

TITLE

Hot eats and cool creeks: juvenile Pacific salmonids use mainstem prey while in thermal refuges

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## 1 ABSTRACT

2 Thermal refuges form important habitat for coldwater fishes in the face of rising temperatures.  
3 As fish become concentrated in refuges, food resources may become depleted. In this study, we  
4 used invertebrate drift sampling and fish density surveys to quantify potential in-refuge food  
5 limitation, temperature-sensitive radio-tagging studies to quantify thermal habitat use, and  
6 isotopic analyses to determine diet sources for juvenile Pacific salmonids using thermal refuges  
7 on California's Klamath River. Juvenile salmonids using refuges formed by tributary junctions  
8 with the mainstem river obtained the majority (range = 47-97%) of their diet from mainstem prey  
9 sources. Mean steelhead body temperatures were significantly cooler ( $\sim 3.5^{\circ}\text{C}$ ) than diet-inferred  
10 foraging temperatures. Thus, while fish seek cooler habitat for physiological benefits, they rely  
11 primarily on mainstem prey. Moreover, consistently high densities of fish in refuges (mean = 3.5  
12 fish  $\text{m}^{-2}$ ) could lead to density-dependent food limitation. Thus, mobile consumers like fish can  
13 exploit existing heterogeneity associated with coldwater refuges by gaining thermal benefits  
14 from a food-limited coldwater habitat while deriving the majority of their prey from the warm  
15 mainstem river.

16  
17 KEYWORDS: Chinook salmon; Klamath River; radio telemetry; steelhead; thermal refuges

## 18 INTRODUCTION

19 Habitat heterogeneity provides organisms with a choice of disparate habitats, and mobile  
20 consumers can integrate across a heterogeneous landscape, thereby balancing trade-offs in  
21 resources (Bridges 2002, Hohausova et al. 2003, Scheuerell and Schindler 2003, Sims et al.  
22 2006, Loose and Dawidowicz 2014). For coldwater fish, thermal refuges provide physiological  
23 relief from stressful in-stream temperatures, but they are also heterogeneous environments where  
24 the distribution of other resources within and surrounding a refuge (e.g., prey availability,  
25 predation risk) could influence the relative costs and benefits of refuge use. Thermal refuges,  
26 coldwater habitat that allows coldwater fish to escape the sub-lethal and lethal effects of hot  
27 temperatures (Torgersen et al. 2012), are often formed by tributaries that are colder than the  
28 mainstem river and create thermally heterogeneous mixing zones at their confluences. Most  
29 studies on thermal refuges focus on their thermal benefits (Matthews et al. 1994, Nielsen et. al  
30 1994, Torgersen et al. 1999, Ebersole et. al 2001); fish exploit in-stream thermal heterogeneity  
31 by moving into cooler water (i.e., thermal refuges) to relieve heat stress and reduce metabolic  
32 demand (Berman and Quinn 1991, Baird and Krueger 2003). Thermal refuges form increasingly  
33 important habitat as river temperatures continue to rise (Isaak et al. 2011, Ruesch et al. 2012,  
34 Davis et al. 2013), and these habitats have the potential to enable population persistence in what  
35 may eventually become thermally uninhabitable ecosystems (Sutton et al. 2007, Loarie et al.  
36 2008). However, there may be trade-offs to refuge use; for example, adult steelhead (*O. mykiss*)  
37 using coolwater refuges in the Columbia River basin experienced increased harvest mortality  
38 (Keefer et al. 2009). A better understanding of how individuals use thermal refuges may help us  
39 understand the potential trade-offs and limits of these habitats, and the consequences for the  
40 dynamics and persistence of populations in a warming world.

41 While fish gain thermal benefits from coolwater refuges, these areas may be sub-optimal  
42 in terms of prey availability (Sutton et al. 2007). Studies on fish using thermal refuges in warm  
43 rivers have recorded cyclic movements out of refuges into thermally stressful mainstem habitat,  
44 presumably to gain some non-thermal benefit (Kaeding 1996, Ebersole et al. 2001, Belchik 2003,  
45 Brewitt and Danner 2014). In-stream prey availability in river ecosystems is highly variable  
46 (Allan and Russek 1985, Shearer et. al 2002, Neale et. al 2008). If prey are more abundant in  
47 areas where temperatures are sub-optimal, individuals may forage in warmer water but move to  
48 cooler water to digest, thereby decreasing metabolic costs (Bevelhimer and Adams 1993, Sims  
49 et. al 2006).

50 Competition for prey resources between individuals using refuges likely varies as a result  
51 of both naturally heterogeneous in-stream prey availability and fish density (Shearer et al. 2002,  
52 Rosenfeld et al. 2005, Hayes et al. 2007). Artificial stream channel experiments simulating  
53 refuges have found that density-dependent effects can lead to decreased growth rates, and higher  
54 mortality and emigration rates (Keeley 2001, Armstrong and Griffiths 2001, Imre et al. 2004).  
55 Within-refuge fish densities increase with rising mainstem temperatures, often reaching high  
56 levels during hot summer months (Sutton et al. 2007, Mather et al. 2008, Brewitt and Danner  
57 2014). We hypothesize that salmonids in the Klamath Basin gain thermal benefits from a food-  
58 limited coldwater habitat while deriving the majority of their prey from the much warmer food-  
59 rich but thermally stressful mainstem river. In this study, we examine how steelhead and  
60 Chinook salmon respond to the heterogeneous thermal and prey landscape surrounding thermal  
61 refuges at tributary confluences of a seasonally warm river system.

62 In the Klamath River in northern California, steelhead trout (*O. mykiss*) and Chinook  
63 salmon (*O. tshawytscha*) populations are reduced to approximately 5% of historical levels, and

64 juvenile salmonids increasingly rely on refuges for suitable thermal habitat during hot summer  
65 months (Nehlsen et al. 1991, Brewitt and Danner 2014). Given possible trade-offs to refuge use,  
66 including density-dependent food limitation, we sought to identify mechanisms of food  
67 limitation in refuges and to assess the diet and thermal habitat use of juvenile salmonids using  
68 thermal refuges. We therefore asked, 1) Are there differences in invertebrate prey availability  
69 between the tributaries and the larger mainstem system? 2) Are fish densities within refuges  
70 higher than in the adjacent mainstem river? 3) What proportion of juvenile steelhead and  
71 Chinook salmon diet is derived from the mainstem river versus tributary, and are there any  
72 seasonal, size-dependent, or species differences in diet? And 4) Are juvenile steelhead foraging  
73 primarily in the same areas they are holding (i.e., thermal habitat use)?

74

## 75 METHODS

76 We used a multi-faceted approach to quantify the thermal and prey landscapes  
77 surrounding thermal refuges (see Table 1). We sampled invertebrate drift (2010 and 2011) to  
78 quantify fish prey sources and assess whether invertebrate energy delivery rates ( $\text{Joules m}^{-2} \text{s}^{-1}$ )  
79 could be a mechanism of food limitation within thermal refuges. To assess how fish use the  
80 landscape surrounding thermal refuges at the individual and population level, we used snorkel  
81 surveys (2012) to estimate fish density in and out of refuges, temperature-sensitive radio tagging  
82 studies (2010-2012) to quantify thermal habitat use, and isotopic analyses (2012) to estimate  
83 juvenile salmonid prey sources.

84

85 *Study system*

86 The Klamath River in northern California is 423 km long, and drains approximately  
87 41,440 km<sup>2</sup> of southern Oregon and northern California (National Research Council 2008) (Fig.  
88 1). It has six mainstem dams, the lowest of which, Iron Gate Dam (rkm 306), acts as a migration  
89 barrier to anadromous fish, cutting off hundreds of kilometers of native anadromous salmonid  
90 habitat (Nehlsen et al. 1991). The dam, along with large-scale watershed alteration (e.g.,  
91 irrigation), contributes to elevated water temperatures for the downstream habitat (Bartholow  
92 2005, National Research Council 2008). During the summer and fall, the Iron Gate Reservoir  
93 undergoes seasonal thermal stratification, and the dam releases warm surface water rather than  
94 releasing colder water from below the thermocline (National Research Council 2004).

95 Below this barrier, the Klamath River supports migrations of steelhead trout (*O. mykiss*),  
96 Chinook salmon (*O. tshawytscha*), coho salmon (*O. kisutch*), and chum salmon (*O. keta*).  
97 Chinook salmon are the most abundant anadromous fish in the basin, with both fall- and spring-  
98 run life-history types, but populations have declined and are far below historical levels. Both  
99 summer and winter steelhead runs have declined from historical levels, and coho salmon were  
100 listed as threatened under the U.S. Endangered Species Act in 1997 (National Research Council  
101 2008). Juvenile steelhead rear in the river for 1-3 years, and juvenile Chinook salmon rear for 5-  
102 12 months, before out-migrating to the ocean. During summer months the Klamath mainstem  
103 river reaches temperatures that can be thermally inhospitable to salmonids—mean daily  
104 mainstem temperatures at the study sites ranged from 15-25°C between July-September 2010-  
105 2012—and juveniles behaviorally thermoregulate by seeking out thermal refuges, usually at  
106 tributary confluences (Sutton et al. 2007, Brewitt and Danner 2014).

107 We conducted this research on the lower Klamath River in July-September 2010-2011,  
108 and July-August 2012. We chose four study sites on the river below Iron Gate Dam, each located

109 at a major coldwater tributary confluence: Beaver Creek (rkm 259), Grider Creek (rkm 210), Fort  
110 Goff Creek (rkm 204), and Thompson Creek (rkm 198). We chose these tributaries based on the  
111 presence of coolwater refuges during summer and early fall months, and the presence of juvenile  
112 steelhead and Chinook salmon. Hereafter, we refer to ‘refuges’ as the coolwater area that  
113 includes both the tributary and thermal mixing zone (i.e., the area where mainstem and tributary  
114 water mix, creating an area of heterogeneous temperature).

### 116 *Water temperatures*

117 To determine the difference in mean water temperature between the tributaries and  
118 mainstem river, we recorded water temperatures at 15-minute intervals throughout July-August  
119 2012 at each study site. We used 2-4 Hobo pendant data loggers (Onset Computer Corporation,  
120 Pocasset, MA;  $\pm 0.5^{\circ}\text{C}$ ) in both the coldwater tributary and mainstem river at point locations  
121 approximately 10 m upstream of each confluence. Recorded temperatures therefore represent the  
122 minimum and maximum temperatures available to fish. All data loggers were placed near the  
123 riverbed to minimize thermal input from direct sunlight, at depths ranging from 0.5–2 m.

### 125 *Mainstem versus tributary invertebrate energy delivery rates*

126 To assess whether fish may be experiencing food limitation in thermal refuges, we  
127 collected invertebrate drift samples and calculated energy delivery rates ( $\text{Joules m}^{-2} \text{second}^{-1}$ ) as  
128 an estimate of food availability in both the mainstem river and coldwater tributaries. We  
129 collected monthly samples (June-August) at all four study sites in 2010, and weekly samples  
130 (July-August) at Beaver Creek (farthest upstream) in 2011; samples were collected from the  
131 mainstem and tributary approximately 15 m upstream of the confluence, and upstream of where

132 high densities of fish were observed in the tributaries (Fig. 2a). We suspended mesh drift nets  
 133 (23x31 cm, 500  $\mu$ m) approximately 5 cm above the substrate for 5-10 minutes, and measured  
 134 water velocity directly in front of the net at 60% of water column depth at both the beginning and  
 135 end of the sample period to estimate mean flow. We took samples at dawn and dusk to capture  
 136 temporal variation in drift, and collected approximately two samples per thermal environment  
 137 during each sampling event to capture spatial variation in drift (Appendix: Table A1&A2).  
 138 Invertebrate samples were identified, counted, and measured to the nearest millimeter. We  
 139 estimated invertebrate biomass (mg dry mass) from taxon-specific length–mass regressions  
 140 (Rogers et al. 1977, Hodar 1996, Burgherr and Meyer 1997, Kawabata and Urabe 1998, Benke et  
 141 al. 1999, Miserendino 2001, Sabo et al. 2002, Baumgartner and Rothhaupt 2003), and calculated  
 142 invertebrate energy content (Joules / mg) for each sample using taxon-specific caloric values in  
 143 the literature (Cummins and Wuycheck 1971, Driver et al. 1974, Driver 1981, Ciancio and  
 144 Pacual 2006).

145 We calculated energy delivery rates (*EDR*; Joules  $\text{m}^{-2}$   $\text{second}^{-1}$ ) as an estimate of  
 146 salmonid prey availability, defined as the product of energy density and water velocity:

$$147 \quad (1) \quad EDR = (E / V) * v$$

148 where *E* is the total energy in a sample, *V* is the water volume of the sample, and *v* is the mean  
 149 water velocity in front of the drift net. Energy delivery rate explicitly takes into account water  
 150 velocity, making it a better metric than energy density for assessing how much food per unit time  
 151 is reaching a fish holding in a specific location. Since there was a high degree of variability in  
 152 the number of samples collected per month at each study site, we used a meta-analytical  
 153 approach to assess whether there was any difference between mainstem and tributary drift  
 154 delivery rates across sites and years. We treated each group of monthly samples taken at a site as

155 a separate analysis, and determined the effect size between mainstem and tributary energy  
156 delivery rates; an effect size of zero indicates no difference. While invertebrate drift data were  
157 taken in 2010 and 2011, and all isotopic samples and fish density data were collected in 2012, we  
158 are using these drift data simply to draw inferences about general patterns of relative food supply  
159 in thermal refuges.

160

### 161 *Fish density in and out of refuges*

162 We quantified fish densities in the mainstem river and refuge (mixing zone and tributary)  
163 to assess whether fish density could be a possible source of in-refuge food limitation. We  
164 conducted weekly snorkel surveys at three times of day (9:00, 12:00, 16:00) at the Beaver Creek  
165 study site throughout August 2012. We established nine 1x2 m<sup>2</sup> plots, three in the tributary (T),  
166 four in the mixing zone (MZ), and two in the mainstem (MS) (Fig. 2a); all plots were marked  
167 with orange flagging on corner rocks. To estimate fish densities, the snorkeler approached the  
168 plot from downstream to avoid spooking the fish, and held a position in the water near the plot  
169 for approximately ten minutes. We calculated mean steelhead and Chinook salmon densities for  
170 all plots across the month. While the limited spatial extent of these surveys may have  
171 underestimated mainstem river salmonid densities, where fish may be more patchily distributed,  
172 this survey design was adequate for establishing relative densities of fish in thermal refuges and  
173 the adjacent mainstem river.

174

### 175 *Quantifying juvenile salmonid diet sources*

176 We used stable isotope analyses to quantify the proportion of mainstem versus tributary  
177 prey contributing to the diet of juvenile steelhead and Chinook salmon using thermal refuges at

178 the two upstream study sites (Beaver and Grider Creek confluences) in 2012. We chose two  
179 sampling periods (early July and late August) in order to assess whether there were any seasonal  
180 (i.e., temperature-dependent) changes in fish diet. We chose these sampling periods because  
181 mainstem temperatures rise with decreased flows and increased air temperatures, and are  
182 therefore usually cooler in early July than in late August.

183 To determine stable isotope values of aquatic prey, we collected benthic invertebrate  
184 samples from the tributary and mainstem at both sites. We chose taxa from the benthic samples  
185 that were representative of the most common invertebrate families found in fish diets and in the  
186 drift for both thermal environments (tributary and mainstem), and that represented a range of  
187 functional groups (see Appendix). We performed invertebrate sampling twice during the two-  
188 week period prior to fish sampling in early July and late August 2012, to capture the integration  
189 window for fish fin tissue (Heady and Moore 2012). During each sampling event, we collected  
190 samples of benthic invertebrates by scrubbing the substrate at 4-7 point locations between 5-15  
191 m upstream of the confluence, and collecting aquatic invertebrates with a 500  $\mu\text{m}$  net. We  
192 identified invertebrates to family under a stereomicroscope, and analyzed 2-3 samples of each  
193 taxon.

194 We took caudal fin tissue samples to measure stable isotope ratios for juvenile steelhead  
195 and Chinook salmon. Fish were caught within 50 m of the tributary confluence (in either the  
196 tributary, thermal mixing zone, or mainstem) using a combination of angling, electro-fishing, and  
197 seining at Beaver Creek in July (n = 30 steelhead; n = 18 Chinook salmon) and August (n = 23  
198 steelhead; n = 7 Chinook salmon), and Grider Creek in July (n = 14 steelhead) and August (n =  
199 18 steelhead) 2012. A small subset of the fish at Beaver Creek (n = 6) were caught in early  
200 August for radio tagging, but these fish showed no statistical differences in inferred diet from the

201 fish caught in July, and were therefore grouped with the early July fish. We weighed and  
202 measured (fork length; FL) all fish prior to taking fin clips.

203 We used stable isotopes of carbon ( $\delta^{13}\text{C}$ ) and nitrogen ( $\delta^{15}\text{N}$ ) to investigate the  
204 contribution of mainstem versus tributary prey to the diet of juvenile salmonids. Prior to isotopic  
205 analyses, invertebrate samples were frozen, and all samples were subsequently dried at 15°C in a  
206 drying oven. For small invertebrates (e.g., chironomids), multiple individuals were pooled into  
207 one sample and homogenized. All samples were analyzed using a PDZ Europa ANCA-GSL  
208 elemental analyzer interfaced with a PDZ Europa 20-20 isotope ratio mass spectrometer (Sercon  
209 Ltd., Cheshire, UK) at the University of California, Davis Stable Isotope Facility. Isotopic  
210 composition is expressed in  $\delta$  values (parts per thousand (‰) different from a standard),  
211 calculated as:

$$212 \quad (2) \quad \delta X = [(R_{\text{sample}}/R_{\text{standard}}) - 1] * 1000$$

213 where X is the element, R is the ratio of the heavy to light element, and the standards are Vienna  
214 Pee Dee Belemnite limestone (V-PDB) and air for carbon and nitrogen, respectively.

215 We used MixSIAR, a Bayesian stable isotope mixing model (Stock and Semmens 2013),  
216 to determine the probability distributions for the relative contribution of different prey sources to  
217 salmonid diet. Within MixSIAR, Gibbs sampling was performed for each of three parallel  
218 chains. We used a burn-in phase of 10,000 iterations, and ran the model for 100,000 iterations,  
219 retaining every 15<sup>th</sup> posterior sample, resulting in 6,000 independent draws for the posterior  
220 distribution. We used Gelman-Rubin diagnostics to confirm model convergence (Gelman and  
221 Rubin 1992). We ran the model separately for each study site, and included sampling month as a  
222 categorical variable in the model, and individuals as a random effect. We incorporated a trophic

223 discrimination factor of  $3.2 \pm 0.2$  for  $\delta^{15}\text{N}$  and  $1.9 \pm 0.5$  for  $\delta^{13}\text{C}$  ( $\pm 1\text{SD}$ ) into the mixing model  
224 (McCutchan et al. 2003).

225 We collected juvenile steelhead diet samples to qualitatively assess whether fish diet  
226 composition was similar to the species composition of the drift. Fish were sampled by hook and  
227 line from Beaver and Fort Goff sites in 2010 ( $n = 22$ ) and from Fort Goff in 2011 ( $n = 12$ ).  
228 (Appendix: Table A3 and Fig. A3&A4).

### 229 *Fish thermal habitat versus foraging habitat*

231 We used a two-part approach to assess whether juvenile steelhead were foraging  
232 primarily in the same areas they were holding (i.e., thermal habitat use). First, we used an  
233 individual-level analysis for the small number of steelhead ( $n = 9$ ) for which we had both body  
234 temperature data and isotopic diet analyses; we compared mean thermal habitat use derived from  
235 radio-tag data to the mean water temperature where individuals were foraging, inferred from  
236 isotopic diet analyses (hereafter, diet-inferred foraging temperatures). Since this first analysis  
237 only allowed us to analyze a small number of individuals, we then used a population-level  
238 analysis to address the same question, comparing diet-inferred foraging temperatures for all  
239 steelhead for which we had isotopic diet data (July-August 2012;  $n = 53$  at Beaver Creek;  $n = 32$   
240 at Grider Creek) to thermal habitat use across three years of temperature sensitive radio-tagging  
241 data (June-August 2010-2012;  $n = 76$  at Beaver Creek;  $n = 40$  at Grider Creek).

242 We used temperature-sensitive radio tags to track the body temperature of juvenile  
243 steelhead caught at Beaver Creek and Grider Creek study sites. Size limitations prevented  
244 tagging juvenile Chinook salmon. We surgically implanted the tags (Lotek's MST-720T  
245 temperature-sensor transmitter tags; 1.3g dry weight;  $\pm 0.8^\circ\text{C}$ ) following the tagging protocol

246 described in Brewitt and Danner (2014). The life expectancy of the radio tags was 42 days. We  
247 used data-logging receivers (Lotek SRX\_400A and SRX600) to log fish body temperatures at 5-  
248 second intervals for any tagged fish within approximately 100 m of the tributary confluence.

249 For the individual-level analysis of steelhead radio-tagged at Beaver Creek in 2012, we  
250 compared the proportion of time spent in mainstem water (derived from radio-tag data) to the  
251 mean contribution of mainstem prey sources to fish diet (derived from isotopic diet analyses).  
252 For this analysis, we used only fish for which we had both temperature data and isotopic diet  
253 data, and that remained at the refuge for a minimum of seven days post-tagging ( $n = 9$ ). We used  
254 a mass-balance equation to translate time-series of fish body temperatures into the mean  
255 proportion of time an individual held in mainstem water ( $P_m$ ). The equation we used was:

256 (3) 
$$P_m = \text{mean} ((T_f - T_t) / (T_m - T_t))$$

257 where  $T_f$  represents time-series of fish temperatures, and  $T_t$  and  $T_m$  represent simultaneous time-  
258 series of tributary and mainstem water temperatures, respectively. In making this comparison, we  
259 are assuming that these individuals' behavior was similar before and after tagging, since the fish  
260 temperature time-series represent data collected in the two weeks after fin samples were taken  
261 for isotopic diet analysis; this constraint was due to our inability to recapture radio tagged fish  
262 post-tagging. We believe this is a reasonable assumption, since time-series of fish temperature  
263 were fairly consistent for each individual tagged, until the fish left the study area.

264 We then used a population-level analysis to determine whether the diet-inferred foraging  
265 temperatures of all fish for which we had isotopic diet data (2012;  $n = 53$  at Beaver Creek;  $n =$   
266  $32$  at Grider Creek) matched the holding temperatures of all fish for which we had radio-tagging  
267 data (2010-2012;  $n = 76$  at Beaver Creek;  $n = 40$  at Grider Creek). We used a mass-balance  
268 equation to calculate the water temperature where fish were foraging (diet-inferred foraging

269 temperature;  $T_d$ ) based on their isotope-inferred diet. We used time-series of mainstem and  
270 tributary temperatures from the two weeks prior to fish sampling to characterize the water  
271 temperature profile experienced by individuals during the fin tissue integration window (Heady  
272 and Moore 2012), and multiplied the time-series through by the isotope-inferred proportion  
273 mainstem diet for each fish. The diet-inferred foraging temperature for an individual fish can  
274 therefore be expressed as:

$$275 \quad (4) \quad T_d = \text{mean} ((D_m * T_m) + ((1 - D_m) * T_t))$$

276 where  $D_m$  is the proportion of mainstem diet for an individual, and  $T_t$  and  $T_m$  represent time-  
277 series of mainstem and tributary temperatures. Thus,  $T_d$  represents a metric of the temperature  
278 experienced by the fish when they were obtaining their prey. For the purposes of this calculation,  
279 we assumed that mainstem and tributary prey in the mixing zone are being mixed just like water,  
280 since steelhead primarily feed on invertebrates in the water column (i.e., drift) that use passive  
281 dispersal mechanisms. In addition, we are assuming that local prey production in the mixing  
282 zone does not contribute significantly to fish diet, given that the volume of the mixing zone is  
283 minimal compared to the larger mainstem and tributary water bodies. While  $T_d$  is a useful index  
284 of potential foraging temperatures, there may be sources of uncertainty (e.g., non-random daily  
285 foraging patterns) that we have not taken into consideration in these estimates.

286 To quantify thermal habitat use for the population of fish using the thermal refuge at  
287 Beaver Creek and Grider Creek study sites, we used data from a larger temperature-sensitive  
288 radio-tagging study (Brewitt and Danner 2014). We calculated the fish body temperature  
289 distribution for the sample of steelhead tagged at each site during the study period 2010-2012 at  
290 Beaver Creek (n = 76) and 2010-2011 at Grider Creek (n = 40), using all fish detections sub-  
291 sampled at 5-minute intervals, and weighting all fish equally. We used a Kolmogorov-Smirnov

292 two-sample test to test for differences between the thermal and foraging temperature  
293 distributions at each site.

294

## 295 RESULTS

296 *Water temperatures*

297 Mainstem temperatures were consistently warmer than tributary temperatures at Beaver  
298 Creek (mean difference =  $6.8 \pm 0.5^\circ\text{C}$ ) and Grider Creek (mean difference =  $7.1 \pm 0.9^\circ\text{C}$ )  
299 throughout July and August 2012 (Appendix: Fig. A1). Mean mainstem temperatures for the two  
300 weeks prior to fish isotope sampling in early July and late August 2012 were  $22.5^\circ\text{C}$  and  $22.0^\circ\text{C}$   
301 at Beaver Creek, and  $21.8^\circ\text{C}$  and  $21.6^\circ\text{C}$  at Grider Creek, respectively. In contrast, mean  
302 temperatures in the coldwater refuges during these same periods ranged from  $15.5 - 19.5^\circ\text{C}$  at  
303 Beaver Creek and  $13.1 - 18.8^\circ\text{C}$  at Grider Creek.

304

305 *Mainstem versus tributary invertebrate energy delivery rates*

306 Invertebrate energy delivery rates were highly variable across sites, and were not  
307 consistently higher in the larger mainstem river than the tributaries. In 2010, energy delivery  
308 rates ranged from  $0.9-43.3 \text{ Joules m}^{-2} \text{ s}^{-1}$  in the mainstem and  $0.4-37.5 \text{ Joules m}^{-2} \text{ s}^{-1}$  in the  
309 tributary across the four study sites (Appendix: Fig. A2). In 2011, energy delivery rates at Beaver  
310 Creek site ranged from  $13.2-86.7 \text{ Joules m}^{-2} \text{ s}^{-1}$  in the mainstem and  $11.6-828.9 \text{ Joules m}^{-2} \text{ s}^{-1}$  in  
311 the tributary. The mean ( $\pm$  SD) effect size between the mainstem and tributaries across all sites  
312 and years was  $0.102 \pm 1.18 \text{ Joules m}^{-2} \text{ s}^{-1}$ ; an effect size of zero indicates no difference. Thus,  
313 there was no significant difference between mainstem and tributary prey supply per unit area.

314

315 *Fish density in and out of refuges*

316 Steelhead and Chinook salmon densities were approximately eight times higher in the  
317 refuge than in the mainstem river at Beaver Creek throughout August 2012 (Fig. 2b,c). Mean  
318 ( $\pm$ SD) juvenile steelhead densities (fish m<sup>-2</sup>), aggregated across hourly and weekly observations,  
319 were higher in the tributary (mean = 2.0  $\pm$  1.3) and mixing zone (mean = 1.9  $\pm$  1.6) than in the  
320 mainstem plots (mean = 0.4  $\pm$  0.4). The plot with the consistently highest density of steelhead  
321 (4.0 fish m<sup>-2</sup>) was located near the top of the mixing zone, just below the tributary confluence  
322 with the mainstem, and had a mean temperature of 19.8°C. Mean ( $\pm$ SD) juvenile Chinook  
323 salmon densities were also higher in the tributary (mean = 1.7  $\pm$  2.3) and mixing zone (mean =  
324 1.5  $\pm$  1.9) than in the mainstem plots (mean = 0  $\pm$  0.1). The plot with the consistently highest  
325 density of Chinook (3 fish m<sup>-2</sup>) was located at the downstream portion of the mixing zone, in an  
326 area of lower velocity flow, and had a mean temperature of 20.5°C. Fish density within a thermal  
327 environment did not vary by time of day for either steelhead (F = 1.97; p = 0.16) or Chinook  
328 salmon (F = 0.047; p = 0.83).

329  
330 *Quantifying juvenile salmonid diet sources*

331 Mainstem invertebrate prey sources were  $\delta^{15}\text{N}$ -enriched and  $\delta^{13}\text{C}$ -enriched compared to  
332 tributary invertebrate prey sources. Juvenile steelhead and Chinook salmon isotope signatures  
333 ranged between tributary and mainstem prey source signatures, indicating diet variation between  
334 individuals (Fig. 3a,b). There were no seasonal differences in juvenile steelhead or Chinook  
335 salmon diet between the two sampling periods (early July and late August).

336 Juvenile salmonids using thermal refuges obtained over 50% of their diet from mainstem  
337 prey sources (Fig. 4). The mean proportion mainstem diet for juvenile steelhead caught in the

338 mainstem (Beaver =  $0.82 \pm 0.13$  standard deviation (SD); Grider =  $0.97 \pm 0.02$ ) and mixing zone  
339 (Beaver =  $0.75 \pm 0.13$ ; Grider =  $0.94 \pm 0.11$ ) was much higher than for tributary-caught steelhead  
340 (Beaver =  $0.57 \pm 0.20$ ; Grider =  $0.60 \pm 0.38$ ). However, tributary-caught steelhead and Chinook  
341 salmon ( $0.47 \pm 0.15$ ) still obtained approximately half their diet from mainstem prey sources  
342 (Fig. 4a,b).

343 Tributary-caught steelhead had a smaller mean fork length (Beaver =  $148 \pm 40$  mm;  
344 Grider =  $134 \pm 34$  mm) than steelhead caught in the mixing zone (Beaver =  $168 \pm 26$  mm; Grider  
345  $218 \pm 31$  mm) or mainstem (Beaver =  $212 \pm 80$  mm; Grider =  $203 \pm 71$  mm). The proportion of  
346 diet obtained from mainstem sources increased with increasing steelhead body size (fork length)  
347 ( $R^2 = 0.30$  and  $0.23$  for Beaver and Grider Creeks, respectively; linear regression).

348

#### 349 *Fish thermal habitat versus foraging habitat*

350 Steelhead body temperatures describing thermal habitat use were significantly cooler  
351 ( $\sim 3.5^\circ\text{C}$ ) than diet-inferred steelhead foraging temperatures, indicating a mismatch in thermal  
352 and foraging habitat for fish using thermal refuges. Fish temperatures for the nine radio-tagged  
353 steelhead at Beaver Creek indicate that fish used a range of thermal habitat (range in mean body  
354 temperature across sample period:  $15.7^\circ\text{C} - 22.5^\circ\text{C}$ ). Based on these body temperatures and river  
355 temperatures over the same time period, the mean ( $\pm$  SD) proportion of time individuals spent in  
356 mainstem water ( $0.22 \pm 0.23$ ) was relatively low, whereas the mean proportion of prey these  
357 same individuals obtained from mainstem sources ( $0.72 \pm 0.15$ ) was higher than would be  
358 expected if fish were feeding in the area in which they were holding based on body temperatures  
359 (Fig. 5).

360 In the population-level analysis comparing thermal habitat use and diet-inferred foraging  
361 temperatures, fish body temperatures at both Beaver Creek (mean =  $17.5 \pm 2.3^{\circ}\text{C}$ ) and Grider  
362 Creek (mean =  $17.8 \pm 2.3^{\circ}\text{C}$ ) were significantly cooler than diet-inferred foraging temperatures  
363 at Beaver Creek (mean =  $20.7 \pm 1.2^{\circ}\text{C}$ ) and Grider Creek (mean =  $21.0 \pm 2.6^{\circ}\text{C}$ ) (Kolmogorov-  
364 Smirnov two-sample test,  $p < 0.001$  at both sites; Fig. 6).

365

## 366 DISCUSSION

367 We found that while juvenile steelhead and Chinook salmon consistently used thermal  
368 refuges to behaviorally thermoregulate, they obtained the majority of their prey from mainstem  
369 sources, even during thermally stressful summer months when the mainstem neared upper  
370 incipient lethal temperatures. Steelhead body temperatures centered around  $18\text{-}19^{\circ}\text{C}$ , the optimal  
371 growth temperature given sufficient food availability (Myrick and Cech 2000); this suggests that  
372 steelhead use refuges to thermoregulate and possibly optimize growth, but can still obtain much  
373 of their prey from warmer mainstem water (mean diet-inferred foraging temperature  $\cong 21\text{-}22^{\circ}\text{C}$ ).  
374 Thus, juvenile salmonids can exploit the heterogeneity at tributary junctions forming thermal  
375 refuges by using refuges to thermoregulate while still accessing the mainstem river prey base.

376 Our results suggest that juvenile salmonids using coolwater refuges shift laterally  
377 between the refuges and the mainstem river in order to access mainstem prey. While we did not  
378 measure fish movement directly, steelhead body temperatures used as a proxy for location  
379 indicate that fish move in and out of thermal refuges (Brewitt and Danner 2014). The high  
380 proportion of mainstem prey in isotope-inferred fish diets therefore implies that juvenile  
381 steelhead and Chinook salmon move out of refuges to obtain mainstem prey. There is evidence  
382 of fish in other systems using diel vertical or horizontal migration as a strategy to maximize

383 access to food availability and metabolic efficiency across heterogeneous thermal landscapes  
384 (Wurtsbaugh and Neverman 1988, Bevelhimer and Adams 1993, Armstrong et al. 2013). For  
385 example, Armstrong and Schindler (2013) found that juvenile coho salmon forage in colder  
386 water but shift to warmer water to digest, thereby increasing digestive capacity; conversely, Sims  
387 et. al (2006) found that a benthic shark (*Scyliorhinus canicula*) lowered daily metabolic costs by  
388 hunting in warmer water and shifting to cooler water to digest.

389 Juvenile salmonid movement out of thermal refuges into sub-optimal mainstem  
390 temperatures suggests that refuges may be food-limited, likely due to high fish densities causing  
391 competition for prey. Invertebrate energy delivery rates were highly variable, and there was no  
392 consistent difference between energy delivery rates in the tributaries and mainstem river;  
393 invertebrate drift densities alone are therefore unlikely to drive fish to consistently forage outside  
394 of thermal refuges. However, fish densities were approximately eight times higher in the refuge  
395 (mean = 3.5 fish m<sup>-2</sup>) than the adjacent mainstem river throughout August 2012, and competition  
396 for prey would therefore be much higher in the refuge than the mainstem. These densities were  
397 much higher than stream-dwelling steelhead densities in other systems (Everest and Chapman  
398 1972, Harvey and Nakamoto 1996); for example, Keeley and McPhail (1998) measured juvenile  
399 steelhead densities of approximately 1.9 fish m<sup>-2</sup> (mean FL  $\cong$  70 mm) in two Canadian rivers. As  
400 mainstem temperatures rise, movement of individuals into thermal refuges will increase in-  
401 refuge fish densities, likely reducing per capita prey availability. Density-dependent processes  
402 may therefore be driving individuals to venture into warmer water to access more prey; however,  
403 access to the mainstem prey base could allow thermal refuges to support higher densities of fish.

404 Steelhead size appears to mediate how individuals negotiate the variability in temperature  
405 and prey availability in refuges, as indicated by size-based variation in steelhead isotope-inferred

406 diet. Larger juvenile steelhead (FL ~ 200 mm) caught in the mainstem and mixing zone were  
407 more likely to rely on mainstem prey (~75-95% mean proportion mainstem prey) than smaller  
408 steelhead (FL ~ 150 mm) caught in the tributary, who had a lower proportion (~60% mean  
409 proportion mainstem prey) of mainstem prey in their isotope-inferred diet composition (see Fig.  
410 4). Given sufficient food availability, the mixing zone and boundary habitat (i.e., boundary  
411 between the refuge and mainstem river) is likely optimal habitat for fish growth; mixing zone  
412 temperatures were often at the bioenergetic optima for steelhead (~18-19°C) (Brett et al. 1982;  
413 Heady 2012; Myrick and Cech 2000). Larger juveniles may be holding dominant positions near  
414 the edge of thermal refuges where temperature and prey availability can be optimized, while  
415 smaller fish hold in cooler tributary waters. Both observational and radio-tagging studies on  
416 juvenile steelhead in Klamath River refuges have observed fish (especially larger juveniles)  
417 concentrated along the edge of refuges (Sutton et al. 2007, Brewitt and Danner 2014). An  
418 alternate hypothesis is that larger individuals are able to make longer feeding forays into the  
419 mainstem river before incurring thermal stress, since it takes longer for the body temperature of  
420 larger fish to equilibrate to ambient temperatures (Pepino et al. 2015). Balancing resource needs  
421 may therefore be easier for larger juvenile steelhead that can establish dominant positions on the  
422 mixing zone boundary habitat, and likely forage in the mainstem river at longer time scales,  
423 while smaller tributary-based juveniles that still need access to mainstem prey would need to  
424 move further to gain sufficient resources.

425 Juvenile steelhead and Chinook salmon did not always hold in the coldest areas of the  
426 refuge, and both species tended to cluster in areas that met their species-specific habitat  
427 requirements (Everest and Chapman 1972, Quinn 2005). Chinook salmon were caught in either  
428 the tributary or the lower mixing zone (~50 m below the confluence) in slower velocity areas,

429 rather than the higher velocity riffle forming the top of the mixing zone where larger steelhead  
430 were found. Juvenile Chinook salmon also exhibited size-based variation in holding position  
431 within the refuge; Chinook salmon caught in the lower mixing zone ( $\sim 20.5^{\circ}\text{C}$ ) were larger (mean  
432 FL = 88.3 mm) than those caught in the tributary ( $\sim 16.8^{\circ}\text{C}$ ) (mean FL = 83.3 mm). The  
433 preference of larger Chinook salmon for warmer water suggests that, as with steelhead, larger  
434 individuals may be holding dominant positions nearer the boundary of thermal refuges where  
435 temperature and prey availability can be optimized; given sufficient prey availability, Chinook  
436 salmon growth potential is optimal at approximately  $19^{\circ}\text{C}$  (Brett et al. 1982, McCullough 1999).  
437 Maintaining habitat heterogeneity in thermal refuges could provide the necessary temperature  
438 and prey resources for different species and size-classes of juveniles present, and facilitate co-  
439 existence due to differential habitat selection and foraging strategies.

440 Our results emphasize that not only are thermal refuges at tributary junctions important  
441 for fish seeking thermal respite, but the efficacy of refuges may depend on food web dynamics in  
442 both tributary and mainstem habitats. Even if the mainstem river is apparently too hot for fish to  
443 reside in, it still provides key food resources for fish in thermal refuges. While the scope of this  
444 study was limited to the Klamath River, the isotopic values for fish at both study sites make a  
445 compelling case that fish using thermal refuges are foraging in the mainstem; moreover, the  
446 population-level analysis of steelhead shows a clear mismatch between diet-inferred foraging  
447 temperatures and thermal habitat use (Fig. 6), indicating that fish are disproportionately foraging  
448 in the mainstem river. Rivers along the west coast are experiencing warming trends similar to the  
449 Klamath River (Knowles and Cayan 2002, Payne et al. 2004, Bartholow 2005, Isaak et al. 2012),  
450 making thermal refuges essential habitat for the over-summer survival of coldwater fish such as  
451 stream-rearing salmonids. As mainstem temperatures rise, any density-dependent food limitation

452 in refuges will likely be exacerbated by higher in-refuge fish densities (Ebersole et. al 2001,  
453 Belchik 2003, Sutton et al. 2007, Brewitt and Danner 2014), as well as by low tributary flows  
454 that could cut off connectivity from the mainstem river (Van Kirk and Naman 2009). While  
455 coldwater tributaries are also susceptible to climate-induced warming, proper riparian shading  
456 can maintain cooler temperatures and protect thermal refuges formed by tributary junctions with  
457 the mainstem river (Boughton et. al 2012). Management that targets maintaining robust thermal  
458 refuges at tributary junctions with the mainstem river will provide essential habitat for juvenile  
459 salmonids seeking to exploit a heterogeneous thermal and prey landscape.

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## LITERATURE CITED

- Achord, S., P. S. Levin, and R. W. Zabel. 2003. Density-dependent mortality in Pacific salmon: the ghost of impacts past? *Ecology Letters* 6:335–342.
- Armstrong, J., and S. Griffiths. 2001. Density-dependent refuge use among over-wintering wild Atlantic salmon juveniles. *Journal of Fish Biology* 58:1524–1530.
- Armstrong, J. B. and D. E. Schindler. 2013. Going with the flow: spatial distributions of juvenile coho salmon track an annually shifting mosaic of water temperature. *Ecosystems* 16(8):1429–41.
- Armstrong, J., D. Schindler, C. Ruff, G. T. Brooks, K. E. Bentley, and C. E. Torgersen. 2013. Diel horizontal migration in streams: juvenile fish exploit spatial heterogeneity in thermal and trophic resources. *Ecology* 94:2066–2075.
- Baird, O. E., and C. C. Krueger. 2003. Behavioral thermoregulation of brook and rainbow trout: comparison of summer habitat use in an Adirondack River, New York. *Transactions of the American Fisheries Society* 132:1194–1206.
- Bartholow, J. M. 2005. Recent water temperature trends in the Lower Klamath River, California. *North American Journal of Fisheries Management* 25:152–162.
- Baumgartner, D. and K. Rothhaupt. 2003. Predictive length–dry mass regressions for freshwater invertebrates in a pre-alpine lake littoral. *Hydrobiology* 88(5): 453–463.

- Belchik, M. 2003. Use of thermal refugial areas on the Klamath River by juvenile salmonids; Summer 1998. Yurok Tribal Fisheries Program Technical Report 8-FG-20-17510. Klamath, California, USA.
- Benke, A.C., A.D. Huryn, L.A. Smock, and J.B. Wallace. 1999. Length-mass relationships for freshwater macroinvertebrates in North America with particular reference to the southeastern United States. *Journal of the North American Benthological Society* 18:308–343.
- Bentley, K. T., D. E. Schindler, T. J. Cline, J. B. Armstrong, D. Macias, L. R. Ciepiela, and R. Hilborn. 2014. Predator avoidance during reproduction: diel movements by spawning sockeye salmon between stream and lake habitats. *Journal of Animal Ecology*. In press.
- Berman, C., and T. P. Quinn. 1991. Behavioural thermoregulation and homing by spring chinook salmon, *Oncorhynchus tshawytscha* (Walbaum), in the Yakima River. *Journal of Fish Biology* 39:301–312.
- Bevelhimer, M. S., and S. M. Adams. 1993. A bioenergetics analysis of diel vertical migration by kokanee salmon, *Oncorhynchus nerka*. *Canadian Journal of Fisheries and Aquatic Sciences* 50:2336–2349.
- Blumstein, D., and D. Pelletier. 2005. Yellow-bellied marmot hiding time is sensitive to variation in costs. *Canadian Journal of Zoology* 367:363–367.
- Boughton, D. A., C. Hatch, and E. Mora, Identifying distinct thermal components of a creek. *Water Resources Research*. 48(9):W09506.

- Breau, C., R. A. Cunjak, and G. Bremset. 2007. Age-specific aggregation of wild juvenile Atlantic salmon *Salmo salar* at cool water sources during high temperature events. *Journal of Fish Biology* 71:1179–1191.
- Brett, J., W. Clarke, J. Shelbourn, and P. Station. 1982. Experiments on thermal requirements for growth and food conversion efficiency of juvenile chinook salmon *Oncorhynchus tshawytscha*. Canadian Technical Report of Fisheries and Aquatic Sciences No. 1127. Nanaimo, British Columbia, Canada.
- Brewitt, K. S. and E. M. Danner. 2014. Spatio-temporal temperature variation influences juvenile steelhead (*Oncorhynchus mykiss*) use of thermal refuges. *Ecosphere*. 5(7):1-14.
- Burgherr, P., and E.I. Meyer. 1997. Regression analysis of linear body dimensions vs. dry mass in stream macroinvertebrates. *Archiv für Hydrobiologie* 139:101–112.
- Bridges, C. M. 2002. Tadpoles balance foraging and predator avoidance: Effects of predation, pond drying, and hunger. *Journal of Herpetology* 36:627–634.
- Ciancio, J. and M. Pacual. 2006. Energy density of freshwater Patagonian organisms. *Ecología Austral* 16:xxx-xxx.
- Christy, T. 2003. Calif. Dept. of Fish and Wildlife and Pacific States Marine Fisheries. Biogeographic Information and Observation System (BIOS). Map layer Cdfg\_100k\_2003\_6.shp. Retrieved Nov 11, 2011 from <http://bios.dfg.ca.gov>

- Cummins, K. W., and J. C. Wuycheck. 1971. Caloric equivalents for investigations in ecological energetics. *Internationale Vereinigung für Theoretische und Angewandte Limnologie, Mitteilung* 1818:1–158.
- Davis, J., A. Pavlova, R. Thompson, and P. Sunnucks. 2013. Evolutionary refugia and ecological refuges: key concepts for conserving Australian arid zone freshwater biodiversity under climate change. *Global Change Biology* 19:1970–1984.
- Deas, M., S. K. Tanaka, and J. C. Vaughn. 2006. Klamath River thermal refugia study: Flow and temperature characterization. Watercourse Engineering, Inc., prepared for U.S. Bureau of Reclamation in cooperation with the Karuk Tribe and Yurok Tribe. Klamath Falls, Oregon.
- Dhondt, A. A., B. Kempnaers, and F. Adriaensen. 1992. Density-dependent clutch size caused by habitat heterogeneity. *Journal of Animal Ecology* 61:643–648.
- Driver, E.A., L.G. Sugden, and R.J. Kovach. 1974. Calorific, chemical and physical values of potential duck foods. *Freshwater Biology* 4(3):281–292.
- Driver, E.A. 1981. Calorific values of pond invertebrates eaten by ducks. *Freshwater Biology* 11(6):579–581.
- Ebersole, J., W. Liss, and C. Frissell. 2001. Relationship between stream temperature, thermal refugia and rainbow trout *Oncorhynchus mykiss* abundance in arid-land streams in the northwestern United States. *Ecology of Freshwater Fish* 10:1–10.

- Ebersole, J., W. J. Liss, and C. A. Frissell. 2003. Thermal heterogeneity, stream channel morphology, and salmonid abundance in northeastern Oregon streams. *Canadian Journal of Fisheries and Aquatic Sciences* 60:1266–1280.
- Everest, F., and D. Chapman. 1972. Habitat selection and spatial interaction by juvenile chinook salmon and steelhead trout in two Idaho streams. *Journal of the Fisheries Research Board of Canada* 29:91–100.
- Gelman, A., and D. B. Rubin. 1992. Inference from iterative simulation using multiple sequences. *Statistical Science* 7:457–472.
- Grant, J. W. A., S. Ó. Steingrímsson, E. R. Keeley, and R. A. Cunjak. 1998. Implications of territory size for the measurement and prediction of salmonid abundance in streams. *Canadian Journal of Fisheries and Aquatic Sciences* 55:181–190.
- Greene, C., and T. Beechie. 2004. Consequences of potential density-dependent mechanisms on recovery of ocean-type Chinook salmon (*Oncorhynchus tshawytscha*). *Canadian Journal of Fisheries and Aquatic Sciences* 61:590–602.
- Gregory, J., and J. Griffith. 1996. Aggressive behaviour of underyearling rainbow trout in simulated winter concealment habitat. *Journal of Fish Biology* 49:237–245.
- Hanson, P., T. Johnson, J. Kitchell, and D.E. Schindler. 1997. *Fish bioenergetics 3.0*. University of Wisconsin Sea Grant Institute, Madison, Wisconsin, USA.
- Harvey, B. C. and R. J. Nakamoto. 1996. Effects of Steelhead Density on Growth of Coho

Salmon in a Small Coastal California Stream. Transactions of the American Fisheries Society. 125:237-243.

Hayes, J. W., N. F. Hughes, and L. H. Kelly. 2007. Process-based modelling of invertebrate drift transport, net energy intake and reach carrying capacity for drift-feeding salmonids. Ecological Modelling 207:171–188.

Heady, W., and J. Moore. 2012. Tissue turnover and stable isotope clocks to quantify resource shifts in anadromous rainbow trout. Oecologia 172:21–34.

Heady, W. N. 2012. Relationships among environment, movement, growth and survival of coastal rainbow trout (*Oncorhynchus mykiss*). Dissertation. University of California Santa Cruz, Santa Cruz, California, USA.

Hodar, J. 1996. The use of regression equations for estimation of arthropod biomass in ecological studies. Acta Oecologia 17:421–433.

Hohausova, E., G. Copp, and P. Jankovsky. 2003. Movement of fish between a river and its backwater: diel activity and relation to environmental gradients. Ecology of Freshwater Fish 12:107–117.

Huntsman, B. M., and J. T. Petty. 2014. Density-dependent regulation of brook trout population dynamics along a core-periphery distribution gradient in a complex Appalachian watershed. PLoS ONE 9:e91673.

- Imre, I., J. W. A. Grant, and E. R. Keeley. 2004. The effect of food abundance on territory size and population density of juvenile steelhead trout (*Oncorhynchus mykiss*). *Oecologia* 138:371–8.
- Isaak, D. J., S. Wollrab, D. Horan, and G. Chandler. 2012. Climate change effects on stream and river temperatures across the northwest U.S. from 1980–2009 and implications for salmonid fishes. *Climatic Change* 113:499–524.
- Jobling, M. 1994. *Fish Bioenergetics*. Chapman & Hill, London.
- Kaeding, L. 1996. Summer use of coolwater tributaries of a geothermally heated stream by rainbow and brown trout, *Oncorhynchus mykiss* and *Salmo trutta*. *American Midland Naturalist* 135:283–292.
- Kawabata, K. and J. Urabe 1998. Length–weight relationships of eight freshwater planktonic crustacean species in Japan. *Freshwater Biology* 39(2): 199–205.
- Keefer, M. L., C. A. Peery, and B. High. 2009. Behavioral thermoregulation and associated mortality trade-offs in migrating adult steelhead (*Oncorhynchus mykiss*): variability among sympatric populations. *Canadian Journal of Fisheries and Aquatic Sciences* 66:1734–1747.
- Keeley, E., and J. McPhail. 1998. Food abundance, intruder pressure, and body size as determinants of territory size in juvenile steelhead trout (*Oncorhynchus mykiss*). *Behaviour* 135:65–82.
- Keeley, E. R. 2001. Demographic responses to food and space competition by juvenile steelhead trout. *Ecology* 82:1247.

- Kiffney, P., C. Greene, J. Hall, and J. Davies. 2006. Tributary streams create spatial discontinuities in habitat, biological productivity, and diversity in mainstem rivers. *Canadian Journal of Fisheries and Aquatic Sciences* 63:2518–2530.
- Knowles, N. and D. R. Cayan. 2002. Potential effects of global warming on the Sacramento/San Joaquin watershed and the San Francisco estuary. *Geophysical Research Letters*. 29(18):38-1 - 38-4.
- Lawler, J. J., D. White, R. P. Neilson, and A. R. Blaustein. 2006. Predicting climate-induced range shifts: model differences and model reliability. *Global Change Biology* 12:1568–1584.
- Loarie, S. R., B. E. Carter, K. Hayhoe, S. McMahon, R. Moe, C. A. Knight, and D. D. Ackerly. 2008. Climate change and the future of California's endemic flora. *PLoS ONE* 3:e2502.
- Loose, C. J., and P. Dawidowicz. 2014. Trade-offs in diel vertical migration by zooplankton: the costs of predator avoidance. *Ecology* 75:2255–2263.
- Mather, M. E., D. L. Parrish, C. A. Campbell, J. R. McMenemy, and J. M. Smith. 2008. Summer temperature variation and implications for juvenile Atlantic salmon. *Hydrobiologia* 603:183–196.
- Matthews, K. R., N. H. Berg, D. L. Azuma, and T. R. Lambert. 1994. Cool water formation and trout habitat use in a deep pool in the Sierra Nevada, California. *Transactions of the American Fisheries Society*. 123:549–564.

- McCullough, D. A. 1999. A review and synthesis of effects of alterations to the water temperature regime on freshwater life stages of salmonids, with special reference to Chinook Salmon. U.S. Environmental Protection Agency. Report 910-R-99-010, Seattle.
- McCutchan, J., W. Lewis, C. Kendall, and C. McGrath. 2003. Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos* 2:378–390.
- Miserendino, M.L. 2001. Length-mass relationships for macroinvertebrates in freshwater environments of Patagonia (Argentina). *Ecología Austral* 11:3-8.
- Myrick, C. A., and J. J. Cech. 2000. Temperature influences on California rainbow trout physiological performance. *Fish Physiology and Biochemistry* 22:245–254.
- Myrick, C., and J. Cech. 2005. Effects of temperature on the growth, food consumption, and thermal tolerance of age-0 nimbus-strain steelhead. *North American Journal of Aquaculture* 67:324–330.
- Mac Nally, R., A. F. Bennett, J. R. Thomson, J. Q. Radford, G. Unmack, G. Horrocks, and P. A. Vesk. 2009. Collapse of an avifauna: climate change appears to exacerbate habitat loss and degradation. *Diversity and Distributions* 15:720–730.
- National Research Council. 2004. Endangered and threatened fishes in the Klamath River basin. National Academies Press, Washington DC, USA.
- National Research Council. 2008. Hydrology, ecology, and fishes of the Klamath River basin. National Academies Press, Washington DC, USA.

- Nehlsen, W., J. E. Williams, and J. A. Lichatowich. 1991. Pacific salmon at the crossroads: stocks at risk from California, Oregon, Idaho, and Washington. *Fisheries* 16:4–21.
- Pärn, H., T. H. Ringsby, H. Jensen, and B.-E. Sæther. 2011. Spatial heterogeneity in the effects of climate and density-dependence on dispersal in a house sparrow metapopulation. *Proceedings of the Royal Society B* 279:144–152.
- Payne, J. T., A. W. Wood, A. F. Hamlet, R. N. Palmer, and D. P. Lettenmaier. 2004. Mitigating the effects of climate change on the water resources of the Columbia River Basin. *Climatic Change*. 62(1):233–56.
- Pearson, R. G., and T. P. Dawson. 2003. Predicting the impacts of climate change on the distribution of species: Are bioclimate envelope models useful? *Global Ecology and Biogeography* 12:361–371.
- Pepino, M., K. Goyer, and P. Magnan. 2015. Heat transfer in fish: are short excursions between habitats a thermoregulatory behaviour to exploit resources in an unfavourable thermal environment? *Journal of Experimental Biology* 218: 3461-3467.
- Petty, J. T., J. L. Hansbarger, B. M. Huntsman, and P. M. Mazik. 2012. Brook trout movement in response to temperature, flow, and thermal refugia within a complex Appalachian riverscape. *Transactions of the American Fisheries Society* 141:1060–1073.
- Quinn T. P. 2005. The behavior and ecology of Pacific salmon and trout. American Fisheries Society. Bethesda, Maryland, in association with University of Washington Press. Seattle, Washington.

- Rangeley, R., and D. Kramer. 1998. Density-dependent antipredator tactics and habitat selection in juvenile pollock. *Ecology* 79:943–952.
- Richter, A., and S. A. Kolmes. 2005. Maximum temperature limits for chinook, Coho, and Chum salmon, and steelhead trout in the Pacific Northwest. *Reviews in Fisheries Science* 13:23–49.
- Rogers, L. E., R. L. Buschbom, and C. R. Watson. 1977. Length-weight relationships of shrub-steppe invertebrates. *Annals of the Entomological Society of America* 70:51–53.
- Rosenfeld, J. S., T. Leiter, G. Lindner, and L. Rothman. 2005. Food abundance and fish density alters habitat selection, growth, and habitat suitability curves for juvenile coho salmon (*Oncorhynchus kisutch*). *Canadian Journal of Fisheries and Aquatic Sciences* 62:1691–1701.
- Ruesch, A. S., C. E. Torgersen, J. J. Lawler, J. D. Olden, E. E. Peterson, C. J. Volk, and D. J. Lawrence. 2012. Projected climate-induced habitat loss for salmonids in the John Day River network, Oregon, U.S.A. *Conservation Biology* 26:873–82.
- Sabo, J. L., J. L. Bastow, and M. E. Power. 2002. Length-mass relationships for adult aquatic and terrestrial invertebrates in a California watershed. *Journal of the North American Benthological Society* 21:336–343.
- Scheuerell, M. D., and D. E. Schindler. 2003. Diel vertical migration by juvenile sockeye salmon: empirical evidence for the antipredation window. *Ecology* 84:1713–1720.

- Shearer, K., J. Hayes, and J. Stark. 2002. Temporal and spatial quantification of aquatic invertebrate drift in the Maruia River, South Island, New Zealand. *New Zealand Journal of Marine and Freshwater Research* 36:529–536.
- Sims, D. W., V. J. Wearmouth, E. J. Southall, J. M. Hill, P. Moore, K. Rawlinson, N. Hutchinson, G. C. Budd, D. Righton, J. D. Metcalfe, J. P. Nash, and D. Morritt. 2006. Hunt warm, rest cool: bioenergetic strategy underlying diel vertical migration of a benthic shark. *Journal of Animal Ecology* 75:176–190.
- Stock, B. C., and B. X. Semmens. 2013. MixSIAR GUI user manual, version 1.0. Accessible online at: <http://conserver.iugo-cafe.org/user/brice.semmens/MixSIAR>
- Sutton, R., M. Deas, S. Tanaka, T. Soto, and A. Corum. 2007. Salmonid observations at a Klamath River thermal refuge under various hydrological and meteorological conditions. *River Research and Applications* 23:775–785.
- Thomas, C. D., A. M. A. Franco, and J. K. Hill. 2006. Range retractions and extinction in the face of climate warming. *Trends in Ecology and Evolution* 21:415–6.
- Torgersen, C., J. Ebersole, and D. Keenan. 2012. Primer for identifying cold-water refuges to protect and restore thermal diversity in riverine landscapes. EPA scientific guidance handbook 910-C-12-001. U.S. Environmental Protection Agency, Seattle, Washington, USA.
- Travis, J. M. J. 2003. Climate change and habitat destruction: a deadly anthropogenic cocktail. *Proceedings of the Royal Society B* 270:467–73.

Van Kirk, R. W. and S. W. Naman. 2009. Relative Effects of Climate and Water Use on Base-Flow Trends in the Lower Klamath Basin. *Journal of the American Water Resources Association*. 44(4): 1035-1052.

Vitousek, P. M. 1997. Human domination of Earth's ecosystems. *Science* 277:494–499.

Willis, K. J., and S. A. Bhagwat. 2009. Biodiversity and climate change. *Science* 326:806–807.

Wurtsbaugh, W., and D. Neverman. 1988. Post-feeding thermotaxis and daily vertical migration in a larval fish. *Nature* 333:846–848.

**Table I.** Summary of field methods and modeled variables.

Variable	Method	Year and Study Site	Purpose of data
Water temperature (°C)	Collected using in-stream temperature loggers in the tributary and mainstem river.	2012 (S1&2) <sup>a</sup>	To quantify the difference in water temperature between the coldwater tributary and mainstem river; recorded temperatures represent the minimum and maximum temperatures available to fish.
Energy delivery rate (Joules m <sup>-2</sup> s <sup>-1</sup> ) of invertebrate prey	Modeled using data from invertebrate drift sampling in the tributary and mainstem river (see eq. 1).	2010 (S1-4) 2011 (S1)	To quantify energy delivery rates in the different thermal habitats at refuges (tributary and adjacent mainstem river), in order to assess potential sources of in-refuge food limitation.
Fish density (fish m <sup>-2</sup> )	Collected using snorkel surveys in the tributary, mixing zone, and mainstem river.	2012 (S1)	To quantify fish density in the different thermal habitats at refuges, in order to assess potential sources of in-refuge food limitation.
Isotope-inferred fish diet	Mixing model analysis using data from fish tissue samples and invertebrate prey sources (collected via benthic sampling in tributary and mainstem at thermal refuges).	2012 (S1&2)	To quantify the proportion of mainstem vs. tributary invertebrate prey contributing to the diet of fish using thermal refuges.
Fish body temperatures (°C)	Collected using continuous recordings of steelhead body temperatures from temperature-sensitive radio tags.	2010-2012 (S1&2)	To quantify thermal habitat use for juvenile steelhead using thermal refuges, so as to assess whether fish were foraging primarily in the same areas that they were holding.
Diet-inferred foraging temperatures (°C)	Modeled using isotope-inferred diet and water temperature data (see eq. 4).	2010-2012 (S1&2)	To model the water temperatures where fish were foraging, so as to assess whether fish using thermal refuges were foraging primarily in the same areas that they are holding.

<sup>a</sup> Sites indicated as follows: S1 (Beaver Creek), S2 (Grider Creek), S3 (Fort Goff Creek), and S4 (Thompson Creek)

## FIGURE CAPTIONS

Figure 1. Study sites located at tributary confluences on the Klamath River. (1 = Beaver Creek; 2 = Grider Creek; 3 = Fort Goff Creek; 4 = Thompson Creek). Inset shows location of study area within the watershed. Map layer from the California Department of Fish and Game, Pacific States Marine Fisheries Commission (Christy 2003).

Figure 2. (A) Map of snorkel survey plots (open squares) and invertebrate drift sampling locations (filled triangles) at Beaver Creek confluence. (B) Steelhead and (C) Chinook salmon densities ( $m^{-2}$ ) in plots within each thermal environment at Beaver Creek refuge in August 2012. Boxplots are coded by thermal environment (tributary (blue) = A, B, C; mixing zone (purple) = D, E, F, G; mainstem (red) = H, I). Labels above boxplots indicate mean water temperatures in each plot across the sampling period.

Figure 3. Isotope signatures of  $\delta^{13}C$  and  $\delta^{15}N$  for juvenile salmonids and mainstem (red) and tributary (blue) invertebrate prey sources (mean  $\pm$  SD). (A) Beaver Creek steelhead ( $n = 53$ ) indicated by solid gray points and Chinook ( $n = 25$ ) indicated by open squares. (B) Grider Creek steelhead ( $n = 32$ ) indicated by solid gray points. Isotope signatures shown are unadjusted, but it worth noting that trophic enrichment is approximately 3.2 for  $\delta^{15}N$  and 1.9 for  $\delta^{13}C$ .

Figure 4. Posterior estimates from MixSIAR of the contribution of mainstem prey sources to fish diet plotted against fish body size (fork length). Mean diet estimates and 90% credible interval (CI) for individual steelhead at (A) Beaver Creek, (B) Grider Creek, and (C) Chinook at Beaver

Creek. Symbols are coded by the thermal environment in which individuals were caught (tributary = blue squares; mixing zone = purple triangles; mainstem = red circles).

Figure 5. Posterior estimates of the mean contribution of mainstem prey sources to fish diet versus the proportion of time spent in mainstem water for individual juvenile steelhead radio tagged at Beaver Creek, for which there were >7 days of temperature data (n = 9).

Figure 6. Distribution of fish body temperatures (blue) and diet-inferred foraging temperatures (green) for steelhead sampled at (A) Beaver Creek and (B) Grider Creek in 2012. Thermal distributions are derived from temperature-sensitive radio tag data, and diet-inferred foraging temperature distributions are derived from the mass balance equation (Eq. 4).

## FIGURES

Figure 1.

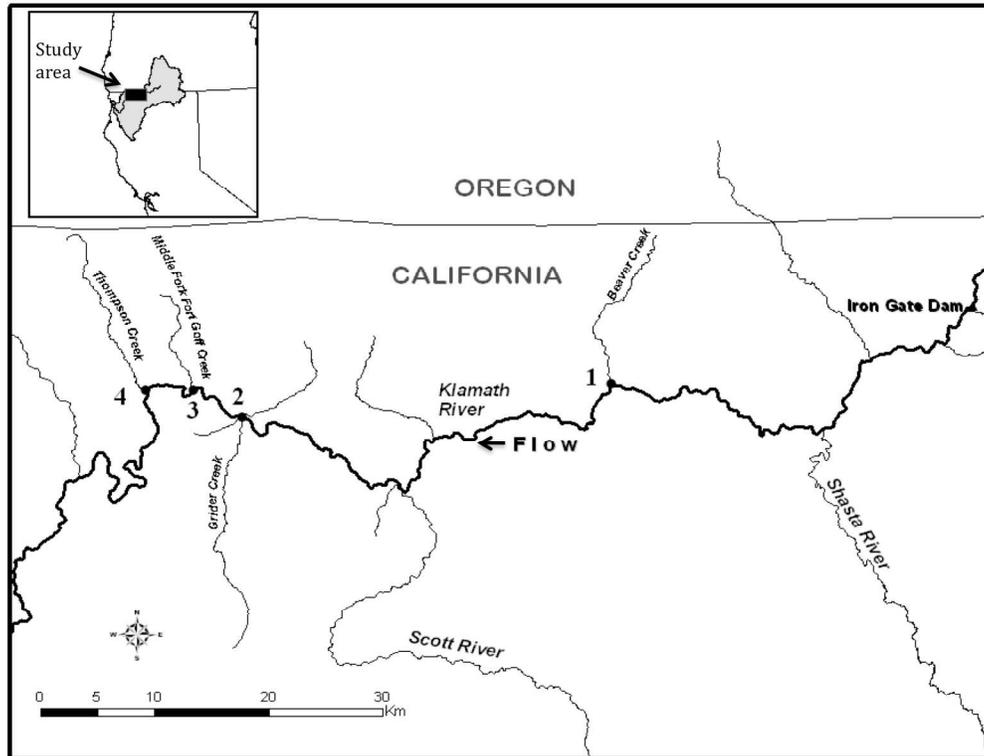


Figure 2.

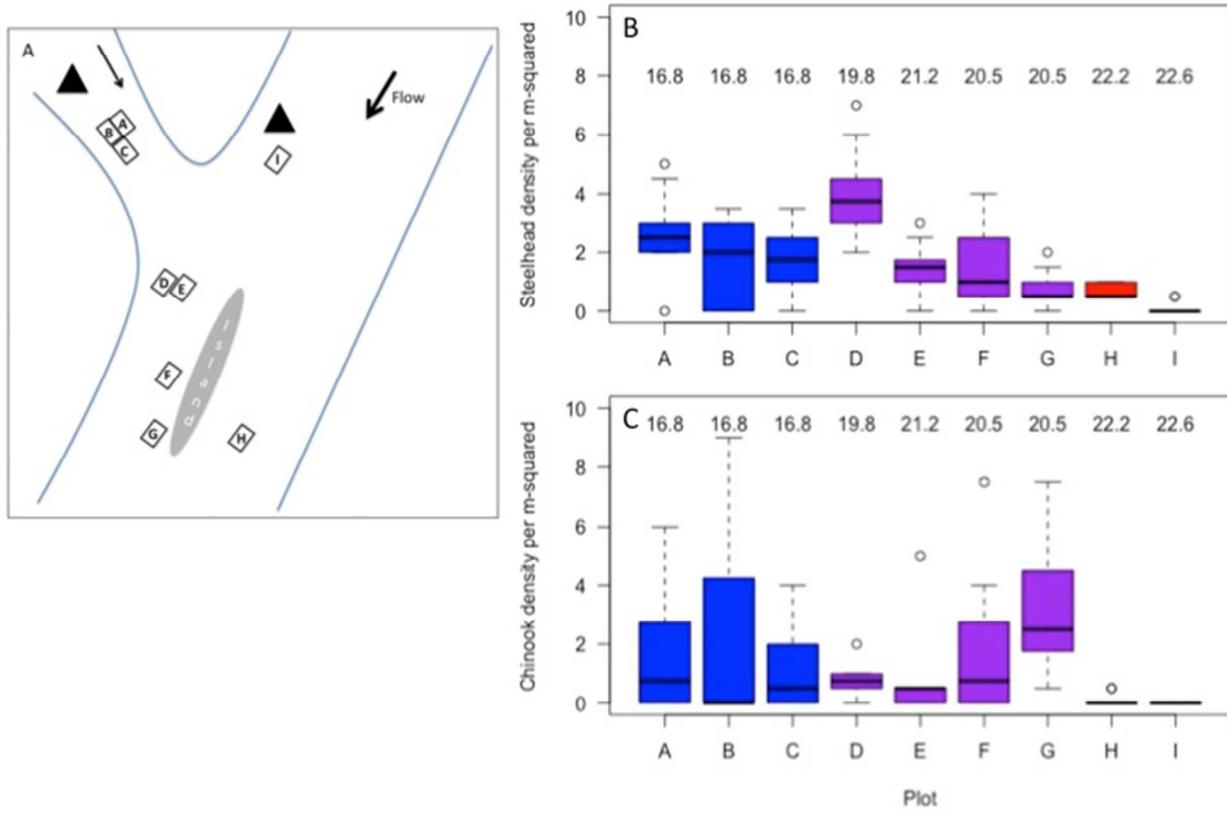


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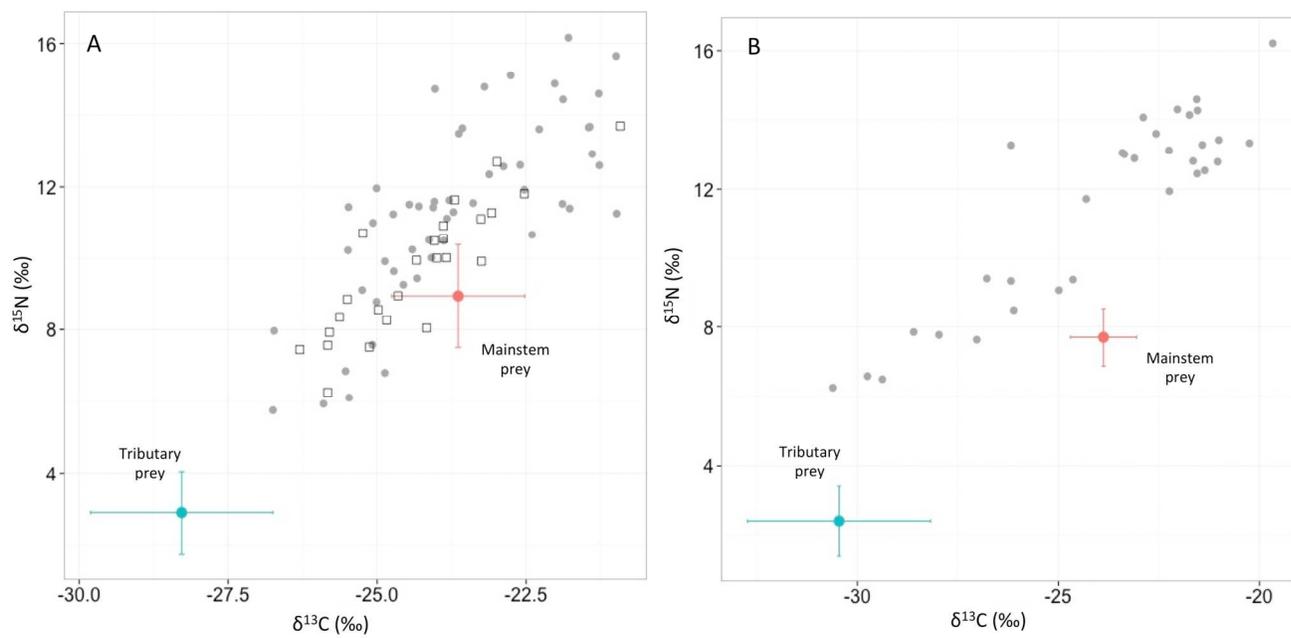


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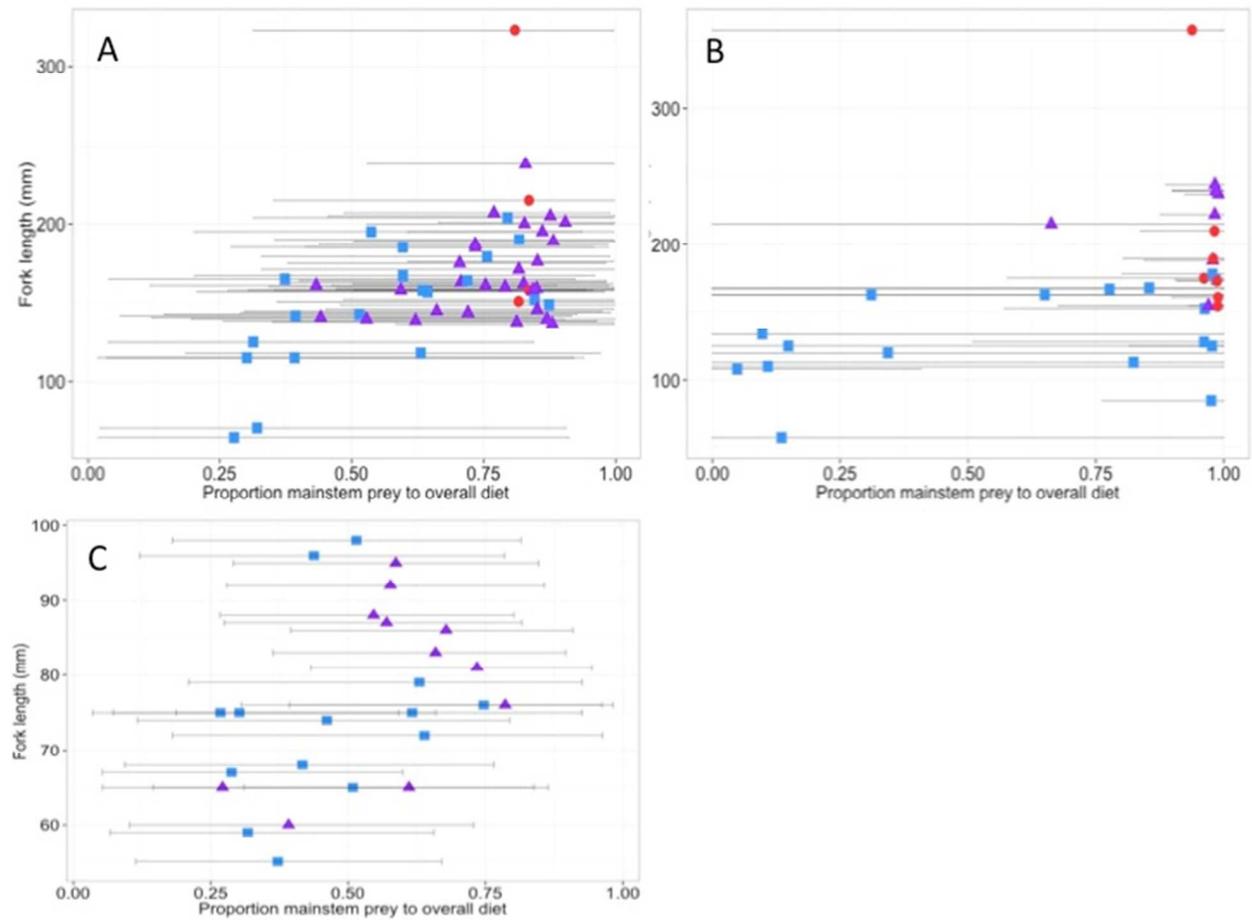


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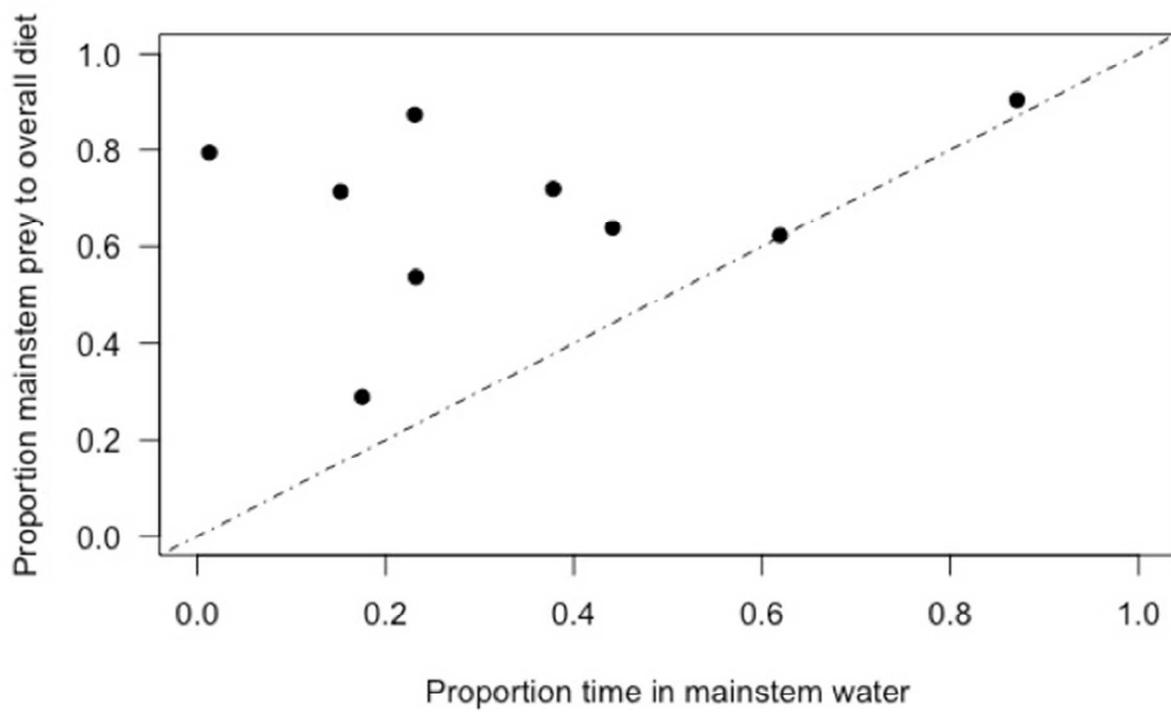
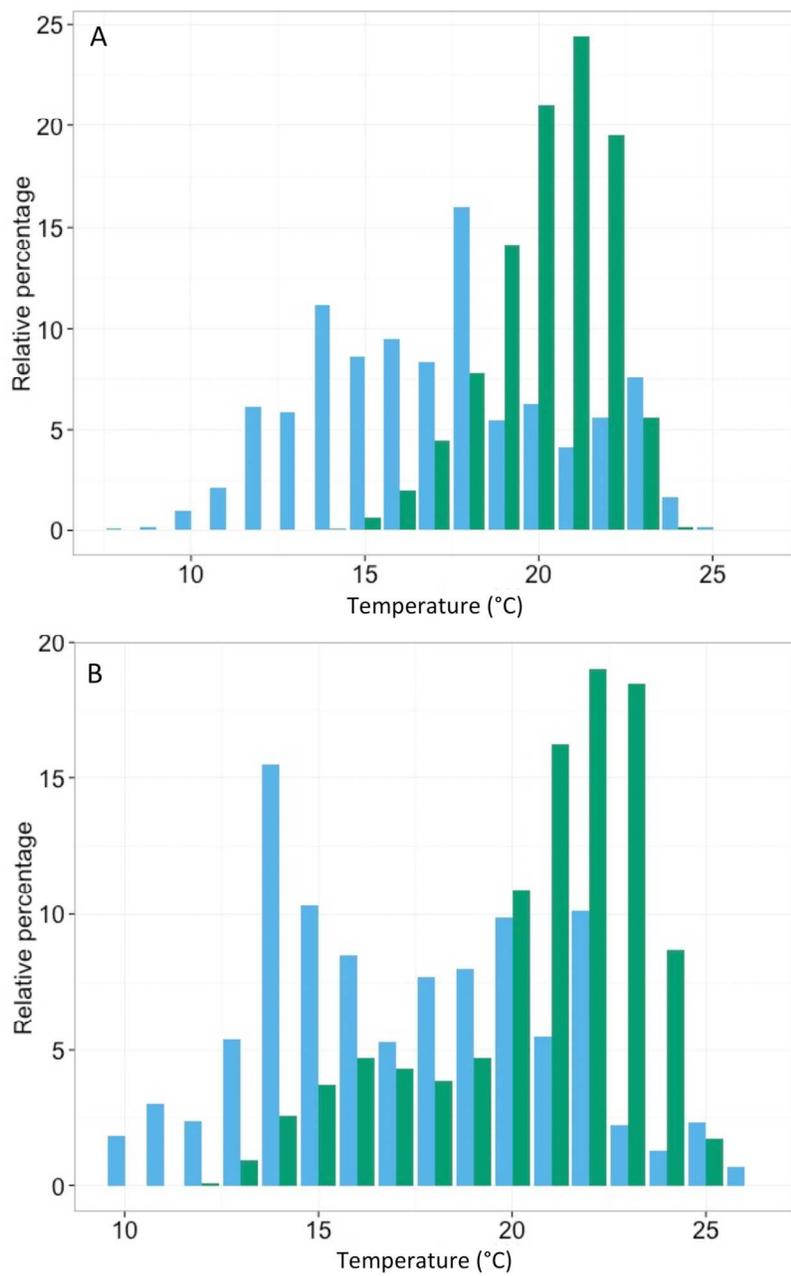


Figure 6.



## APPENDIX FIGURE CAPTIONS

Figure A1. Mainstem (red) and tributary (blue) water temperatures at (A) Beaver Creek and (B) Grider Creek in 2012. Black dashed vertical lines indicate fish sampling events, and gray shaded rectangles indicate the two week period prior to fish sampling when benthic invertebrates were collected.

Figure A2. Invertebrate energy delivery rates (mean  $\pm$  SD) at all sampled sites in 2010 and 2011 (mainstem = red; tributary = blue).

Figure A3. Relative abundance of invertebrate species (identified to family) in ten-minute drift samples (mainstem = red; tributary = blue). Taxa included represent most abundant represented in drift (mean abundance across samples  $>40$  per family).

Figure A4. Relative abundance of invertebrate species (identified to family) in juvenile steelhead diets in 2010 and 2011.

## APPENDIX FIGURES

Fig. A1

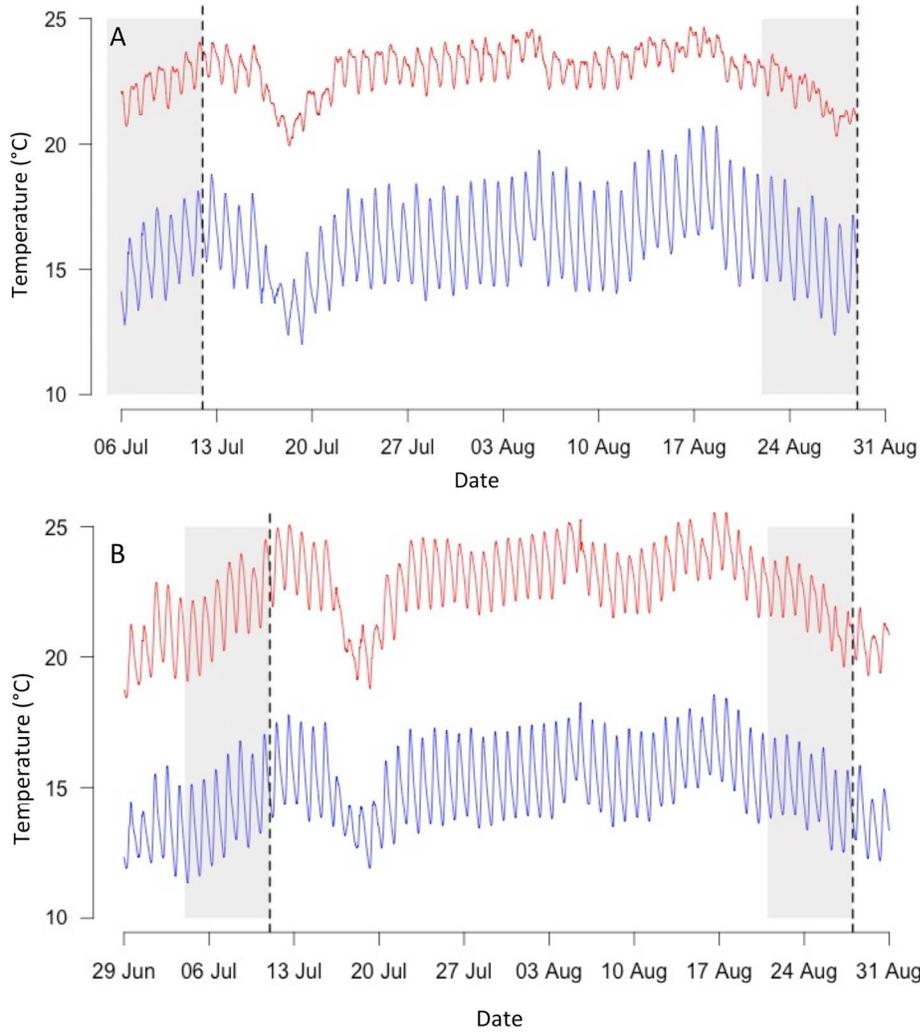


Figure A2.

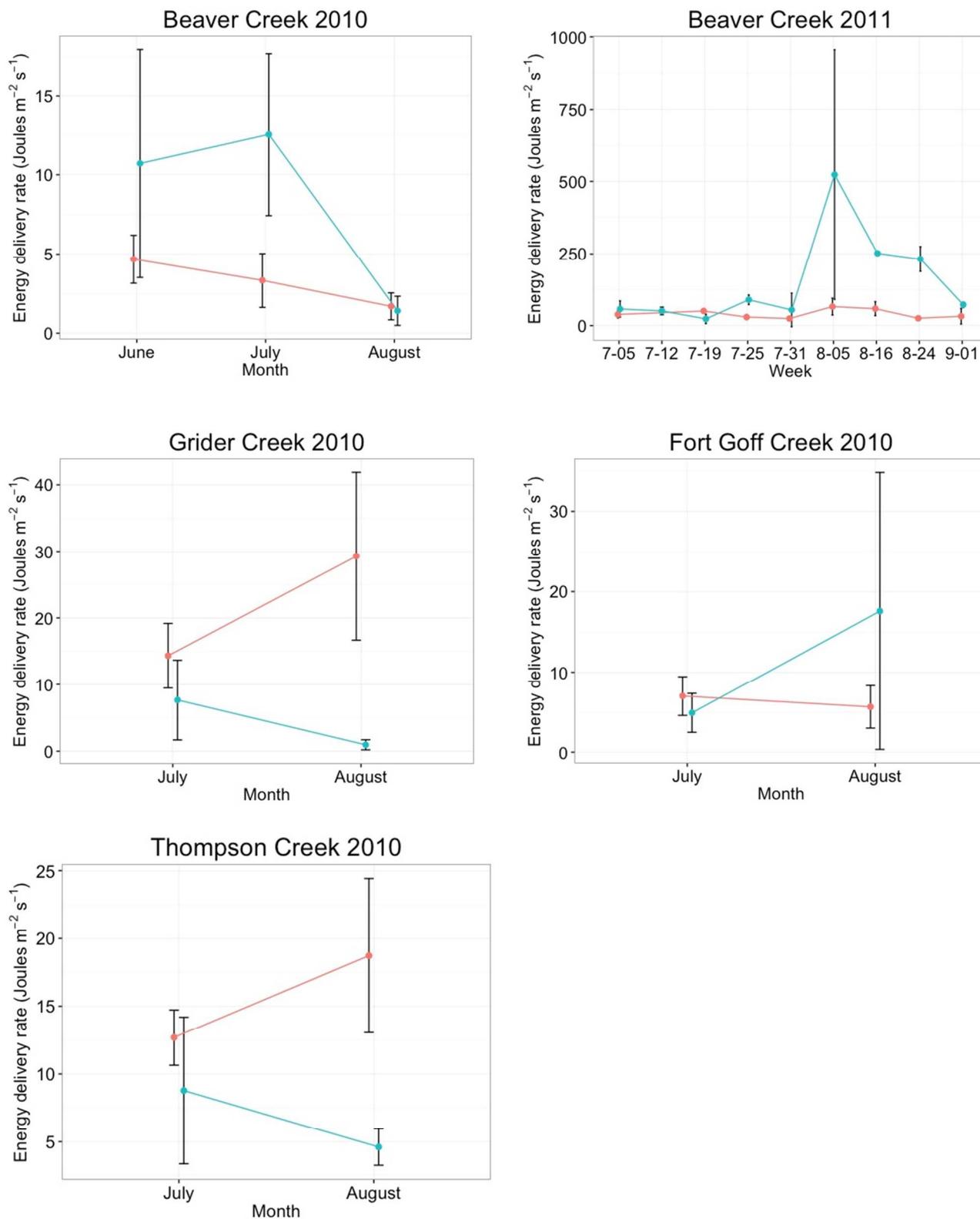


Figure A3.

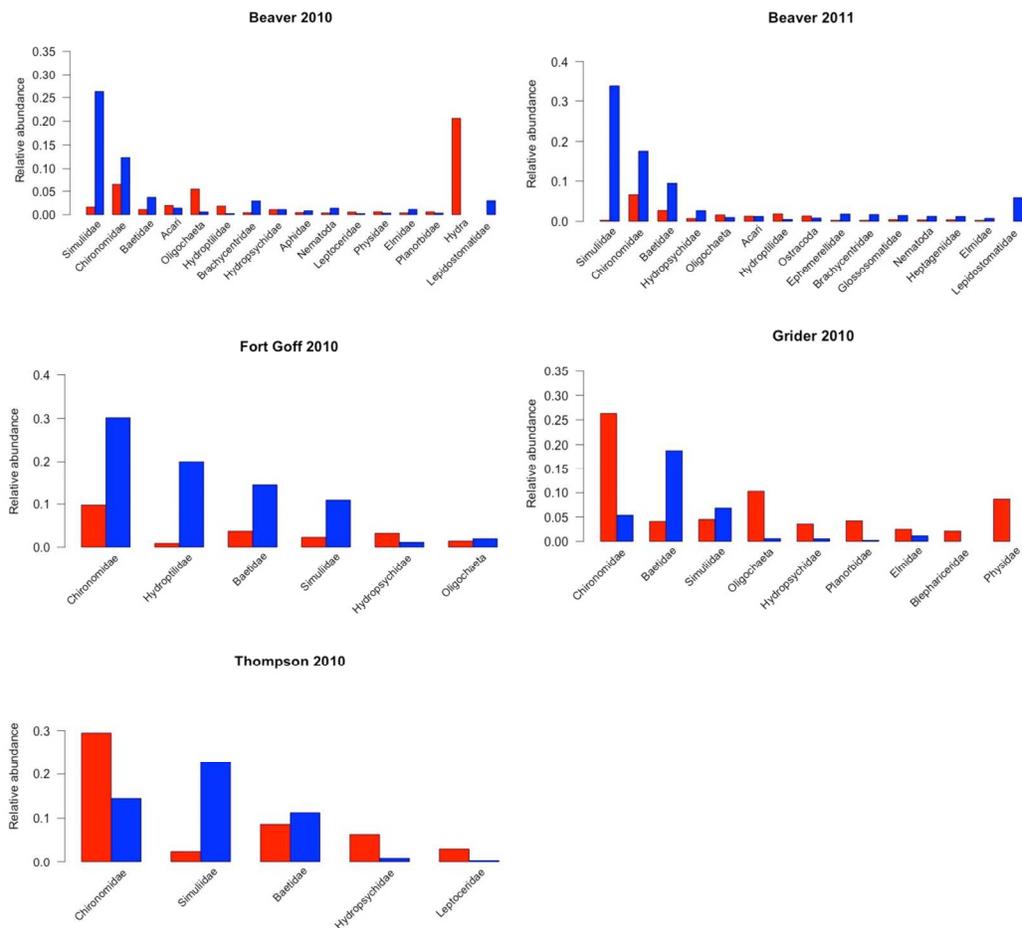
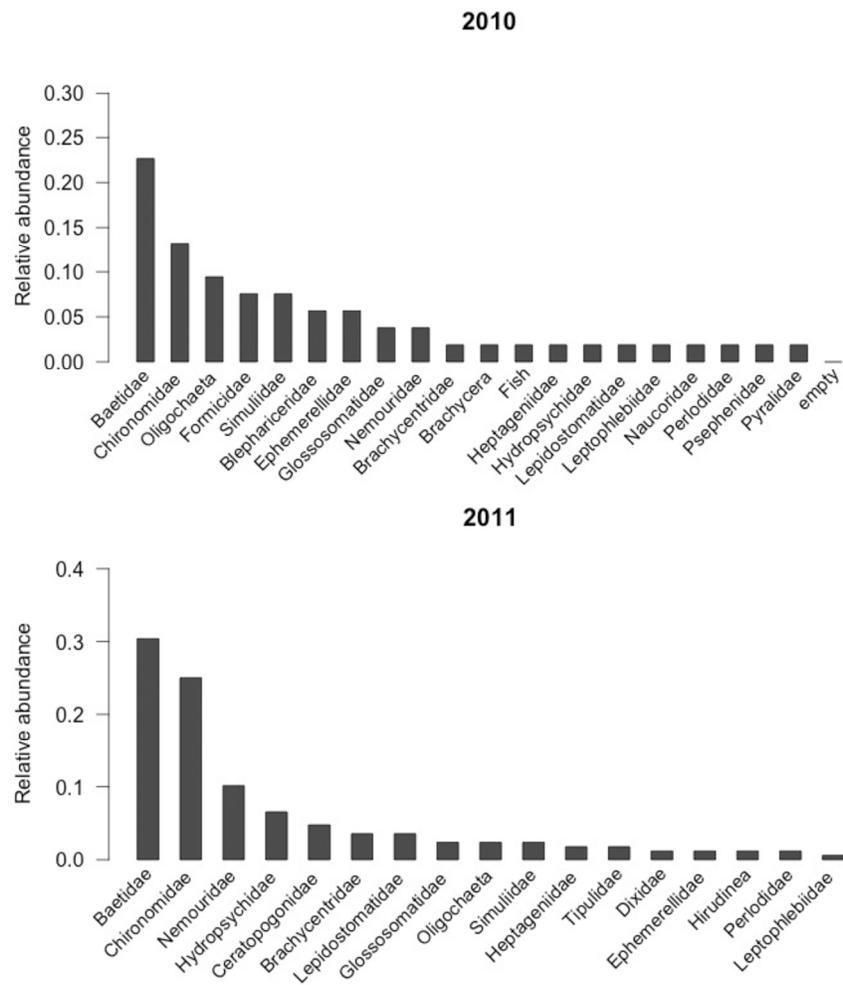


Figure A4.



## APPENDIX TABLES

Table A1. Sample sizes for invertebrate drift samples in 2010. Samples were collected monthly at each site; thermal environment indicates samples taken from either the mainstem river (MS) or tributary (T).

Site	Sample Month	Thermal Environment	N
Beaver	August	MS	4
Beaver	August	T	3
Beaver	July	MS	6
Beaver	July	T	6
Beaver	June	MS	6
Beaver	June	T	6
Fort Goff	August	MS	4
Fort Goff	August	T	3
Fort Goff	July	MS	4
Fort Goff	July	T	4
Grider	August	MS	4
Grider	August	T	4
Grider	July	MS	4
Grider	July	T	4
Thompson	August	MS	4
Thompson	August	T	4
Thompson	July	MS	2
Thompson	July	T	4

Table A2. Sample sizes for invertebrate drift samples in 2011. Samples were collected weekly (Week indicates sampling date), at both dawn and dusk, but only the dawn samples were analyzed due to processing time constraints.

Site	Week	Thermal Environment	N
Beaver	7-05	MS	2
Beaver	7-05	T	2
Beaver	7-12	T	2
Beaver	7-19	MS	1
Beaver	7-19	T	2
Beaver	7-25	MS	2
Beaver	7-25	T	2
Beaver	8-01	MS	2
Beaver	8-01	T	2
Beaver	8-05	MS	2
Beaver	8-05	T	2
Beaver	8-16	MS	2
Beaver	8-16	T	2
Beaver	8-24	MS	2
Beaver	8-24	T	2
Beaver	9-01	MS	2
Beaver	9-01	T	2

Table A3. Sample sizes for diet samples of juvenile steelhead taken in 2010 and 2011.

<b>Year and Site</b>	<b>Date</b>	<b>N</b>
2010		
Beaver	29-Jun	2
Beaver	27-Jul	10
Fort Goff	4-Aug	10
2011		
Fort Goff	14-Oct	12

Table A4. Invertebrate families selected from benthic samples and used to determine isotopic signatures of juvenile salmonid prey sources.

<b>Mainstem samples</b>	<b>Tributary samples</b>
Chironomidae	Chironomidae
Baetidae	Baetidae
Hydrophychidae	Hydrophychidae
Simuliidae	Simuliidae
Brachycentridae	Glossosomatidae
	Perlidae