmon River, USA during March 2011.

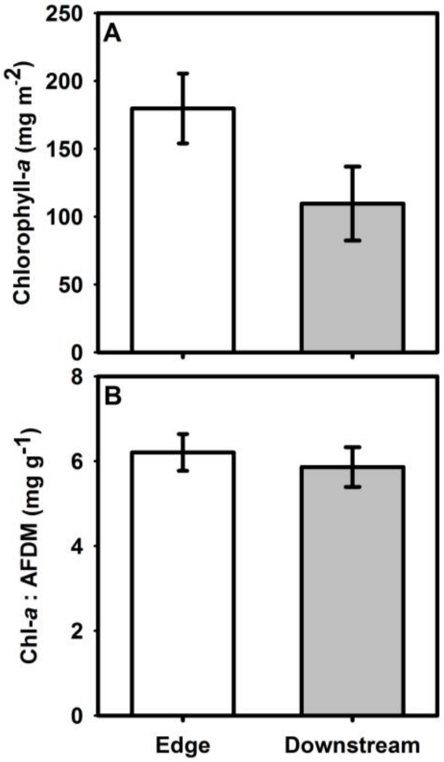

Figure 4. Concentration of chlorophyll-*a* (mg $m^{-2}$) (A) and chl-*a* : AFDM (mg $g^{-1}$) (B) of biofilm on rocks collected from edge habits ($n = 20$) during May 2011 and from rocks collected from edge habitats downstream of an elk carcass ($n = 4$) during March 2011, 1 day after it was deposited in Big Creek, Middle Fork Salmon River, USA.

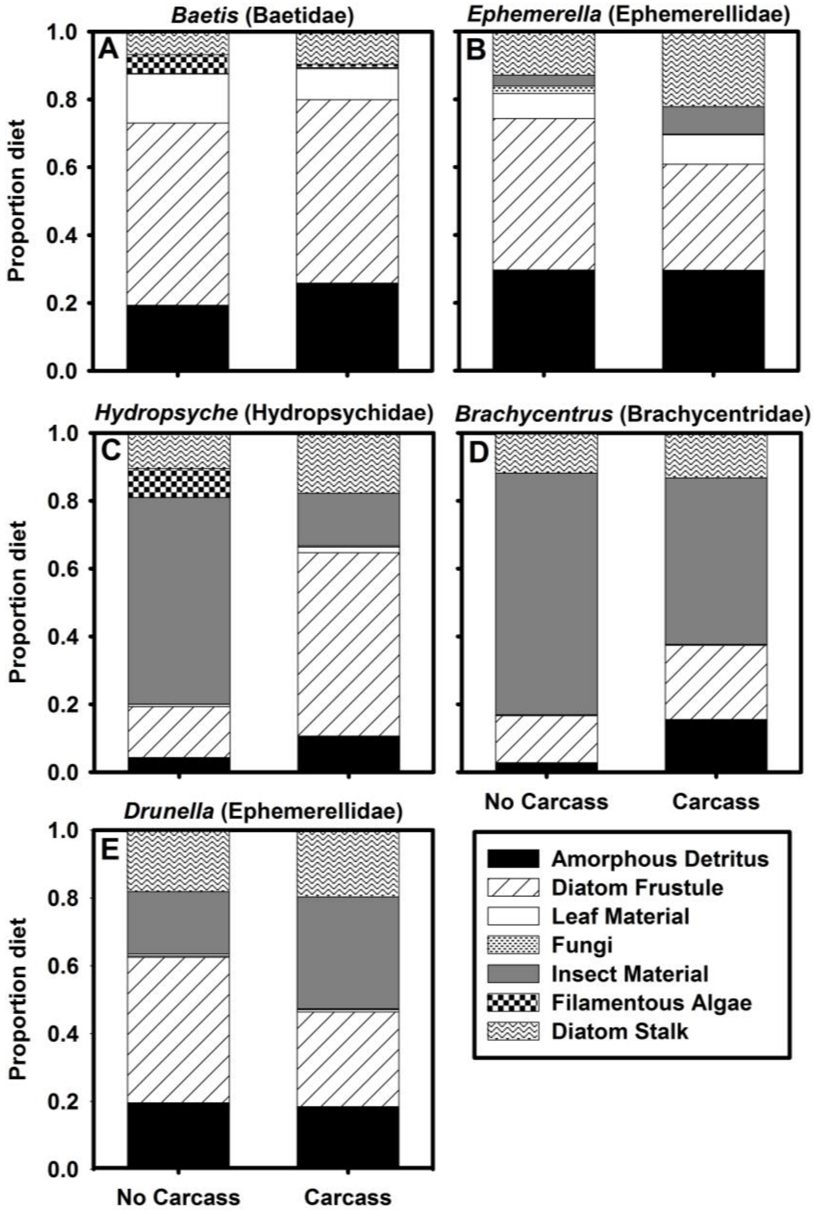

Figure 5. Diets of aquatic insects collected from a submerged elk carcass in May 2011, 43 days since initial kill, (right side of each graph) and insects collected from sites without a carcass in March 2011 (left side of each graph) from Big Creek in Idaho, USA. Stacked bars represent mean ($n = 3 – 6$) proportion of food-particle types in gut-content of *Baetis* (A), *Ephemerella* (B), *Hydropsyche* (C), *Brachycentrus* (D), and *Drunella* (E).

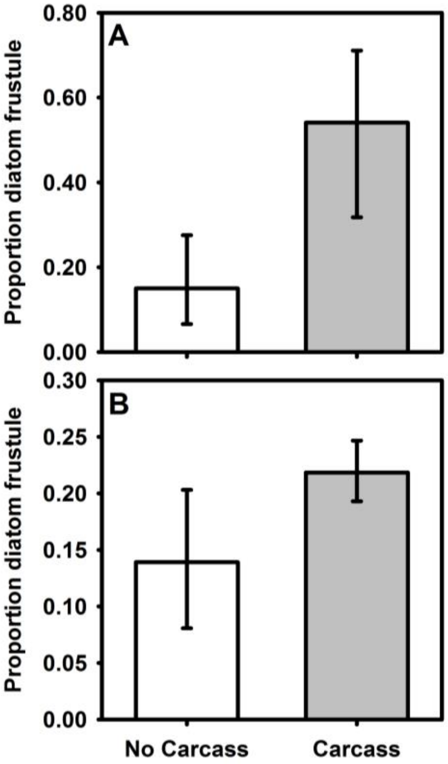

Figure 6. Means (± 95% CI) of proportions of diatom frustules in the gut-contents of *Hydropsyche* (*n* = 5) (A) and *Brachycentrus* (*n* = 3) (B). White vertical bars represent the proportion diatom frustules in gut-contents of aquatic insects collected from sites without a carcass in March 2011. Grey vertical bars represent the proportion diatom frustules in gut-contents of aquatic insects collected from submerged elk carcass in May 2011.

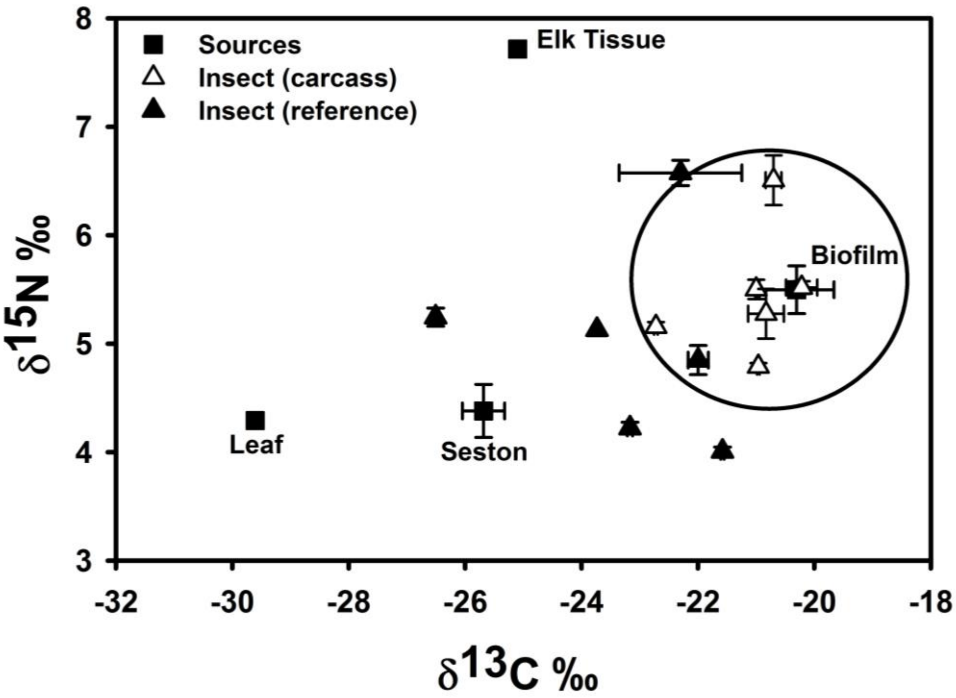

Figure 7. Mean δ13C and δ15N isotope values (±1 SE) of potential food sources (black squares, [elk tissue (*n* = 3), biofilm (*n* = 10), seston (*n* = 11), and leaf (*n* = 2)]), aquatic insects collected from elk carcass in May 2011 (open triangles), and aquatic insects collected from sites without a carcass in March 2011 (black triangles). Black circle indicates isotope values of aquatic insects collected from submerged elk carcass were similar to biofilm. In contrast, isotope values of aquatic insects collected from sites without a carcass were similar to a mixture of biofilm and seston.

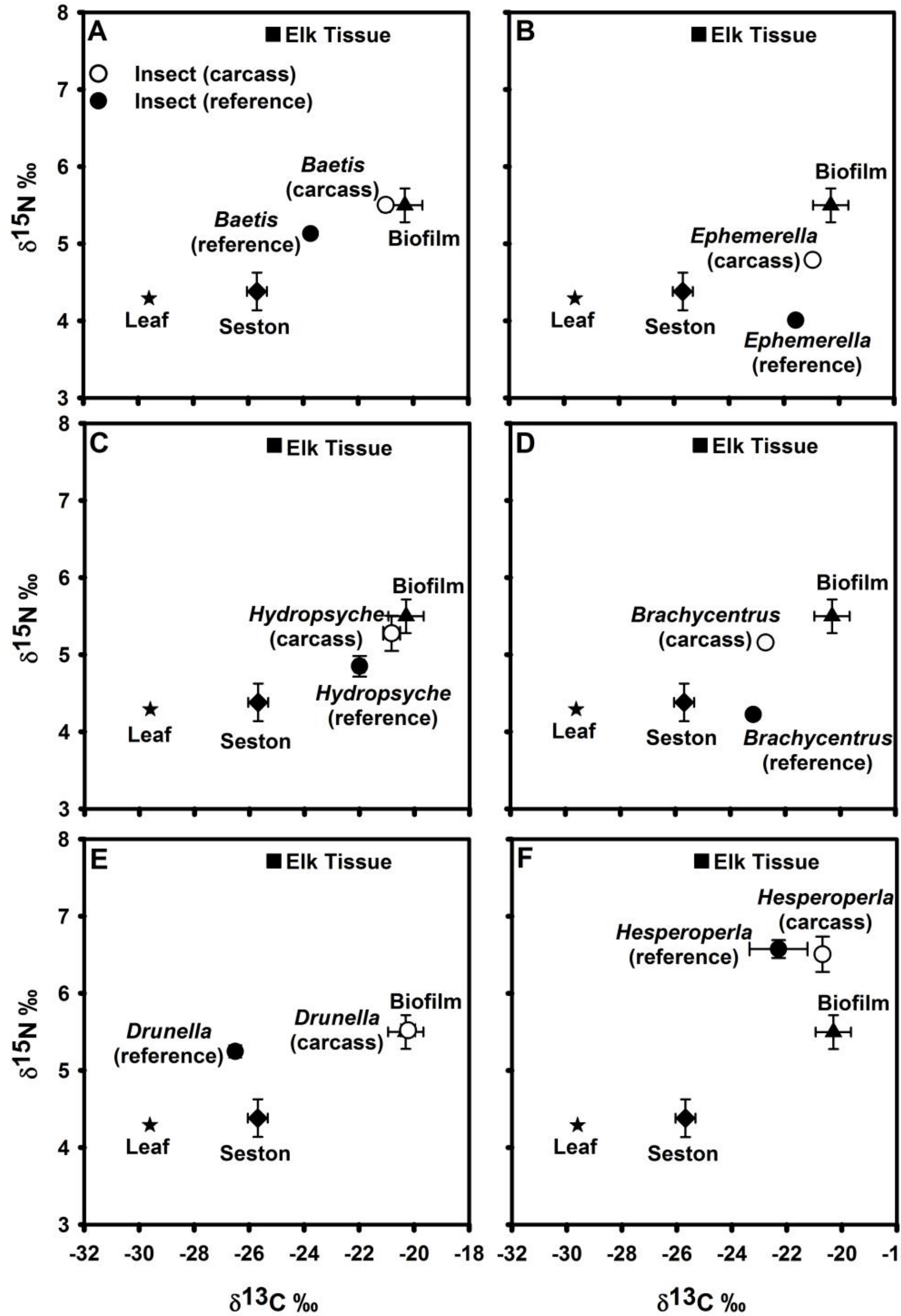

Figure 8. Mean δ13C and δ15N isotope values (±1 SE) of food sources (elk tissue [black square], biofilm [black triangle], seston [black diamond], and leaf [black star]) and aquatic insects collected from an elk carcass in May 2011 (open circles), and aquatic insects collected from sites without a carcass in March 2011 (black circles) of *Baetis* (A), *Ephemerella* (B), *Hydropsyche* (C), *Brachycentrus* (D), *Drunella* (E), and *Hesperoperla* (F).

Appendix A. Mean (± 1 SE) ash-free dry mass (AFDM, g $m^{-2}$), chlorophyll-*a* (chl-*a*, mg $m^{-2}$), and the ratio of chl-*a* :AFDM (mg $g^{-1}$) of biofilm collected from Big Creek, Middle Fork Salmon River of central Idaho, USA during winter (Dec. 2010 – Mar. 2011) and summers (July, 2008 – 2011).

| Date | Habitat | (n) | AFDM (g $m^{-2}$) | Chl-*a* (mg $m^{-2}$) | Chl-*a* : AFDM (mg $g^{-1}$) |
|---|---|---|---|---|---|
| Dec. 2010 | Thalweg | 17 | 21.9791<br>(2.5522) | 186.4437<br>(28.4605) | 7.8795<br>(0.6538) |
| Dec. 2010 | Edge | 16 | 35.2051<br>(7.8939) | 158.1584<br>(21.0445) | 5.5407<br>(0.5004) |
| Jan. 2011 | Thalweg | 18 | 15.0488<br>(2.8631) | 139.9507<br>(24.8881) | 10.9431<br>(1.5045) |
| Jan. 2011 | Edge | 18 | 20.1676<br>(3.4735) | 127.4495<br>(22.6302) | 8.3085<br>(1.4985) |
| Feb. 2011 | Thalweg | 19 | 7.7695<br>(1.3439) | 81.9671<br>(10.9986) | 11.3164<br>(0.7478) |
| Feb. 2011 | Edge | 18 | 8.5769<br>(1.8447) | 75.0637<br>(12.9173) | 9.6065<br>(0.8562) |
| Mar. 2011 | Thalweg | 18 | 24.2926<br>(3.8503) | 163.0862<br>(28.4438) | 6.8538<br>(0.4595) |
| Mar. 2011 | Edge | 18 | 25.7468<br>(3.1247) | 156.5197<br>(20.4911) | 6.1482<br>(0.4755) |
| July 2011 | Thalweg | 5 | 4.2805<br>(0.6768) | 8.9244<br>(1.5135) | 2.1237<br>(0.1814) |
| July 2010 | Thalweg | 5 | 2.8968<br>(0.8364) | 10.1269<br>(1.8439) | 2.1237<br>(0.1814) |
| July 2009 | Thalweg | 5 | 10.1438<br>(1.2201) | 17.4427<br>(1.7906) | 1.7913<br>(0.2083) |
| July 2008 | Thalweg | 5 | 3.4923<br>(0.7501) | 6.3382<br>(1.7366) | 1.7775<br>(0.2379) |

Appendix B. Aquatic insect gut-contents (raw proportions). Mean proportions (± 1 SE) by area of food types in gut-contents aquatic insects (scrapers) collected from mainstem of Big Creek, Middle Fork Salmon River of central Idaho, USA during winter (Dec. 2010 – Mar. 2011).

| Functional group / Taxon | Date | (n) | Amorphous detritus | Diatom frustules | Diatom stalks | Leaf material | Fungi | Wood | Insect material | Filamentous algae |
|---|---|---|---|---|---|---|---|---|---|---|
| Scrapers | | | | | | | | | | |
| *Rithrogena* | Dec. 2010 | 6 | 0.7166<br>(0.0646) | 0.1394<br>(0.0304) | 0.0644<br>(0.0518) | 0.0513<br>(0.0268) | 0.0207<br>(0.0055) | 0 | 0 | 0.0077<br>(0.0070) |
| | Jan. 2011 | 6 | 0.4748<br>(0.0624) | 0.3713<br>(0.0831) | 0.0351<br>(0.0351) | 0.1127<br>(0.0250) | 0.0061<br>(0.0028) | 0 | 0 | 0 |
| | Feb. 2011 | 6 | 0.4184<br>(0.0334) | 0.4184<br>(0.0293) | 0.0151<br>(0.0151) | 0.0793<br>(0.0250) | 0.0191<br>(0.0072) | 0 | 0.0371<br>(0.0371) | 0 |
| | Mar. 2011 | 6 | 0.3520<br>(0.0919) | 0.3520<br>(0.0350) | 0 | 0.0922<br>(0.0662) | 0.0157<br>(0.0055) | 0 | 0.2240<br>(0.1327) | 0 |
| *Bibiocephala* | Dec. 2010 | 7 | 0.4004<br>(0.0784) | 0.4205<br>(0.0572) | 0.1323<br>(0.0492) | 0.0469<br>(0.0275) | 0 | 0 | 0 | 0 |
| | Jan. 2011 | 7 | 0.3295<br>(0.0253) | 0.5676<br>(0.0396) | 0.0834<br>(0.0534) | 0.0166<br>(0.0073) | 0.0029<br>(0.0021) | 0 | 0 | 0 |
| | Feb. 2011 | 7 | 0.3052<br>(0.0664) | 0.5412<br>(0.0664) | 0.1305<br>(0.0385) | 0.0079<br>(0.0048) | 0.0153<br>(0.0097) | 0 | 0 | 0 |
| | Mar. 2011 | 7 | 0.3909<br>(0.0734) | 0.5401<br>(0.0569) | 0.0412<br>(0.0338) | 0.0278<br>(0.0181) | 0 | 0 | 0 | 0 |

Appendix C. Aquatic insect gut-contents (raw proportions). Mean proportions (± 1 SE) by area of food types in gut-contents aquatic insects (collector-gatherers) collected from mainstem of Big Creek, Middle Fork Salmon River of central Idaho, USA during winter (Dec. 2010 – Mar. 2011).

| Functional group / Taxon | Date | (n) | Amorphous detritus | Diatom frustules | Diatom stalks | Leaf material | Fungi | Wood | Insect material | Filamentous algae |
|---|---|---|---|---|---|---|---|---|---|---|
| Collector-gatherers | | | | | | | | | | |
| *Baetis* | Dec. 2010 | 6 | 0.4392<br>(0.0165) | 0.3740<br>(0.0251) | 0.1303<br>(0.0420) | 0.0450<br>(0.0290) | 0.0115<br>(0.0056) | 0 | 0 | 0 |
| | Jan. 2011 | 6 | 0.3444<br>(0.0240) | 0.5458<br>(0.0439) | 0.0372<br>(0.0150) | 0.0235<br>(0.0075) | 0.0088<br>(0.0053) | 0 | 0.0<br>(0) | 0.0403<br>(0.0393) |
| | Feb. 2011 | 6 | 0.3784<br>(0.0496) | 0.4960<br>(0.0625) | 0.0526<br>(0.0370) | 0.0606<br>(0.0159) | 0.0124<br>(0.0049) | 0 | 0 | 0 |
| | Mar. 2011 | 6 | 0.1932<br>(0.0292) | 0.5372<br>(0.0839) | 0.0672<br>(0.0496) | 0.1448<br>(0.0649) | 0.0014<br>(0.0006) | 0 | 0 | 0.0562<br>(0.0562) |
| non-Tanypodinae Chironomidae | Dec. 2010 | 6 | 0.2772<br>(0.0547) | 0.4554<br>(0.0547) | 0.1524<br>(0.0674) | 0.0989<br>(0.0267) | 0.0077<br>(0.0063) | 0 | 0.0011<br>(0.0011) | 0.0073<br>(0.0073) |
| | Jan. 2011 | 6 | 0.3924<br>(0.0493) | 0.3970<br>(0.0336) | 0.0201<br>(0.0201) | 0.1417<br>(0.0398) | 0.0488<br>(0.0219) | 0 | 0 | 0 |
| | Feb. 2011 | 6 | 0.3079<br>(0.0704) | 0.4993<br>(0.0844) | 0 | 0.1456<br>(0.0376) | 0.0472<br>(0.0252) | 0 | 0 | 0 |
| | Mar. 2011 | 6 | 0.2231<br>(0.0394) | 0.6787<br>(0.0437) | 0 | 0.0765<br>(0.0183) | 0.0217<br>(0.0127) | 0 | 0 | 0 |

Appendix D. Aquatic insect gut-contents (raw proportions). Mean proportions (± 1 SE) by area of food types in gut-contents aquatic insects (collector-filterers) collected from mainstem of Big Creek, Middle Fork Salmon River of central Idaho, USA during winter (Dec. 2010 – Mar. 2011).

| Functional group / Taxon | Date | (n) | Amorphous detritus | Diatom frustules | Diatom stalks | Leaf material | Fungi | Wood | Insect material | Filamentous algae |
|---|---|---|---|---|---|---|---|---|---|---|
| Collector-filterers | | | | | | | | | | |
| *Simulium* | Dec. 2010 | 6 | 0.7378<br>(0.0214) | 0.1466<br>(0.0141) | 0 | 0.0701<br>(0.0094) | 0.0498<br>(0.0169) | 0 | 0 | 0.0018<br>(0.0018) |
| | Jan. 2011 | 6 | 0.4694<br>(0.0760) | 0.2455<br>(0.0440) | 0.1210<br>(0.0612) | 0.1463<br>(0.0391) | 0.0179<br>(0.0063) | 0 | 0 | 0 |
| | Feb. 2011 | 3 | 0.3234<br>(0.0525) | 0.3234<br>(0.0527) | 0 | 0.1982<br>(0.0669) | 0.0800<br>(0.0769) | 0 | 0 | 0 |
| | Mar. 2011 | 6 | 0.4339<br>(0.0566) | 0.4339<br>(0.0761) | 0 | 0.2325<br>(0.0987) | 0.0369)<br>(0.0173) | 0 | 0 | 0 |
| *Arctopsyche* | Dec. 2010 | 6 | 0.1191<br>(0.0286) | 0.1298<br>(0.0321) | 0.1022<br>(0.0694) | 0.0070<br>(0.0029) | 0.0006<br>(0.0004) | 0 | 0.6353<br>(0.0802) | 0.0053<br>(0.0032) |
| | Jan. 2011 | 6 | 0.0614<br>(0.0120) | 0.1353<br>(0.0443) | 0 | 0.0001<br>(0.0001) | 0.0013<br>(0.0011) | 0 | 0.7969<br>(0.0348) | 0.0051<br>(0.0051) |
| | Feb. 2011 | 6 | 0.0664<br>(0.0170) | 0.0905<br>(0.0200) | 0.0418<br>(0.0289) | 0.0279<br>(0.0279) | 0.0008<br>(0.0007) | 0 | 0.7726<br>(0.0739) | 0 |
| | Mar. 2011 | 6 | 0.0903<br>(0.0139) | 0.1127<br>(0.0215) | 0.1193<br>(0.0468) | 0.0024<br>(0.0015) | 0 | 0 | 0.6692<br>(0.0535) | 0.0060<br>(0.0060) |

Appendix E. Aquatic insect gut-contents (raw proportions). Mean proportions (± 1 SE) by area of food types in gut-contents aquatic insects (shredder) collected from mainstem of Big Creek, Middle Fork Salmon River of central Idaho, USA during winter (Dec. 2010 – Mar. 2011).

| Functional group / Taxon | Date | (n) | Amorphous detritus | Diatom frustules | Diatom stalks | Leaf material | Fungi | Wood | Insect material | Filamentous algae |
|---|---|---|---|---|---|---|---|---|---|---|
| Shredder | | | | | | | | | | |
| *Pteronarcys* | Dec. 2010 | 7 | 0.1771<br>(0.0442) | 0.0874<br>(0.0230) | 0.2084<br>(0.0511) | 0.2438<br>(0.0712) | 0.0004<br>(0.0003) | 0.1905<br>(0.0446) | 0.0881<br>(0.0403) | 0.0044<br>(0.0044) |
| | Jan. 2011 | 7 | 0.1901<br>(0.0289) | 0.2413<br>(0.0535) | 0.1652<br>(0.0475) | 0.1485<br>(0.0354) | 0.0030<br>(0.0020) | 0.1033<br>(0.0397) | 0.0931<br>(0.0511) | 0.0554<br>(0.0539) |
| | Feb. 2011 | 7 | 0.1382<br>(0.0313) | 0.2239<br>(0.0767) | 0.0113<br>(0.0066) | 0.1457<br>(0.0448) | 0.0244<br>(0.0171) | 0.4155<br>(0.1005) | 0.0410<br>(0.0177) | 0 |
| | Mar. 2011 | 7 | 0.1444<br>(0.0191) | 0.2774<br>(0.0515) | 0.0715<br>(0.0505) | 0.2269<br>(0.0457) | 0.0037<br>(0.0019) | 0.2491<br>(0.0481) | 0.0271<br>(0.0164) | 0 |

Appendix F. Aquatic insect gut-contents (raw proportions). Mean proportions (± 1 SE) by area of food types in gut-contents aquatic insects collected from an elk carcass in May 2011 and from similar locations without a carcass in March 2011 from the mainstem of Big Creek, Middle Fork Salmon River of central Idaho, USA.

| Taxon | Date | (n) | Amorphous detritus | Diatom frustules | Diatom stalks | Leaf material | Fungi | Wood | Insect material | Filamentous algae |
|---|---|---|---|---|---|---|---|---|---|---|
| *Baetis* | Carcass – May 2011 | 6 | 0.2586 (0.0484) | 0.5404 (0.0815) | 0.0967 (0.0586) | 0.0914 (0.0395) | 0.0032 (0.0025) | 0 | 0 | 0.0095 (0.0095) |
| *Baetis* | No Carcass – Mar. 2011 | 6 | 0.1932 (0.0292) | 0.5372 (0.0839) | 0.0672 (0.0496) | 0.1448 (0.0649) | 0.0014 (0.0006) | 0 | 0 | 0.0562 (0.0562) |
| *Brachycentrus* | Carcass – May 2011 | 3 | 0.1547 (0.1209) | 0.2185 (0.0155) | 0.1326 (0.0866) | 0 | 0.0032 (0.0032) | 0 | 0.4910 (0.1891) | 0 |
| *Brachycentrus* | No Carcass – Mar. 2011 | 3 | 0.0272 (0.0032) | 0.1392 (0.0354) | 0.1188 (0.0403) | 0.0014 (0.0014) | 0.0014 (0.0014) | 0 | 0.7120 (0.0164) | 0 |
| *Drunella spinifera* | Carcass – May 2011 | 3 | 0.1847 (0.0263) | 0.2792 (0.0993) | 0.1971 (0.1034) | 0.0063 (0.0032) | 0.0031 (0.0031) | 0 | 0.3298 (0.1661) | 0 |
| *Drunella spinifera* | No Carcass – Mar. 2011 | 3 | 0.1952 (0.0695) | 0.4196 (0.0994) | 0.1817 (0.0631) | 0.0039 (0.0025) | 0.0055 (0.0055) | 0 | 0.1821 (0.1115) | 0 |
| *Ephemerella* | Carcass – May 2011 | 6 | 0.2963 (0.0348) | 0.3122 (0.0439) | 0.2216 (0.0413) | 0.0863 (0.0289) | 0.0022 (0.0014) | 0 | 0.0814 (0.0335) | 0 |
| *Ephemerella* | No Carcass – Mar. 2011 | 6 | 0.2966 (0.0300) | 0.4468 (0.0645) | 0.1286 (0.0323) | 0.0734 (01208) | 0.0216 (0.0106) | 0 | 0.0330 (0.0129) | 0 |
| *Hydropsyche* | Carcass – May 2011 | 5 | 0.1062 (0.0211) | 0.5408 (0.1161) | 0.1779 (0.0536) | 0.0167 (0.0057) | 0.0034 (0.0020) | 0 | 0.1549 (0.1489) | 0 |
| *Hydropsyche* | No Carcass – Mar. 2011 | 5 | 0.0424 (0.0203) | 0.1500 (0.0636) | 0.1055 (0.0753) | 0.0073 (0.0035) | 0.0005 (0.0003) | 0 | 0.6092 (0.1422) | 0.0850 (0.0458) |

Appendix G. Mean (± 1 SE) stable isotopes (δ13C and δ15N per mil ‰) and C / N values (% C / % N) of aquatic insects collected from an elk carcass in May 2011 and from similar habitats without a carcass in March 2011 from the mainstem of Big Creek, Middle Fork Salmon River of central Idaho, USA.

| Sample type | Date | (n) | δ15N ‰ | δ13C ‰ | % C / % N |
|---|---|---|---|---|---|
| *Baetis* – Carcass | May 2011 | 3 | 5.50 (0.09) | -21.00 (0.02) | 6.97 (0.06) |
| *Baetis* – No carcass | March 2011 | 1 | 5.13 | -23.73 | 6.56 |
| *Brachycentrus* – Carcass | May 2011 | 3 | 5.16 (0.04) | -22.72 (0.03) | 6.40 (0.01) |
| *Brachycentrus* – No carcass | March 2011 | 3 | 4.22 (0.05) | -23.17 (0.05) | 6.09 (0.04) |
| *Drunella* – Carcass | May 2011 | 3 | 5.52 (0.06) | -20.22 (0.27) | 4.61 (0.14) |
| *Drunella* – No carcass | March 2011 | 3 | 5.24 (0.08) | -26.51 (0.02) | 5.12 (0.02) |
| *Ephemerella* – Carcass | May 2011 | 3 | 4.79 (0.03) | -20.96 (0.02) | 6.47 (0.06) |
| *Ephemerella* – No Carcass | March 2011 | 3 | 4.01 (0.04) | -21.58 (0.03) | 6.33 (0.03) |
| *Hydropsyche* – Carcass | May 2011 | 3 | 5.28 (0.23) | -20.83 (0.31) | 5.31 (0.24) |
| *Hydropsyche* – No carcass | March 2011 | 3 | 4.85 (0.13) | -22.00 (0.17) | 5.07 (0.15) |
| *Hesperoperla* – Carcass | May 2011 | 3 | 6.51 (0.23) | -20.70 (0.14) | 4.87 (0.18) |
| *Hesperoperla* – No carcass | March 2011 | 3 | 6.57 (0.12) | -22.30 (1.05) | 4.67 (0.21) |

Appendix H. Mean (± 1 SE) stable isotopes (δ13C and δ15N per mil ‰) and C / N values (% C / % N) of potential food sources (elk muscle tissue, biofilm, seston (CTOM + FTOM), and leaf) collected from the mainstem of Big Creek, Middle Fork Salmon River of central Idaho, USA.

| Sample type | Date | (n) | δ15N ‰ | δ13C ‰ | % C / % N |
|---|---|---|---|---|---|
| Elk muscle tissue | March 2011 | 6 | 5.42 (0.02) | -25.40 (0.06) | 3.31 (0.01) |
| Biofilm | Dec. 2010 – May 2011 | 10 | 3.20 (0.22) | -20.60 (0.64) | 8.40 (0.33) |
| Seston (CTOM + FTOM) | Feb. – Mar. 2011 | 11 | 2.08 (0.24) | -25.98 (0.36) | 15.68 (0.92) |
| Leaf | Dec. 2010 | 2 | 1.99 (0.01) | -29.91 (0.0005) | 42.80 (1.004) |