Winter feeding, growth and condition of brown trout *Salmo trutta* in a groundwater-dominated stream.

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Abstract

Winter can be a stressful period for stream dwelling salmonid populations, often resulting in reduced growth and survival. Stream water temperatures have been identified as a primary mechanism driving reductions in fitness during winter. However, groundwater inputs can moderate water temperature and may reduce winter severity. Additionally, seasonal reductions in prey availability may contribute to decreased growth and survival, although few studies have examined food webs supporting salmonids under winter conditions. This study employed diet, stable isotope, and mark-recapture techniques to examine winter (November through March) feeding, growth, and condition of brown trout *Salmo trutta* in a groundwater-dominated stream (Badger Creek, Minnesota USA). Growth occurred during the winter season and was greater for fish \( \leq 150 \text{mm} \) (mean = 4.1 mg*g\(^{-1}\)*day\(^{-1}\)) than for those 151 - 276mm (mean = 1.0 mg*g\(^{-1}\)*day\(^{-1}\)). Overall condition from early winter to late winter did not vary for fish \( \leq 150 \text{mm} \) (Mean relative weight (Wr) = 89.5) and increased for those 151 - 276mm (Mean Wr = 85.8 early, 89.4 late). Although composition varied both temporally and by individual, brown trout diets were dominated by aquatic invertebrates, primarily Amphipods, Dipterans, and Trichopterans. Stable isotope analysis supported the observations of dominant prey taxa in stomach contents, and indicated the winter food web was supported by a combination of allochthonous inputs and aquatic macrophytes. Brown trout in Badger Creek likely benefited from the thermal regime and increased prey abundance present in this groundwater-dominated stream during winter.
Keywords: Brown trout, winter, diet, growth, stable isotope analysis
Introduction

Winter can be a stressful time for stream dwelling salmonids, as evidenced by reduced growth rate, condition, and survival (Quinn and Peterson 1996; Schultz and Conover 1999; Post and Parkinson 2001). Winter ice formation can be a significant stressor for stream dwelling trout. Surface and anchor ice can decrease the amount of available habitat for trout through reductions in physical space and the formation of ice dams (Brown et al. 2011; Chisholm et al. 1987). Biro et al. (2004) found overwinter mortality of age-0 fish (60-80%) because of depleted lipid reserves was a primary limiting factor for rainbow trout *Oncorhynchus mykiss* recruitment. Winter conditions often cause stream dwelling salmonids to alter behaviors and habitat preferences, and can lead to reductions in foraging and general activity levels (Hussko et al. 2007).

Brown trout *Salmo trutta* are a stream dwelling salmonid that inhabit a range of habitats encompassing a wide variety of physical variation in winter stream conditions. As such, brown trout populations can experience a range of winter severity dependent on the physical characteristics of a particular stream. Brown trout in the Credit River, Ontario (Canada) and the River Dodder (Ireland) experienced depressed growth rates and reductions in condition over winter (Cunjak and Power 1987; Cunjak et al. 1987; Kelly-Quinn and Bracken 1990). Age-0 brown trout had significantly reduced monthly survival rates during winter than during summer (0.65 vs. 0.99) in a small southeastern Norwegian stream (Lund et al. 2003).

In contrast, some studies suggest winter may be no more stressful for brown trout than other seasons. Survival rates of stream-dwelling brown trout in winter were equal to or greater
than other seasons in 11 of 16 cases reviewed by Carlson et al. (2008). Similarly, brown trout experienced positive growth and low over-winter mortality in three groundwater-dominated streams in southeastern Minnesota (USA) (Dieterman et al. 2012). Although winter severity appears to vary, differences in stream thermal regime have the potential to influence the degree to which brown trout may be affected in winter.

Reductions in growth and condition during winter are generally attributed to the effects of decreased temperature on brown trout physiology, with a minimum temperature of ~3.6°C required for growth (Elliot et al. 1995). Water temperatures in surface water-dominated streams closely track air temperatures, and often drop below 3.6°C in temperate locales during winter (Pilgrim et al. 1998). However, the temperature of ground water is approximately equal to mean annual air temperature (Erickson et al. 2000). Ground water input has a buffering effect on stream thermal regime, and can maintain water temperatures within acceptable ranges for brown trout growth even when air temperatures drop below freezing (Power 1999; O’Driscoll and DeWalle, 2004, 2006; Krider et al. 2013). The elevated winter temperatures of ground-water dominated streams may allow brown trout to maintain higher activity levels and more efficient functioning of metabolic processes.

Although water temperature may directly affect fish during winter, reductions in prey availability and quality (e.g., terrestrial invertebrate and aquatic invertebrate emergence and drift) may have additional implications for brown trout growth and condition. Summer diets of brown trout frequently include a significant proportion of terrestrial invertebrates (Kelly-Quinn and Bracken 1990; Bridcut 2000; Kawaguchi and Nakano 2001), but these prey are often
unavailable to trout during the winter. Aquatic invertebrates comprise the bulk of stream trout diets during winter (Cunjak et al. 1987; Kelly-Quinn and Bracken 1990), but aquatic invertebrate abundance can be reduced during the winter season (Newman and Waters 1984; Gislason 1985; Rundio and Lindley 2008). Dieterman et al. (2004) suggested differences in annual growth among brown trout populations in groundwater-dominated southeastern Minnesota streams were driven by differences in prey availability. Thus, declines in aquatic invertebrate availability have the potential to negatively affect stream trout foraging and growth.

The presence of seasonally available aquatic invertebrate species can increase the relative abundance of aquatic invertebrates in groundwater-dominated streams relative to surface-water dominated streams during winter (Bouchard and Ferrington 2009). The contributions of these seasonally available aquatic invertebrates to winter groundwater-dominated stream food webs are not well understood, but an increase in the relative abundance of potential prey may benefit brown trout within these systems.

Analysis of stomach contents has traditionally been used to examine trophic relationships, which allows for quantification of the contribution of specific prey taxa. However, stomach contents offer only a snapshot (dependent on stomach evacuation rate) of long-term patterns in diet. Conversely, stable isotope analysis (SIA) offers a time-integrated method of examining trophic relationships between consumers and their prey by examining ratios of stable carbon ($\delta^{13}$C) and nitrogen ($\delta^{15}$N) isotopes incorporated into the consumer’s tissue (Peterson and Fry 1987). $\delta^{13}$C is commonly used to determine energy sources in fishes (Peterson and Fry 1987), because $\delta^{13}$C signatures of prey are passed on to predators with a
predictable enrichment of ~ 0.4‰. Thus, δ¹³C can be used to track energy flow through the food web and compare the relative importance of various prey taxa. Trophic level can be inferred via δ¹⁵N with about a 3.4 ‰ increase in δ¹⁵N typically observed between predators and prey (Vander Zanden et al. 1997). The combination of taxonomically specific diet data from stomach contents with energy flow information from SIA provides a more complete picture of an organism’s position in the food web (Chipps and Garvey 2002).

The goal of this study was to evaluate the effects of winter conditions (here defined as November through March) on a brown trout population in a groundwater-dominated stream. Brown trout growth and condition were chosen as metrics to examine the effects of winter. Additionally, brown trout diet and relative position in the winter aquatic food web were quantified to examine potential mechanisms affecting growth and condition. The objectives of this study were to: (1) quantify and compare patterns of brown trout growth and condition during the winter season in a groundwater-dominated stream, (2) quantify the contributions of aquatic invertebrates to brown trout diets during winter using stomach contents and stable isotope analysis, and (3) quantify the relative position of brown trout within the winter aquatic food web of a groundwater-dominated stream using stable isotope analysis. Diet composition and growth were tracked by uniquely marked individuals, allowing relationships between diet and growth to be examined.

Methods

Study site
Badger Creek is a groundwater-dominated (i.e. receives enough groundwater input to significantly alter stream thermal regime and prevent over-winter freezing) tributary of the Root River, located in southeastern Minnesota, USA (Krider et al. 2013). The region is characterized by karst geology, including a large number of groundwater-dominated streams that support cold-water fish assemblages. Brown trout are the most abundant fish species in Badger Creek, but native brook trout *Salvelinus fontinalis* and slimy sculpin *Cottus cognatus* are also present. The sampling site consisted of a 125 m reach of stream containing multiple pools, riffles, and runs located in a forested headwater section of Badger Creek. Stream wetted width was ~3 m, and mean depth was < 1 m. Stream water temperature (7-9°C) was measured hourly from November 2011 through March 2012 by a remote logger device in the study site (*HOBO*™, Onset Computer Corporation Pocasset, MA) and remained within ranges suitable for brown trout growth throughout the winter (Elliot et al. 1995). The logger device was positioned 20cm below the surface at the head of a pool in the approximate middle of the sampling reach.

*Fish collection, growth and condition*

Brown trout were collected from Badger Creek on 19 November 2011 (early winter; 131 fish) and 15 March 2012 (late winter; 139 fish) using a Smith Root LR 20B backpack electric fisher (Smith Root, Vancouver, WA). Fish were placed in in-stream holding pens, anesthetized with an immobilizing dose of tricaine methanesulfonate (MS 222; Argent Chemical Laboratories Redmound, WA), weighed ± 1 g and measured ± 1 mm. All fish collected on 19 November were tagged in the anterior portion of the body cavity with 9mm passive integrated transponder (PIT) tags (Biomark Inc.; Idaho, USA) to track growth (mg* g^{-1} *day^{-1} ) and condition between sampling
events. Relative weight (Wr) compares the weight at length of a fish to a regionalized standard for that species and was used as an index of fish condition. Values between 80 and 100 are generally considered acceptable for healthy populations (Anderson & Neumann 1996).

Condition was only analyzed for fish >140mm TL because of limitations of the standard weight equations for lotic brown trout established by Milewski & Brown (1994).

Diet analysis

Gastric lavage was used on 30 fish per sampling date to examine diet composition. The subsample of 30 fish was selected randomly on 19 November, but 23 fish with PIT tags were preferentially selected on 15 March to quantify diet and growth. Stomach contents were preserved in 95% ethanol in the field, and later processed in the laboratory. Aquatic invertebrates were identified to family or genus and counted. Dry weight of aquatic invertebrates in the diet were estimated with equations from Benke et al. (1999) and Méthot et al. (2012). Mean morphological measurements of aquatic invertebrates (body length, shell width) were calculated from sub samples of 20 individuals per taxon randomly selected from brown trout diets and used to estimate dry weight. Dry weight estimates were multiplied by taxa counts to obtain dry weight composition of diet for each fish.

Stable isotope analysis

The ability of SIA to integrate consumer diet history over a broad time interval can provide a comprehensive food web depiction when used with stomach contents. Growth rates can affect assimilation and turnover rates of C\(^{13}\) and N\(^{15}\) (Church et al. 2009), and the specific tissue to use for SIA must be carefully considered. Use of SIA in winter has been rare because
of the potential for low tissue turnover rates. Muscle and fin tissue have C\textsuperscript{13} and N\textsuperscript{15} turnover
rates >140 day half-life, whereas mucus is especially suited to slow growth conditions because
of more rapid turnover rate (~30 day half-life) and continual regeneration (Church et al. 2009,
Hanisch et al. 2010). Thus, use of mucus and fin tissue with differing turnover rates allow for
temporal comparisons of brown trout diet. The faster turnover rate of mucus reflects
consumption during winter, whereas the slower turnover rate of fin tissue reflects material
consumed within winter, autumn, and late summer.

Pectoral fin tissue and mucus were collected following Church et al. (2009) from the
subsample of 30 fish subjected to gastric lavage on 15 March for SIA. Additionally, SIA was
conducted for 20 individuals on 15 March from each of the five most common prey taxa
observed on 19 November, and samples of allochthonous (leaf litter) and autochthonous
(Spirogyra sp., Nasturtium sp.) primary producers collected throughout the sampling reach.

Prior to analysis, fish fin, invertebrate, and plant samples were rinsed with deionized
water, placed in individual aluminum trays and dried at 55°C for 72 h. After drying, samples
were homogenized into a fine powder with a stainless steel rod, and stored in individually
labeled glass scintillation vials. Subsamples were weighed for SIA and placed into individual tin
capsules. Fish mucus was prepared according to the process outlined in Church et al. (2009). A
single, composite sample was prepared for each aquatic invertebrate and plant taxon from
individuals collected throughout the sampling reach, whereas fish fin and mucus samples were
analyzed individually. Samples were sent to the University of California Davis Stable Isotope
Facility (http://stableisotopefacility.ucdavis.edu/index.html) and analyzed for δ\textsuperscript{13}C and δ\textsuperscript{15}N

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using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon Ltd., Cheshire, UK). A subset of samples were analyzed at the University of Minnesota Stable Isotope Laboratory (http://www.geo.umn.edu/orgs/sil/index.html) using a Costech 4010 Elemental Analyzer interfaced to a Finnigan MAT252 Mass Spectrometer (Costech Analytical Technologies Inc., Valencia, CA) as a quality control measure.

**Statistical analysis**

Growth, condition and diet were compared for small (<150 mm) and large (151 - 276 mm) brown trout. These size categories correspond to age 0 and age 1+ fish collected from similar streams in the region (Dieterman et al. 2012). Growth rate was measured directly for PIT tagged individuals by calculating the change in mass between sampling events and compared between small and large brown trout with a Student $t$-test. The relationships between growth and dry weight of prey consumed, as well as growth and brown trout total length (TL) were examined with linear regression. The differences of $W_r$ for small and large brown trout were compared between early and late winter with a paired $t$-test.

Diet composition was examined by size class and sampling date and non-metric multidimensional scaling (NMS) was used to examine patterns in diet composition by number. Nine variables (size class, sample period, and mean consumption of Chironomidae, *Gammarus*, *Physella*, Limnephilidae, *Glossosoma*, Tipulidae, and other invertebrates consumed) were used in the NMS ordination.

Comment [vV1]: How does a Student $t$-test differ from a paired $t$-test at the end of the paragraph?
δ¹⁵N and δ¹³C for small and large brown trout were analyzed separately by tissue (mucus and fin) and compared with an ANOVA (model: N or C = size class). NMS was performed in PC-ORD (V. 6.0). All other analyses were performed in Program R (V 2.15.1). Statistical significance was declared at α = 0.05. Although sample size for some treatment groups was small (n= 10-12), data were evaluated for normality before analyses were conducted with a series of Shapiro-Wilks normality tests (Shapiro and Wilks 1965). All treatment groups were normally distributed.

Results

Growth, condition and water temperature

Growth during the winter was measured for the 23 fish recaptured during the late sampling event. Both small (n=11) and large (n=12) brown trout grew throughout the winter season, and there was a negative relationship between growth and TL (Figure 1). There was a positive relationship between growth and dry weight of prey consumed by fish on the March sampling date (Figure 2A). Small brown trout (mean = 4.09 mg*g⁻¹*day⁻¹) grew significantly faster than large brown trout (mean = 1.00 mg*g⁻¹*day⁻¹; t₂₂=5.19, P<0.001; Figure 2B). Overall condition from early winter to late winter did not vary for small brown trout (Mean Wr = 89.5, Figure 2C) and increased for large brown trout (Mean Wr = 85.8 early, 89.4 late; t₁₁=2.31, P=0.042; Figure 2D). Water temperature was within the range suitable for brown trout growth throughout the study (minimum temp = 5.5°C, maximum temp = 11.1°C, mean temp = 7.8°C).

Diet analysis
Brown trout diets in Badger creek were dominated by aquatic invertebrates, but small numbers of fish (cannibalized brown trout, n=2) and terrestrial invertebrates (i.e. annelid worms) were also present (Table 1). Only 1.6% of all sampled fish had empty stomachs. Brown trout diet composition varied temporally, but was similar between size classes. NMS (Stress = 11.02; Instability < 0.001; Iterations = 71) explained significant variation along the first two axes (Axis 1, $R^2 = 0.468$; Axis 2, $R^2 = 0.191$; Figure 3). Significant variables for Axis 1 included sample period ($r = -0.76$), Chironomidae consumed ($r = -0.75$), other invertebrates consumed ($r = -0.61$), Glossosoma consumed ($r = -0.68$), and Limnephilidae consumed ($r = 0.345$). Significant variables for Axis 2 included Glossosoma consumed ($r = 0.66$), and Chironomidae consumed ($r = -0.56$).

Stable isotope signatures of brown trout varied by tissue type and size class and supported the overall patterns observed in gastric lavage samples. Leaf litter had higher $\delta^{13}C$ (-29.4‰) compared to Spyrogyra (-35.3‰) and Nasturium sp. (-32.0‰). Aquatic invertebrate $\delta^{13}C$ indicated reliance on leaf litter and/or Nasturium sp., with the exception of Glossosoma, which had low values (-37.6‰) consistent with an algal based diet in headwater streams (Finlay 2001). The similarity of brown trout $\delta^{13}C$ to Gammarus, Chironomidae, Physella, and Limnephilidae $\delta^{13}C$ suggests the importance of these taxa to brown trout diets, whereas the dissimilar $\delta^{13}C$ of Glossosoma suggests lesser importance. $\delta^{15}N$ and $\delta^{13}C$ between large (mean $\delta^{15}N = 7.9‰$, $\delta^{13}C = -30.2‰$) and small (mean $\delta^{15}N = 7.2‰$, $\delta^{13}C = -31.1‰$) brown trout were not significantly different for fin tissue samples. However, $\delta^{13}C$ values were significantly different for large (mean $\delta^{13}C = -27.6‰$) and small (mean $\delta^{13}C = -30.2‰$) brown trout mucus samples ($F_{1,23}=10.61$, $P<0.01$) indicating a greater reliance on more enriched prey during winter (e.g. Physella) by large brown trout. Large (mean $\delta^{15}N = 6.3‰$) and small (mean $\delta^{15}N = 5.8‰$)
brown trout $\delta^{15}$N values were not significantly different suggesting both size classes occupy similar trophic levels.

Discussion

Growth

Both small and large brown trout in Badger Creek fed and grew throughout the winter season. Dieterman et al. (2012) also observed overwinter growth (~0.1mm * day$^{-1}$) in three groundwater-dominated southeastern Minnesota streams. In contrast, brown trout did not grow overwinter in the Credit River, a Canadian tributary to Lake Ontario (Cunjak and Power 1987), and in a tributary of the River Dodder, Ireland (Kelly-Quinn and Bracken 1988). Brown trout in West Brook (Massachusetts, USA) also experienced little or no growth between September and March (Carlson et al. 2007). Dissimilar stream temperature regimes in Badger Creek, the Credit River, and West Brook may explain differences in growth. Water temperature in Badger Creek remained significantly warmer (minimum temperature >5.9°C) throughout the winter than in the Credit River (minimum temperature 0.1 °C) and West Brook (minimum temperature < 0.0°C). Water temperatures were not recorded in the River Dodder, but mean January air temperature was 3.9 °C during the study. Although elevated water temperatures prevented ice formation in Badger Creek, Cunjak and Power (1987) documented surface ice cover of up to 22% in the Credit River during their study period. The prevention of ice formation in Badger Creek may have benefited brown trout by eliminating associated reductions in available habitat and foraging opportunities.
Growth in large brown trout may slow as they approach maximum size. Brown trout up to 388mm were collected from the sampling reach during this study. The largest individual used in our analyses was 276mm, whereas the majority of large brown trout were between 150mm and 250mm. Growth rates for large brown trout in Badger Creek may have decreased as trout approached their maximum size; however, the largest fish used in analyses was only ~70% of the TL of the largest fish captured from within the sampling reach.

Mature brown trout invest substantial amounts of energy into gamete production, and sexual maturation may have influenced growth and condition of large fish in our study. Brown trout in southeastern Minnesota typically spawn in October, and the majority of redds are constructed by early November (Stefanik and Sandheinrich 1999; Doug Dieterman, Minnesota DNR, unpublished data). Late spawning between sampling events may have reduced growth rates of some mature fish because of gamete production and increased activity levels. However, variation in growth rates of large brown trout was considerably less than in small (immature) brown trout, suggesting that most large brown trout had similar resources available for growth.

Condition of brown trout in Badger Creek remained stable or increased during winter for small and large fish (late winter mean Wr = 90.3 for small and 89.4 for large fish). In contrast, brown trout experienced a substantial decrease in condition by the end of winter in the Credit River (Cunjak et al. 1987), and River Dodder (Kelly-Quinn and Bracken 1990) where stream thermal regimes approached freezing. As an autumn spawning species, mature brown trout
trout condition should be reduced following spawning. Surprisingly, no significant change in condition was observed for small brown trout, and large brown trout increased condition between sampling dates in Badger Creek. The ability of brown trout to maintain condition and recoup potential body mass losses from spawning may have reduced demand on energy reserves and provided trout with an advantage to continue growth in spring.

Diet

Diets of both small and large brown trout were dominated by aquatic invertebrates, although the abundance of specific taxa varied by trout size class and sampling date. Empty stomachs were rare in our study (1.6%), but rates as high as 15% were observed in the River Dodder during winter, suggesting possible differences in prey availability (Kelly-Quinn and Bracken 1990). Brown trout often display size selectivity, preferentially feeding on larger prey items (Newman and Waters 1984). In Badger Creek, large-bodied taxa (Gammarus, Limnephilidae, Tipulidae and Physella) comprised the majority of prey consumed by dry weight during both early and late winter. However, smaller bodied prey such as Glossosoma and Chironomidae were often abundant in the diet, especially during late winter. Small and large brown trout consumed a greater abundance of small-bodied prey items in March than November.

The increase in small-bodied prey in brown trout diets during late winter may reflect shifts in aquatic invertebrate abundances between early and late winter. An increase in the abundance of small-bodied prey items may have increased their attractiveness to foraging brown trout, and large emergences of Chironomidae were observed before and during March.
sampling. Alternatively, stream trout can affect the aquatic invertebrate community’s abundance and composition through predation pressure (Lepori et al. 2012). Brown trout predation pressure may have reduced the abundance of large-bodied prey taxa during winter in Badger Creek, forcing brown trout to consume greater numbers of small-bodied prey. Notably, although brown trout showed a substantial increase in the relative number of Glossosoma and Chironomidae consumed in late winter, these taxa contributed little dry weight in the diet because of their small size.

The significant relationship between growth and the mass of prey consumed may indicate that prey availability in winter has the potential to constrain brown trout growth in Badger Creek. Bioenergetics modeling of brown trout populations in southeastern Minnesota suggested that prey quality and availability may limit growth in groundwater-dominated streams (Dieterman et al. 2004). Dry weights of prey used in this analysis were obtained from diet samples collected on a single sampling date, whereas growth rates incorporate changes in mass from November through March. The ability of a snapshot of the diet to represent brown trout consumption over the entire time period during which growth was measured is a legitimate concern. Additionally, the lack of prey density estimates did not allow comparisons between prey availability and consumption in early and late winter. However, the relationship between prey consumption and overwinter growth is an interesting observation that warrants further investigation. [Stable isotope analysis and winter food web]
The stable isotope analyses generally supported the results of stomach content observations. Brown trout diets in Badger Creek were dominated by aquatic invertebrates during winter, primarily by taxa using allochthonous or aquatic macrophyte based food sources. The relatively enriched brown trout $\delta^{13}C$ (-30.1‰ to -27.6‰) indicate *Gammarus* (-31.6‰), Chironomidae (-30.8‰), and *Physella* (-26.5‰) may be important prey taxa. *Glossosoma* do not appear to contribute significantly to brown trout diet, as evidenced by the $\delta^{13}C$ of *Glossosoma* (-37.6‰) and the low biomass of *Glossosoma* consumed by brown trout (mean dry weight= 0.4mg) compared to *Gammarus* (mean dry weight= 5.14mg) or *Physella* (mean dry weight= 3.09mg). Although *Glossosoma* are often abundant in small streams, they are typically not primary taxa in predator diets, as their stone cases may render them less vulnerable to predation (McNeely et al. 2007).

$\delta^{13}C$ of the selected prey taxa and brown trout suggest that energy in the Badger Creek food web may have been derived from a combination of autochthonous and allochthonous sources, primarily leaf litter (-29.4‰) and aquatic macrophytes (*Nasturtium sp.*; -32.0‰). *Watercress* (*Nasturtium sp.*) was abundant throughout the sampling site during the early winter and late winter sampling events, and contributed to primary production for the winter aquatic food web. Autochthonous algal growth (*Spirogyra*; -35.3‰) likely played a lesser role in the Badger Creek food web as consumer $\delta^{13}C$ was more enriched than would be expected from an algal-based diet. Groundwater input within the site may have contributed to aquatic macrophyte growth by maintaining higher water temperatures and preventing the formation of ice cover, which allowed light to reach aquatic macrophytes during winter.
Brown trout often become piscivorous as they grow, sometimes as early as 150mm TL (Jonnson et al. 1999; Garman and Nielson 1982). Although only two fish (both cannibalized brown trout) were found in the 60 diet samples, the large amount of energy supplied by a single instance of piscivory makes the relative importance of fish prey to brown trout diets difficult to determine from stomach content data alone. If piscivory were an important component of brown trout diet in Badger Creek there should have been more than one trophic level of separation between brown trout and primary consumers, but brown trout $\delta^{15}N$ indicated one trophic level (~3.4 ‰) or less separation above Gammarus, Glossosoma, Limnephilidae, Chironomidae and Physella. Additionally, there was no difference in $\delta^{15}N$ between small and large brown trout, which would accompany an ontogenetic shift to piscivory. $\delta^{15}N$ of brown trout from Badger Creek supported the stomach content observation that piscivory was rare, and aquatic invertebrates were the primary prey source for small and large brown trout.

Two tissue types with differing turnover rates for C and N allowed for temporal comparisons of diet of large and small brown trout. The faster turnover rate of mucus (half-life ~30 days) reflects consumption occurring during winter, whereas the slower turnover rate of fin tissue (>140 days) reflects material consumed in late summer, autumn and winter (Church et al. 2009). Large brown trout had more enriched mucus $\delta^{13}C$ than small brown trout, but $\delta^{15}N$ was not significantly different between size classes or tissue types. The difference in mucus $\delta^{13}C$ suggests diets of large and small brown trout may have diverged during winter, possibly due to Physella being more prominent in late winter diets of large brown trout. Alternatively, the differences may reflect the importance of other taxa that were not collected for SIA (e.g.
Tipulidae), or a combination of Physella and other aquatic invertebrate taxa. As no corresponding increase in Physella consumption was observed in late winter stomach contents, a shift to Tipulidae or other unidentified taxa is better supported by the data. Although higher trophic level prey taxa have been observed in brown trout diets in other southeastern Minnesota streams (e.g. sculpin, Rana sp., fish eggs; W. French, unpublished data), these prey likely did not contribute substantially to brown trout diets in Badger Creek, as there was no corresponding enrichment in brown trout δ15N.

Conclusions

Although winter can be stressful for brown trout in some systems, trout in groundwater-dominated streams may benefit from stabilized annual temperature regimes and increased prey availability. Fish mucus was a useful tissue to evaluate temporal variation in SIA signatures during a period of reduced growth, especially when combined with fin tissue, which has a slow turnover rate. Brown trout in a groundwater-dominated stream continued to feed, maintained or increased their condition, and grew during the winter. Allochthonous inputs and aquatic macrophytes were the most significant sources of primary production in the winter aquatic food web of Badger Creek, supporting the majority of aquatic invertebrates and brown trout. These findings illustrate the need for further research of dynamics of trout and aquatic invertebrates of groundwater-dominated streams in winter, particularly the effects of varying amounts of groundwater input on trout population dynamics and aquatic winter food webs.

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Figure Captions

Figure 1. Relationship between growth rate (mg*g⁻¹*day⁻¹) and total length (TL) of brown trout recaptured in Badger creek on 15 March 2012.

Figure 2. (A) Relationship between growth rate (mg*g⁻¹*day⁻¹) and dry weight (mg*g trout⁻¹) of prey consumed., (B) Mean growth rates of large (150 - 276mm TL) and small (<150mm TL) brown trout, (C) Mean relative weight (Wr) of small (<150mm TL) brown trout in early winter and late winter. (D) Mean relative weight (Wr) of large (>150 – 276mm TL) brown trout in early winter and late winter. All data derived from marked and recaptured fish in Badger Creek. * indicates P<0.05.

Figure 3. Non-metric multidimensional scaling ordination of diet composition for small (<150mm) and large (151 - 276mm) brown trout in Badger Creek. Closed squares represent large fish in early winter; closed circles represent small fish in early winter. Open squares represent large fish in late winter; open circles represent small fish in late winter.

Figure 4. Carbon nitrogen bi-plot of mean (± 1 SD) δ¹³C and δ¹⁵N signatures for small (<150mm TL) and large (151 – 276mm TL) brown trout, and integrated δ¹³C and δ¹⁵N signatures for common invertebrate prey taxa, and primary producers for (A) fin tissue and (B) mucus samples.