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

2   **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 $\leq$ 150mm (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 $\leq$ 150mm (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.

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68 **Keywords:** Brown trout, winter, diet, growth, stable isotope analysis

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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

91   than other seasons in 11 of 16 cases reviewed by Carlson et al. (2008). Similarly, brown trout

92   experienced positive growth and low over-winter mortality in three groundwater-dominated

93   streams in southeastern Minnesota (USA) (Dieterman et al. 2012). Although winter severity

94   appears to vary, differences in stream thermal regime have the potential to influence the

95   degree to which brown trout may be affected in winter.

96   Reductions in growth and condition during winter are generally attributed to the effects

97   of decreased temperature on brown trout physiology, with a minimum temperature of ~3.6°C

98   required for growth (Elliot et al. 1995). Water temperatures in surface water-dominated

99   streams closely track air temperatures, and often drop below 3.6°C in temperate locales during

100   winter (Pilgrim et al. 1998). However, the temperature of ground water is approximately equal

101   to mean annual air temperature (Erickson et al. 2000). Ground water input has a buffering

102   effect on stream thermal regime, and can maintain water temperatures within acceptable

103   ranges for brown trout growth even when air temperatures drop below freezing (Power 1999;

104   O'Driscoll and DeWalle, 2004, 2006; Krider et al. 2013). The elevated winter temperatures of

105   ground-water dominated streams may allow brown trout to maintain higher activity levels and

106   more efficient functioning of metabolic processes.

107   Although water temperature may directly affect fish during winter, reductions in prey

108   availability and quality (e.g., terrestrial invertebrate and aquatic invertebrate emergence and

109   drift) may have additional implications for brown trout growth and condition. Summer diets of

110   brown trout frequently include a significant proportion of terrestrial invertebrates (Kelly-Quinn

111   and Bracken 1990; Bridcut 2000; Kawaguchi and Nakano 2001), but these prey are often

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112   unavailable to trout during the winter. Aquatic invertebrates comprise the bulk of stream trout

113   diets during winter (Cunjak et al. 1987; Kelly-Quinn and Bracken 1990), but aquatic invertebrate

114   abundance can be reduced during the winter season (Newman and Waters 1984; Gislason

115   1985; Rundio and Lindley 2008). Dieterman et al. (2004) suggested differences in annual

116   growth among brown trout populations in groundwater-dominated southeastern Minnesota

117   streams were driven by differences in prey availability.   Thus, declines in aquatic invertebrate

118   availability have the potential to negatively affect stream trout foraging and growth.

119   The presence of seasonally available aquatic invertebrate species can increase the

120   relative abundance of aquatic invertebrates in groundwater-dominated streams relative to

121   surface-water dominated streams during winter (Bouchard and Ferrington 2009). The

122   contributions of these seasonally available aquatic invertebrates to winter groundwater-

123   dominated stream food webs are not well understood, but an increase in the relative

124   abundance of potential prey may benefit brown trout within these systems.

125   Analysis of stomach contents has traditionally been used to examine trophic

126   relationships, which allows for quantification of the contribution of specific prey taxa.

127   However, stomach contents offer only a snapshot (dependent on stomach evacuation rate) of

128   long-term patterns in diet. Conversely, stable isotope analysis (SIA) offers a time-integrated

129   method of examining trophic relationships between consumers and their prey by examining

130   ratios of stable carbon (δ^{13}C) and nitrogen (δ^{15}N) isotopes incorporated into the consumer's

131   tissue (Peterson and Fry 1987). δ^{13}C is commonly used to determine energy sources in fishes

132   (Peterson and Fry 1987), because δ^{13}C signatures of prey are passed on to predators with a

133   predictable enrichment of ~ 0.4‰. Thus, $\delta^{13}C$ can be used to track energy flow through the

134   food web and compare the relative importance of various prey taxa. Trophic level can be

135   inferred via $\delta^{15}N$ with about a 3.4 ‰ increase in $\delta^{15}N$ typically observed between predators and

136   prey (Vander Zanden et al. 1997). The combination of taxonomically specific diet data from

137   stomach contents with energy flow information from SIA provides a more complete picture of

138   an organism's position in the food web (Chipps and Garvey 2002).

139   The goal of this study was to evaluate the effects of winter conditions (here defined as

140   November through March) on a brown trout population in a groundwater-dominated stream.

141   Brown trout growth and condition were chosen as metrics to examine the effects of winter.

142   Additionally, brown trout diet and relative position in the winter aquatic food web were

143   quantified to examine potential mechanisms affecting growth and condition.   The objectives of

144   this study were to: (1) quantify and compare patterns of brown trout growth and condition

145   during the winter season in a groundwater-dominated stream, (2) quantify the contributions of

146   aquatic invertebrates to brown trout diets during winter using stomach contents and stable

147   isotope analysis, and (3) quantify the relative position of brown trout within the winter aquatic

148   food web of a groundwater-dominated stream using stable isotope analysis. Diet composition

149   and growth were tracked by uniquely marked individuals, allowing relationships between diet

150   and growth to be examined.

151   **Methods**

152   *Study site*

153   Badger Creek is a groundwater-dominated (i.e. receives enough groundwater input to

154   significantly alter stream thermal regime and prevent over-winter freezing) tributary of the

155   Root River, located in southeastern Minnesota, USA (Krider et al. 2013). The region is

156   characterized by karst geology, including a large number of groundwater-dominated streams

157   that support cold-water fish assemblages. Brown trout are the most abundant fish species in

158   Badger Creek, but native brook trout *Salvelinus fontinalis* and slimy sculpin *Cottus cognatus* are

159   also present. The sampling site consisted of a 125 m reach of stream containing multiple pools,

160   riffles, and runs located in a forested headwater section of Badger Creek. Stream wetted width

161   was ~3 m, and mean depth was < 1 m. Stream water temperature (7-9°C) was measured hourly

162   from November 2011 through March 2012 by a remote logger device in the study site (HOBO^{TM},

163   Onset Computer Corporation Pocassat, MA) and remained within ranges suitable for brown

164   trout growth throughout the winter (Elliot et al. 1995). The logger device was positioned 20cm

165   below the surface at the head of a pool in the approximate middle of the sampling reach.

166   ***Fish collection, growth and condition***

167   Brown trout were collected from Badger Creek on 19 November 2011 (early winter; 131

168   fish) and 15 March 2012 (late winter; 139 fish) using a Smith Root LR 20B backpack electric

169   fisher (Smith Root, Vancouver, WA). Fish were placed in in-stream holding pens, anesthetized

170   with an immobilizing dose of tricaine methanesulfonate (MS 222; Argent Chemical Laboratories

171   Redmound, WA), weighed $\pm$ 1 g and measured $\pm$ 1 mm. All fish collected on 19 November were

172   tagged in the anterior portion of the body cavity with 9mm passive integrated transponder (PIT)

173   tags (Biomark Inc.; Idaho, USA) to track growth (mg*g^{-1} *day^{-1}) and condition between sampling

174   events. Relative weight (Wr) compares the weight at length of a fish to a regionalized standard

175   for that species and was used as an index of fish condition. Values between 80 and 100 are

176   generally considered acceptable for healthy populations (Anderson & Neumann 1996).

177   Condition was only analyzed for fish $\geq$140mm TL because of limitations of the standard weight

178   equations for lotic brown trout established by Milewski & Brown (1994).

179   *Diet analysis*

180   Gastric lavage was used on 30 fish per sampling date to examine diet composition. The

181   subsample of 30 fish was selected randomly on 19 November, but 23 fish with PIT tags were

182   preferentially selected on 15 March to quantify diet and growth. Stomach contents were

183   preserved in 95% ethanol in the field, and later processed in the laboratory. Aquatic

184   invertebrates were identified to family or genus and counted. Dry weight of aquatic

185   invertebrates in the diet were estimated with equations from Benke et al. (1999) and Méthot et

186   al. (2012). Mean morphological measurements of aquatic invertebrates (body length, shell

187   width) were calculated from sub samples of 20 individuals per taxon randomly selected from

188   brown trout diets and used to estimate dry weight. Dry weight estimates were multiplied by

189   taxa counts to obtain dry weight composition of diet for each fish.

190   *Stable isotope analysis*

191   The ability of SIA to integrate consumer diet history over a broad time interval can

192   provide a comprehensive food web depiction when used with stomach contents. Growth rates

193   can affect assimilation and turnover rates of C^{13} and N^{15} (Church et al. 2009), and the specific

194   tissue to use for SIA must be carefully considered. Use of SIA in winter has been rare because

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195   of the potential for low tissue turnover rates.   Muscle and fin tissue have C^{13} and N^{15} turnover

196   rates >140 day half-life, whereas mucus is especially suited to slow growth conditions because

197   of more rapid turnover rate (~30 day half-life) and continual regeneration (Church et al. 2009,

198   Hanisch et al. 2010). Thus, use of mucus and fin tissue with differing turnover rates allow for

199   temporal comparisons of brown trout diet. The faster turnover rate of mucus reflects

200   consumption during winter, whereas the slower turnover rate of fin tissue reflects material

201   consumed within winter, autumn, and late summer.

202   Pectoral fin tissue and mucus were collected following Church et al. (2009) from the

203   subsample of 30 fish subjected to gastric lavage on 15 March for SIA. Additionally, SIA was

204   conducted for 20 individuals on 15 March from each of the five most common prey taxa

205   observed on 19 November, and samples of allochthonous (leaf litter) and autochthonous

206   (*Spirogyra* sp.*, Nasturtium* sp.*) primary producers collected throughout the sampling reach.

207   Prior to analysis, fish fin, invertebrate, and plant samples were rinsed with deionized

208   water, placed in individual aluminum trays and dried at 55°C for 72 h. After drying, samples

209   were homogenized into a fine powder with a stainless steel rod, and stored in individually

210   labeled glass scintillation vials. Subsamples were weighed for SIA and placed into individual tin

211   capsules. Fish mucus was prepared according to the process outlined in Church et al. (2009). A

212   single, composite sample was prepared for each aquatic invertebrate and plant taxon from

213   individuals collected throughout the sampling reach, whereas fish fin and mucus samples were

214   analyzed individually. Samples were sent to the University of California Davis Stable Isotope

215   Facility (http://stableisotopefacility.ucdavis.edu/index.html) and analyzed for $\delta^{13}C$ and $\delta^{15}N$

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216 using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope

217 ratio mass spectrometer (Sercon Ltd., Cheshire, UK). A subset of samples were analyzed at the

218 University of Minnesota Stable Isotope Laboratory

219 (http://www.geo.umn.edu/orgs/sil/index.html) using a Costech 4010 Elemental Analyzer

220 interfaced to a Finnigan MAT252 Mass Spectrometer (Costech Analytical Technologies Inc.,

221 Valencia, CA) as a quality control measure.

222 *Statistical analysis*

223 Growth, condition and diet were compared for small ($\leq$150 mm) and large (151 -

224 276mm) brown trout. These size categories correspond to age 0 and age 1+ fish collected from

225 similar streams in the region (Dieterman et al. 2012). Growth rate was measured directly for

226 PIT tagged individuals by calculating the change in mass between sampling events and

227 compared between small and large brown trout with a Student t-test. The relationships

228 between growth and dry weight of prey consumed, as well as growth and brown trout total

229 length (TL) were examined with linear regression. The differences of Wr for small and large

230 brown trout were compared between early and late winter with a paired t-test.

231 Diet composition was examined by size class and sampling date and non-metric

232 multidimensional scaling (NMS) was used to examine patterns in diet composition by number.

233 Nine variables (size class, sample period, and mean consumption of Chironomidae, *Gammarus*,

234 *Physella*, Limnephilidae, *Glossosoma*, Tipulidae, and other invertebrates consumed) were used

235 in the NMS ordination.

Comment [vV1]: How does a Student t-test differ from a paired t-test at the end of the paragraph?

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236   δ^{15}N and δ^{13}C for small and large brown trout were analyzed separately by tissue

237   (mucus and fin) and compared with an ANOVA (model: N or C = size class). NMS was

238   performed in PC-ORD (V. 6.0). All other analyses were performed in Program R (V 2.15.1).

239   Statistical significance was declared at α = 0.05.   Although sample size for some treatment

240   groups was small (n= 10-12), data were evaluated for normality before analyses were

241   conducted with a series of Shapiro-Wilks normality tests (Shapiro and Wilks 1965). All

242   treatment groups were normally distributed.

243   **Results**

244   ***Growth, condition and water temperature***

245   Growth during the winter was measured for the 23 fish recaptured during the late

246   sampling event. Both small (*n*=11) and large (*n*=12) brown trout grew throughout the winter

247   season, and there was a negative relationship between growth and TL (Figure 1). There was a

248   positive relationship between growth and dry weight of prey consumed by fish on the March

249   sampling date (Figure 2A). Small brown trout (mean = 4.09 mg*g^{-1} *day^{-1}) grew significantly

250   faster than large brown trout (mean = 1.00 mg*g^{-1} *day^{-1}; t_{22}=5.19, *P*<0.001; Figure 2B). Overall

251   condition from early winter to late winter did not vary for small brown trout (Mean Wr = 89.5,

252   Figure 2C) and increased for large brown trout (Mean Wr = 85.8 early, 89.4 late; t_{11}=2.31, *P*=

253   0.042; Figure 2D). Water temperature was within the range suitable for brown trout growth

254   throughout the study (minimum temp = 5.5°C, maximum temp = 11.1°C, mean temp = 7.8°C).

255   ***Diet analysis***

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256   Brown trout diets in Badger creek were dominated by aquatic invertebrates, but small

257   numbers of fish (cannibalized brown trout, *n*=2) and terrestrial invertebrates (i.e. annelid

258   worms) were also present (Table 1). Only 1.6% of all sampled fish had empty stomachs. Brown

259   trout diet composition varied temporally, but was similar between size classes. NMS (Stress =

260   11.02; Instability < 0.001; Iterations = 71) explained significant variation along the first two axes

261   (Axis 1, R^2= 0.468; Axis 2, R^2 = 0.191; Figure 3). Significant variables for Axis 1 included sample

262   period (r= -0.76), Chironomidae consumed (r= -0.75), other invertebrates consumed (r= -0.61),

263   *Glossosoma* consumed (r= -0.68), and Limnephilidae consumed (r= 0.345). Significant variables

264   for Axis 2 included *Glossosoma* consumed (r= 0.66), and Chironomidae consumed (r= -0.56).

265   Stable isotope signatures of brown trout varied by tissue type and size class and

266   supported the overall patterns observed in gastric lavage samples. Leaf litter had higher δ^{13}C (-

267   29.4‰) compared to *Spyrogyra* (-35.3‰) and *Nasturium* sp. (-32.0‰). Aquatic invertebrate

268   δ^{13}C indicated reliance on leaf litter and/or *Nasturium* sp*.,* with the exception of *Glossosoma*,

269   which had low values (-37.6‰) consistent with an algal based diet in headwater streams (Finlay

270   2001).   The similarity of brown trout δ^{13}C to *Gammarus,* Chironomidae, *Physella,* and

271   Limnephilidae δ^{13}C suggests the importance of these taxa to brown trout diets, whereas the

272   dissimilar δ^{13}C of *Glossosoma* suggests lesser importance. δ^{15}N and δ^{13}C between large (mean

273   δ^{15}N = 7.9‰, δ^{13}C= -30.2‰) and small (mean δ^{15}N = 7.2‰, δ^{13}C= -31.1‰) brown trout were

274   not significantly different for fin tissue samples. However, δ^{13}C values were significantly

275   different for large (mean δ^{13}C = -27.6‰) and small (mean δ^{13}C = -30.2‰) brown trout mucus

276   samples ($F_{1,23}$=10.61, P< 0.01) indicating a greater reliance on more enriched prey during winter

277   (e.g. *Physella*) by large brown trout. Large (mean δ^{15}N = 6.3‰) and small (mean δ^{15}N = 5.8‰)

278 brown trout δ^{15}N values were not significantly different suggesting both size classes occupy

279 similar trophic levels.

280   **Discussion**

281 **Growth**

282   Both small and large brown trout in Badger Creek fed and grew throughout the winter

283 season. Dieterman et al. (2012) also observed overwinter growth (~0.1mm * day^{-1}) in three

284 groundwater-dominated southeastern Minnesota streams. In contrast, brown trout did not

285 grow overwinter in the Credit River, a Canadian tributary to Lake Ontario (Cunjak and Power

286 1987), and in a tributary of the River Dodder, Ireland (Kelly-Quinn and Bracken 1988). Brown

287 trout in West Brook (Massachusetts, USA) also experienced little or no growth between

288 September and March (Carlson et al. 2007). Dissimilar stream temperature regimes in Badger

289 Creek, the Credit River, and West Brook may explain differences in growth. Water temperature

290 in Badger Creek remained significantly warmer (minimum temperature >5.9°C) throughout the

291 winter than in the Credit River (minimum temperature 0.1 °C) and West Brook (minimum

292 temperature < 0.0°C). Water temperatures were not recorded in the River Dodder, but mean

293 January air temperature was 3.9 °C during the study. Although elevated water temperatures

294 prevented ice formation in Badger Creek, Cunjak and Power (1987) documented surface ice

295 cover of up to 22% in the Credit River during their study period. The prevention of ice

296 formation in Badger Creek may have benefited brown trout by eliminating associated

297 reductions in available habitat and foraging opportunities.

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298   Growth in large brown trout may slow as they approach maximum size. Brown trout up

299   to 388mm were collected from the sampling reach during this study. The largest individual

300   used in our analyses was 276mm, whereas the majority of large brown trout were between

301   150mm and 250mm. Growth rates for large brown trout in Badger Creek may have decreased

302   as trout approached their maximum size; however, the largest fish used in analyses was only

303   ~70% of the TL of the largest fish captured from within the sampling reach.

304   Mature brown trout invest substantial amounts of energy into gamete production, and

305   sexual maturation may have influenced growth and condition of large fish in our study. Brown

306   trout in southeastern Minnesota typically spawn in October, and the majority of redds are

307   constructed by early November (Stefanik and Sandheinrich 1999; Doug Dieterman, Minnesota

308   DNR, unpublished data). Late spawning between sampling events may have reduced growth

309   rates of some mature fish because of gamete production and increased activity levels.

310   However, variation in growth rates of large brown trout was considerably less than in small

311   (immature) brown trout, suggesting that most large brown trout had similar resources available

312   for growth.

313   **Condition**

314   Condition of brown trout in Badger Creek remained stable or increased during winter

315   for small and large fish (late winter mean Wr = 90.3 for small and 89.4 for large fish). In

316   contrast, brown trout experienced a substantial decrease in condition by the end of winter in

317   the Credit River (Cunjak et al. 1987), and River Dodder (Kelly-Quinn and Bracken 1990) where

318   stream thermal regimes approached freezing. As an autumn spawning species, mature brown

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319   trout condition should be reduced following spawning. Surprisingly, no significant change in

320   condition was observed for small brown trout, and large brown trout increased condition

321   between sampling dates in Badger Creek. The ability of brown trout to maintain condition and

322   recoup potential body mass losses from spawning may have reduced demand on energy

323   reserves and provided trout with an advantage to continue growth in spring.

324   **Diet**

325   Diets of both small and large brown trout were dominated by aquatic invertebrates,

326   although the abundance of specific taxa varied by trout size class and sampling date. Empty

327   stomachs were rare in our study (1.6%), but rates as high as 15% were observed in the River

328   Dodder during winter, suggesting possible differences in prey availability (Kelly-Quinn and

329   Bracken 1990). Brown trout often display size selectivity, preferentially feeding on larger prey

330   items (Newman and Waters 1984). In Badger Creek, large-bodied taxa (*Gammarus*,

331   Limnephilidae, Tipulidae and *Physella*) comprised the majority of prey consumed by dry weight

332   during both early and late winter. However, smaller bodied prey such as *Glossosoma* and

333   Chironomidae were often abundant in the diet, especially during late winter. Small and large

334   brown trout consumed a greater abundance of small-bodied prey items in March than

335   November.

336   The increase in small-bodied prey in brown trout diets during late winter may reflect

337   shifts in aquatic invertebrate abundances between early and late winter. An increase in the

338   abundance of small-bodied prey items may have increased their attractiveness to foraging

339   brown trout, and large emergences of Chironomidae were observed before and during March

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340   sampling. Alternatively, stream trout can affect the aquatic invertebrate community's

341   abundance and composition through predation pressure (Lepori et al. 2012). Brown trout

342   predation pressure may have reduced the abundance of large-bodied prey taxa during winter in

343   Badger Creek, forcing brown trout to consume greater numbers of small-bodied prey. Notably,

344   although brown trout showed a substantial increase in the relative number of *Glossosoma* and

345   Chironomidae consumed in late winter, these taxa contributed little dry weight in the diet

346   because of their small size.

347   The significant relationship between growth and the mass of prey consumed may

348   indicate that prey availability in winter has the potential to constrain brown trout growth in

349   Badger Creek. Bioenergetics modeling of brown trout populations in southeastern Minnesota

350   suggested that prey quality and availability may limit growth in groundwater-dominated

351   streams (Dieterman et al. 2004). Dry weights of prey used in this analysis were obtained from

352   diet samples collected on a single sampling date, whereas growth rates incorporate changes in

353   mass from November through March. The ability of a snapshot of the diet to represent brown

354   trout consumption over the entire time period during which growth was measured is a

355   legitimate concern. Additionally, the lack of prey density estimates did not allow comparisons

356   between prey availability and consumption in early and late winter.   However, the relationship

357   between prey consumption and overwinter growth is an interesting observation that warrants

358   further investigation.

359   **Stable isotope analysis and winter food web**

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360　　　　　The stable isotope analyses generally supported the results of stomach content

361　　observations. Brown trout diets in Badger Creek were dominated by aquatic invertebrates

362　　during winter, primarily by taxa using allochthonous or aquatic macrophyte based food sources.

363　　The relatively enriched brown trout δ^{13}C (-30.1‰ to -27.6‰) indicate *Gammarus* (-31.6‰),

364　　Chironomidae (-30.8‰), and *Physella* (-26.5‰) may be important prey taxa. *Glossosoma* do

365　　not appear to contribute significantly to brown trout diet, as evidenced by the δ^{13}C of

366　　*Glossosoma* (-37.6‰) and the low biomass of *Glossosoma* consumed by brown trout (mean

367　　dry weight= 0.4mg) compared to *Gammarus* (mean dry weight= 5.14mg) or *Physella* (mean dry

368　　weight= 3.09mg). Although *Glossosoma* are often abundant in small streams, they are typically

369　　not primary taxa in predator diets, as their stone cases may render them less vulnerable to

370　　predation (McNeely et al. 2007).

371　　　　　　δ^{13}C of the selected prey taxa and brown trout suggest that energy in the Badger Creek

372　　food web may have been derived from a combination of autochthonous and allochthonous

373　　sources, primarily leaf litter (-29.4‰) and aquatic macrophytes (*Nasturtium sp.; -32.0‰*).

374　　Watercress (*Nasturtium sp.*) was abundant throughout the sampling site during the early winter

375　　and late winter sampling events, and contributed to primary production for the winter aquatic

376　　food web. Autochthonous algal growth (*Spirogyra; -35.3‰*) likely played a lesser role in the

377　　Badger Creek food web as consumer δ^{13}C was more enriched than would be expected from an

378　　algal-based diet. Groundwater input within the site may have contributed to aquatic

379　　macrophyte growth by maintaining higher water temperatures and preventing the formation of

380　　ice cover, which allowed light to reach aquatic macrophytes during winter.

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381   Brown trout often become piscivorous as they grow, sometimes as early as 150mm TL

382   (Jonnson et al. 1999; Garman and Nielson 1982). Although only two fish (both cannibalized

383   brown trout) were found in the 60 diet samples, the large amount of energy supplied by a

384   single instance of piscivory makes the relative importance of fish prey to brown trout diets

385   difficult to determine from stomach content data alone. If piscivory were an important

386   component of brown trout diet in Badger Creek there should have been more than one trophic

387   level of separation between brown trout and primary consumers, but brown trout δ^{15}N

388   indicated one trophic level (~3.4 ‰) or less separation above *Gammarus, Glossosoma*,

389   Limnephilidae, Chironomidae and *Physella*. Additionally, there was no difference in δ^{15}N

390   between small and large brown trout, which would accompany an ontogenetic shift to

391   piscivory. δ^{15}N of brown trout from Badger Creek supported the stomach content observation

392   that piscivory was rare, and aquatic invertebrates were the primary prey source for small and

393   large brown trout.

394   Two tissue types with differing turnover rates for C and N allowed for temporal

395   comparisons of diet of large and small brown trout. The faster turnover rate of mucus (half-life

396   ~30 days) reflects consumption occurring during winter, whereas the slower turnover rate of fin

397   tissue (>140 days) reflects material consumed in late summer, autumn and winter (Church et al.

398   2009). Large brown trout had more enriched mucus δ^{13}C than small brown trout, but δ^{15}N was

399   not significantly different between size classes or tissue types. The difference in mucus δ^{13}C

400   suggests diets of large and small brown trout may have diverged during winter, possibly due to

401   *Physella* being more prominent in late winter diets of large brown trout. Alternatively, the

402   differences may reflect the importance of other taxa that were not collected for SIA (e.g.

20

Comment [vV2]: In the previous sentence you indicated no significant differences, but in the following sentences you indicate a difference.

403 Tipulidae), or a combination of *Physella* and other aquatic invertebrate taxa. As no

404 corresponding increase in *Physella* consumption was observed in late winter stomach contents,

405 a shift to Tipulidae or other unidentified taxa is better supported by the data. Although higher

406 trophic level prey taxa have been observed in brown trout diets in other southeastern

407 Minnesota streams (e.g. sculpin, *Rana* sp*.,* fish eggs; W. French, unpublished data), these prey

408 likely did not contribute substantially to brown trout diets in Badger Creek, as there was no

409 corresponding enrichment in brown trout δ^{15}N.

410

411 **Conclusions**

412   Although winter can be stressful for brown trout in some systems, trout in groundwater-

413 dominated streams may benefit from stabilized annual temperature regimes and increased

414 prey availability. Fish mucus was a useful tissue to evaluate temporal variation in SIA signatures

415 during a period of reduced growth, especially when combined with fin tissue, which has a slow

416 turnover rate. Brown trout in a groundwater-dominated stream continued to feed, maintained

417 or increased their condition, and grew during the winter. Allochthonous inputs and aquatic

418 macrophytes were the most significant sources of primary production in the winter aquatic

419 food web of Badger Creek, supporting the majority of aquatic invertebrates and brown trout.

420 These findings illustrate the need for further research of dynamics of trout and aquatic

421 invertebrates of groundwater-dominated streams in winter, particularly the effects of varying

422 amounts of groundwater input on trout population dynamics and aquatic winter food webs.

423 **Acknowledgements**

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576

Figure Captions

578   **Figure 1.** Relationship between growth rate $(mg*g^{-1}*day^{-1})$ and total length (TL) of brown trout

579   recaptured in Badger creek on 15 March 2012.

580   **Figure 2.** (A) Relationship between growth rate $(mg*g^{-1}*day^{-1})$ and dry weight $(mg*g\ trout^{-1})$ of

581   prey consumed., (B) Mean growth rates of large (150 - 276mm TL) and small ($\leq$150mm TL)

582   brown trout, (C) Mean relative weight (Wr) of small ($\leq$ 150mm TL) brown trout in early winter

583   and late winter. (D) Mean relative weight (Wr) of large (>150 – 276mm TL) brown trout in early

584   winter and late winter. All data derived from marked and recaptured fish in Badger Creek. *

585   indicates *P*<0.05.

586   **Figure 3.** Non-metric multidimensional scaling ordination of diet composition for small ($\leq$

587   150mm) and large (151 - 276mm) brown trout in Badger Creek. Closed squares represent large

588   fish in early winter; closed circles represent small fish in early winter. Open squares represent

589   large fish in late winter; open circles represent small fish in late winter.

590   **Figure 4.** Carbon nitrogen bi-plot of mean ($\pm$ 1 SD) δ^{13}C and δ^{15}N signatures for small ($\leq$150mm

591   TL) and large (151 – 276mm TL) brown trout, and integrated δ^{13}C and δ^{15}N signatures for

592   common invertebrate prey taxa, and primary producers for (A) fin tissue and (B) mucus

593   samples.

594