

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

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

Résumé : Si les refuges thermiques constituent un important habitat pour les poissons d'eau froide dans un contexte de réchauffement, la concentration de poissons dans les refuges peut entraîner un appauvrissement des ressources de nourriture. Nous avons utilisé l'échantillonnage d'invertébrés à la dérive et des relevés de la densité de poissons pour quantifier la limitation potentielle de nourriture dans les refuges, des études avec des radio-émetteurs sensibles à la température pour quantifier l'utilisation d'habitats thermiques et des analyses isotopiques pour déterminer les sources d'aliments pour des salmonidés du Pacifique juvéniles qui utilisent des refuges thermiques dans le fleuve Klamath, en Californie. Les salmonidés juvéniles utilisant des refuges formés par la confluence d'affluents et du cours principal du fleuve obtenaient la majeure partie de leur alimentation (fourchette = 47 % – 97 %) de sources de proies dans le cours principal. Les températures corporelles moyennes de truites arc-en-ciel anadromes (*Oncorhynchus mykiss*) étaient significativement plus faibles ($\sim 3.5^{\circ}\text{C}$) que les températures d'approvisionnement inférées sur la base du régime alimentaire. Ainsi, alors que les poissons cherchent des habitats plus frais pour les avantages physiologiques qu'ils offrent, leurs proies proviennent principalement du cours principal. De plus, des densités de poissons uniformément élevées dans les refuges (moyenne = $3.5\text{ poissons}\cdot\text{m}^{-2}$) pourraient mener à une limitation de la nourriture dépendante de la densité. Les consommateurs mobiles comme les poissons peuvent donc exploiter l'hétérogénéité existante associée à des refuges d'eau froide en profitant des avantages thermiques d'un habitat d'eau froide où la nourriture est limitée tout en obtenant la majeure partie de leurs proies du cours principal plus chaud. [Traduit par la Rédaction]

Introduction

Habitat heterogeneity provides organisms with a choice of disparate habitats, and mobile consumers can integrate across a heterogeneous landscape, thereby balancing trade-offs in resources (Loose and Dawidowicz 1994; Bridges 2002; Hohnsava et al. 2003; Scheuerell and Schindler 2003; Sims et al. 2006). For cold-water fish, thermal refuges provide physiological relief from stressful in-stream temperatures, but they are also heterogeneous environments where the distribution of other resources within and surrounding a refuge (e.g., prey availability, predation risk) could influence the relative costs and benefits of refuge use. Thermal refuges, cold-water habitat that allows cold-water fish to escape the sublethal and lethal effects of hot temperatures (Torgersen et al. 2012), are often formed by tributaries that are colder than the mainstem river and create thermally heterogeneous mixing zones at their confluences. Most studies on thermal refuges focus

on their thermal benefits (Matthews et al. 1994; Nielsen et al. 1994; Torgersen et al. 1999; Ebersole et al. 2001); fish exploit in-stream thermal heterogeneity by moving into cooler water (i.e., thermal refuges) to relieve heat stress and reduce metabolic demand (Berman and Quinn 1991; Baird and Krueger 2003). Thermal refuges form increasingly important habitat as river temperatures continue to rise (Isaak et al. 2012; Ruesch et al. 2012; Davis et al. 2013), and these habitats have the potential to enable population persistence in what may eventually become thermally uninhabitable ecosystems (Sutton et al. 2007; Loarie et al. 2008). However, there may be trade-offs to refuge use; for example, adult steelhead (*Oncorhynchus mykiss*) using cool-water refuges in the Columbia River basin experienced increased harvest mortality (Keefer et al. 2009). A better understanding of how individuals use thermal refuges may help us understand the potential trade-offs and limits

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Table 1. Summary of field methods and modeled variables.

Variable	Method	Year and study site ^a	Purpose of data
Water temperature (°C)	Collected using in-stream temperature loggers in the tributary and mainstem river	2012 (S1, S2)	To quantify the difference in water temperature between the cold-water tributary and mainstem river; recorded temperatures represent the minimum and maximum temperatures available to fish
Energy delivery rate (J·m ⁻² ·s ⁻¹) of invertebrate prey	Modeled using data from invertebrate drift sampling in the tributary and mainstem river (see eq. 1)	2010 (S1–S4), 2011 (S1)	To quantify energy delivery rates in the different thermal habitats at refuges (tributary and adjacent mainstem river), to assess potential sources of in-refuge food limitation
Fish density (fish·m ⁻²)	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, 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, S2)	To quantify the proportion of mainstem versus tributary invertebrate prey contributing to the diet of fish using thermal refuges
Fish body temperatures (°C)	Collected using continuous recordings of steelhead trout body temperatures from temperature-sensitive radio tags	2010–2012 (S1, S2)	To quantify thermal habitat use by juvenile steelhead trout using thermal refuges, to assess whether fish were foraging primarily in the same areas in which they were holding
Diet-inferred foraging temperatures (°C)	Modeled using isotope-inferred diet and water temperature data (see eq. 4)	2010–2012 (S1, S2)	To model the water temperatures where fish were foraging, to assess whether fish using thermal refuges were foraging primarily in the same areas in which they are holding

^aSites indicated as follows: S1, Beaver Creek; S2, Grider Creek; S3, Fort Goff Creek; and S4, Thompson Creek.

of these habitats, and the consequences for the dynamics and persistence of populations in a warming world.

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

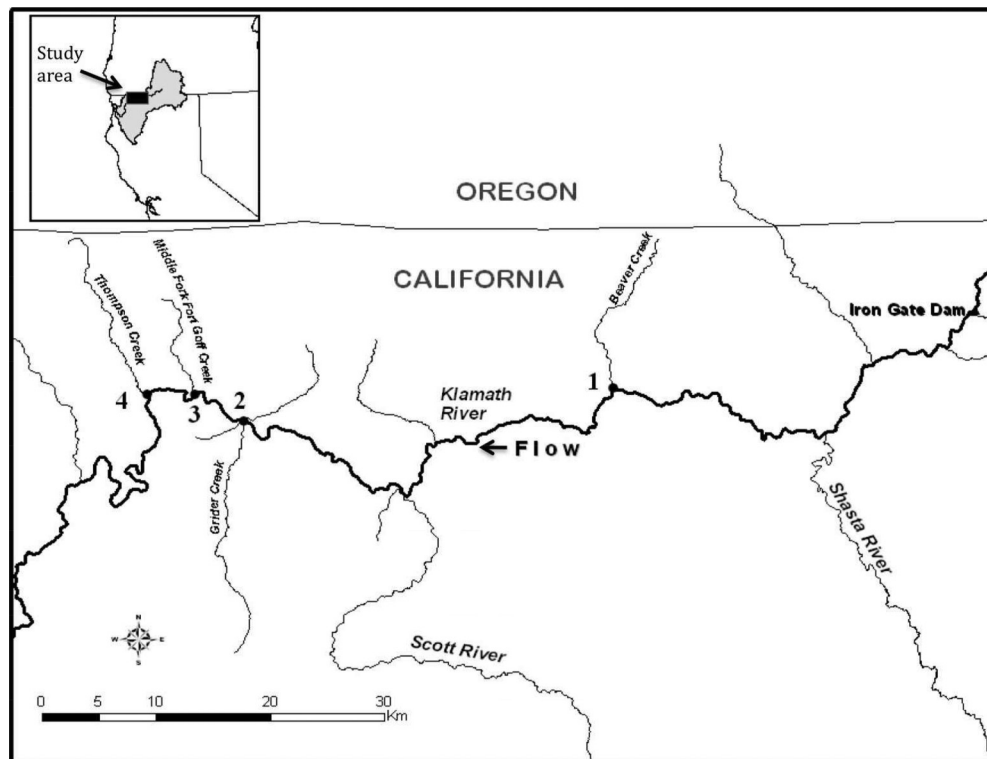
Competition for prey resources between individuals using refuges likely varies as a result of both naturally heterogeneous in-stream prey availability and fish density (Shearer et al. 2002; Rosenfeld et al. 2005; Hayes et al. 2007). Artificial stream-channel experiments simulating refuges have found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

In the Klamath River in northern California, steelhead (*O. mykiss*) and Chinook salmon (*O. tshawytscha*) populations have been reduced to approximately 5% of historical levels, and juvenile salmonids increasingly rely on refuges for suitable thermal habitat during hot summer months (Nehlsen et al. 1991; Brewitt and Danner 2014). Given possible trade-offs to refuge use, including density-dependent food limitation, we sought to identify mechanisms of food limitation in refuges and to assess the diet and thermal habitat use of juvenile salmonids using thermal refuges. We therefore asked the following questions: (i) Are there differences in invertebrate prey availability between the tributaries and the larger mainstem system? (ii) Are fish densities within refuges higher than those in the adjacent mainstem river? (iii) What proportion of juvenile steelhead and Chinook salmon diets is derived from the mainstem river versus tributary, and are there any seasonal, size-dependent, or species differences in diet? (iv) Are juvenile steelhead foraging primarily in the same areas they are holding (i.e., thermal habitat use)?

Methods

We used a multifaceted approach to quantify the thermal and prey landscapes surrounding thermal refuges (see Table 1). We sampled invertebrate drift (2010 and 2011) to quantify fish prey sources and assess whether invertebrate energy delivery rates (J·m⁻²·s⁻¹) could be a mechanism of food limitation within thermal refuges. To assess how fish use the landscape surrounding thermal refuges at the individual and population level, we used snorkel surveys (2012) to estimate fish density in and out of refuges, temperature-sensitive radio-tagging studies (2010–2012) to quantify thermal habitat use, and isotopic analyses (2012) to estimate juvenile salmonid prey sources.

Fig. 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).



Study system

The Klamath River in northern California is 423 km long, and drains ~41 440 km² of southern Oregon and northern California (National Research Council 2008) (Fig. 1). It has six mainstem dams, the lowest of which, Iron Gate Dam (rkm 306), acts as a migration barrier to anadromous fish, cutting off hundreds of kilometres of native anadromous salmonid habitat (Nehlsen et al. 1991). The dam, along with large-scale watershed alteration (e.g., irrigation), contributes to elevated water temperatures for the downstream habitat (Bartholow 2005; National Research Council 2008). During the summer and fall, the Iron Gate Reservoir undergoes seasonal thermal stratification, and the dam releases warm surface water rather than releasing colder water from below the thermocline (National Research Council 2004).

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

We conducted this research on the lower Klamath River in July–September 2010–2011 and July–August 2012. We chose four study sites on the river below Iron Gate Dam, each located at a major cold-water tributary confluence: Beaver Creek (rkm 259), Grider Creek (rkm 210), Fort Goff Creek (rkm 204), and Thompson Creek (rkm 198). We chose these tributaries based on the presence of cool-water refuges during summer and early fall months, and the presence of juvenile steelhead and Chinook salmon. Hereafter, we refer to “refuges” as the cool-water area that includes both the tributary and thermal mixing zone (i.e., the area where mainstem and tributary water mix, creating an area of heterogeneous temperature).

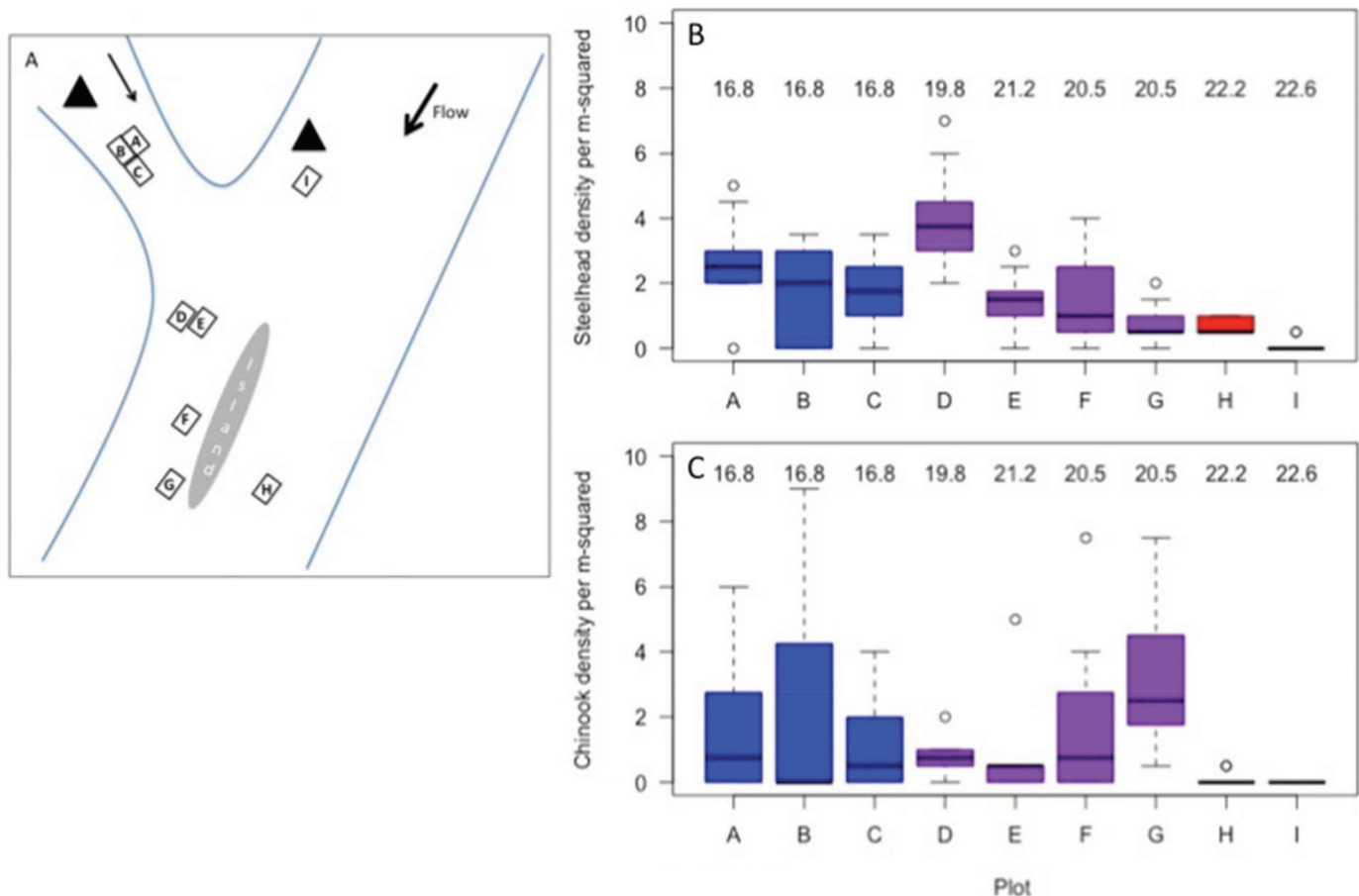
Water temperatures

To determine the difference in mean water temperature between the tributaries and mainstem river, we recorded water temperatures at 15 min intervals throughout July and August 2012 at each study site. We used two to four Hobo pendant data loggers (Onset Computer Corporation, Pocasset, Massachusetts, USA; ±0.5 °C) in both the cold-water tributary and mainstem river at point locations ~10 m upstream of each confluence. Recorded temperatures therefore represent the minimum and maximum temperatures available to fish. All data loggers were placed near the riverbed to minimize thermal input from direct sunlight, at depths ranging from 0.5 to 2 m.

Mainstem versus tributary invertebrate energy delivery rates

To assess whether fish may be experiencing food limitation in thermal refuges, we collected invertebrate drift samples and calculated energy delivery rates (J·m⁻²·s⁻¹) as an estimate of food availability in both the mainstem river and cold-water tributaries. We collected monthly samples (June–August) at all four study sites in 2010, and weekly samples (July–August) at Beaver Creek

Fig. 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 (fish·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. [Colour online.]



(farthest upstream) in 2011; samples were collected from the mainstem and tributary ~15 m upstream of the confluence, and upstream of where high densities of fish were observed in the tributaries (Fig. 2a). We suspended mesh drift nets (23 cm × 31 cm, 500 µm) ~5 cm above the substrate for 5–10 min, and measured water velocity directly in front of the net at 60% of water-column depth at both the beginning and end of the sample period to estimate mean flow. We took samples at dawn and dusk to capture temporal variation in drift, and collected approximately two samples per thermal environment during each sampling event to capture spatial variation in drift (see Tables A1 and A2 in Appendix A). Invertebrate samples were identified, counted, and measured to the nearest millimetre. We estimated invertebrate biomass (mg dry mass) from taxon-specific length–mass regressions (Benke et al. 1999; Miserendino 2001; Sabo et al. 2002), and calculated invertebrate energy content (J·mg⁻¹) for each sample using taxon-specific caloric values in the literature (Cummins and Wuycheck 1971; Driver et al. 1974; Driver 1981; Ciancio and Pacual 2006).

We calculated energy delivery rates (EDR; J·m⁻²·s⁻¹) as an estimate of salmonid prey availability, defined as the product of energy density and water velocity:

$$(1) \quad EDR = \frac{E}{V} \times v$$

where E is the total energy in a sample, V is the water volume of the sample, and v is the mean water velocity in front of the drift net. Energy delivery rate explicitly takes into account water veloc-

ity, making it a better metric than energy density for assessing how much food per unit time is reaching a fish holding in a specific location. Since there was a high degree of variability in the number of samples collected per month at each study site, we used a meta-analytical approach to assess whether there was any difference between mainstem and tributary drift delivery rates across sites and years. We treated each group of monthly samples taken at a site as a separate analysis, and determined the effect size between mainstem and tributary energy delivery rates; an effect size of zero indicates no difference. While invertebrate drift data were taken in 2010 and 2011, and all isotopic samples and fish density data were collected in 2012, we are using these drift data simply to draw inferences about general patterns of relative food supply in thermal refuges.

Fish density in and out of refuges

We quantified fish densities in the mainstem river and refuge (mixing zone and tributary) to assess whether fish density could be a possible source of in-refuge food limitation. We conducted weekly snorkel surveys at three times of day (0900, 1200, 1600) at the Beaver Creek study site throughout August 2012. We established nine 1 m × 2 m plots, three in the tributary (T), four in the mixing zone (MZ), and two in the mainstem (MS) (Fig. 2a); all plots were marked with orange flagging on corner rocks. To estimate fish densities, the snorkeler approached the plot from downstream to avoid spooking the fish and held a position in the water near the plot for ~10 min. We calculated mean steelhead and Chinook salmon densities for all plots across the month. While

the limited spatial extent of these surveys may have underestimated mainstem river salmonid densities, where fish may be more patchily distributed, this survey design was adequate for establishing relative densities of fish in thermal refuges and the adjacent mainstem river.

Quantifying juvenile salmonid diet sources

We used stable isotope analyses to quantify the proportion of mainstem versus tributary prey contributing to the diet of juvenile steelhead and Chinook salmon using thermal refuges at the two upstream study sites (confluences of Beaver and Grider creeks) in 2012. We chose two sampling periods (early July and late August) to assess whether there were any seasonal (i.e., temperature dependent) changes in fish diet. We chose these sampling periods because mainstem temperatures rise with decreased flows and increased air temperatures and are therefore usually cooler in early July than in late August.

To determine stable isotope values of aquatic prey, we collected benthic invertebrate samples from the tributary and mainstem at both sites. We chose taxa from the benthic samples that were representative of the most common invertebrate families found in fish diets and in the drift for both thermal environments (tributary and mainstem) and that represented a range of functional groups (see [Appendix A](#)). We performed invertebrate sampling twice during the 2-week period prior to fish sampling in early July and late August 2012, to capture the integration window for fish fin tissue ([Heady and Moore 2012](#)). During each sampling event, we collected samples of benthic invertebrates by scrubbing the substrate at four to seven point locations between 5 and 15 m upstream of the confluence, and collecting aquatic invertebrates with a 500 μm net. We identified invertebrates to family under a stereomicroscope, and analyzed two or three samples of each taxon.

We took caudal fin tissue samples to measure stable isotope ratios for juvenile steelhead and Chinook salmon. Fish were caught within 50 m of the tributary confluence (in either the tributary, thermal mixing zone, or mainstem) using a combination of angling, electrofishing, and seining at Beaver Creek in July ($n = 30$ steelhead; $n = 18$ Chinook salmon) and August ($n = 23$ steelhead; $n = 7$ Chinook salmon) and Grider Creek in July ($n = 14$ steelhead) and August ($n = 18$ steelhead) 2012. A small subset of the fish at Beaver Creek ($n = 6$) were caught in early August for radio tagging, but these fish showed no statistical differences in inferred diet from the fish caught in July and were therefore grouped with the early-July fish. We weighed and measured (fork length; FL) all fish prior to taking fin clips.

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

$$(2) \quad \delta X = \left(\frac{R_{\text{sample}}}{R_{\text{standard}}} - 1 \right) \times 1000$$

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

We used MixSIAR, a Bayesian stable isotope mixing model ([Stock and Semmens 2013](#)), to determine the probability distribu-

tions for the relative contribution of different prey sources to salmonid diet. Within MixSIAR, Gibbs sampling was performed for each of three parallel chains. We used a burn-in phase of 10 000 iterations and ran the model for 100 000 iterations, retaining every fifteenth posterior sample, resulting in 6000 independent draws for the posterior distribution. We used Gelman–Rubin diagnostics to confirm model convergence ([Gelman and Rubin 1992](#)). We ran the model separately for each study site, with sampling month included as a categorical variable in the model and individuals as a random effect. We incorporated a trophic discrimination factor of 3.2 ± 0.2 for $\delta^{15}\text{N}$ and 1.9 ± 0.5 for $\delta^{13}\text{C}$ (± 1 standard deviation (SD)) into the mixing model ([McCutchan et al. 2003](#)).

We collected diet samples from juvenile steelhead to qualitatively assess whether fish diet composition was similar to the species composition of the drift. Fish were sampled by hook and line from Beaver and Fort Goff sites in 2010 ($n = 22$) and from Fort Goff in 2011 ($n = 12$). ([Table A3](#); [Figs. A3 and A4](#)).

Fish thermal habitat versus foraging habitat

We used a two-part approach to assess whether juvenile steelhead were foraging primarily in the same areas they were holding (i.e., thermal habitat use). First, we used an individual-level analysis for the small number of steelhead ($n = 9$) for which we had both body temperature data and isotopic diet analyses; we compared mean thermal habitat use derived from radio-tag data to the mean water temperature where individuals were foraging, inferred from isotopic diet analyses (hereinafter diet-inferred foraging temperatures). Since this first analysis only allowed us to analyze a small number of individuals, we then used a population-level analysis to address the same question, comparing diet-inferred foraging temperatures for all steelhead for which we had isotopic diet data (July–August 2012; $n = 53$ at Beaver Creek; $n = 32$ at Grider Creek) to thermal habitat use across 3 years of temperature-sensitive radio-tagging data (June–August 2010–2012; $n = 76$ at Beaver Creek; $n = 40$ at Grider Creek).

We used temperature-sensitive radio tags to track the body temperature of juvenile steelhead caught at Beaver Creek and Grider Creek study sites. Size limitations prevented the tagging of juvenile Chinook salmon. We surgically implanted the tags (Lotek's MST-720T temperature-sensor transmitter tags; 1.3 g dry mass; ± 0.8 $^{\circ}\text{C}$) following the tagging protocol described in [Brewitt and Danner \(2014\)](#). The life expectancy of the radio tags was 42 days. We used data-logging receivers (Lotek SRX_400A and SRX 600) to log fish body temperatures at 5 s intervals for any tagged fish within ~ 100 m of the tributary confluence.

For the individual-level analysis of steelhead radio-tagged at Beaver Creek in 2012, we compared the proportion of time spent in mainstem water (derived from radio-tag data) to the mean contribution of mainstem prey sources to fish diet (derived from isotopic diet analyses). For this analysis, we used only fish for which we had both temperature data and isotopic diet data and that remained at the refuge for a minimum of 7 days after tagging ($n = 9$). We used a mass-balance equation to translate time series of fish body temperatures into the mean proportion of time an individual held in mainstem water (P_m). The equation we used was

$$(3) \quad P_m = \text{Mean} \left(\frac{T_f - T_t}{T_m - T_t} \right)$$

where T_f represents time series of fish temperatures, and T_t and T_m represent simultaneous time series of tributary and mainstem water temperatures, respectively. In making this comparison, we are assuming that these individuals' behavior was similar before and after tagging, since the fish temperature time series represent data collected in the 2 weeks after fin samples were taken for isotopic diet analysis; this constraint was due to our inability to recapture radio-tagged fish after tagging. We believe this is a rea-

sonable assumption, since time series of fish temperature were fairly consistent for each individual tagged, until the fish left the study area.

We then used a population-level analysis to determine whether the diet-inferred foraging temperatures of all fish for which we had isotopic diet data (2012; $n = 53$ at Beaver Creek; $n = 32$ at Grider Creek) matched the holding temperatures of all fish for which we had radio-tag data (2010–2012; $n = 76$ at Beaver Creek; $n = 40$ at Grider Creek). We used a mass-balance equation to calculate the water temperature where fish were foraging (diet-inferred foraging temperature; T_d) based on their isotope-inferred diet. We used time series of mainstem and tributary temperatures from the 2 weeks prior to fish sampling to characterize the water temperature profile experienced by individuals during the fin tissue integration window (Heady and Moore 2012) and multiplied the time series through by the isotope-inferred proportion of mainstem diet for each fish. The diet-inferred foraging temperature for an individual fish can therefore be expressed as

$$(4) \quad T_d = \text{Mean}\{(D_m \times T_m) + [(1 - D_m) \times T_t]\}$$

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

To quantify thermal habitat use for the population of fish using the thermal refuge at the Beaver Creek and Grider Creek study sites, we used data from a larger temperature-sensitive radio-tagging study (Brewitt and Danner 2014). We calculated the fish body temperature distribution for the sample of steelhead tagged at each site during the 2010–2012 study period at Beaver Creek ($n = 76$) and 2010–2011 study period at Grider Creek ($n = 40$), using all fish detections subsampled at 5 min intervals and weighting all fish equally. We used a Kolmogorov–Smirnov two-sample test to detect differences between the thermal and foraging temperature distributions at each site.

Results

Water temperatures

Mainstem temperatures were consistently warmer than tributary temperatures at Beaver Creek (mean difference = 6.8 ± 0.5 °C) and Grider Creek (mean difference = 7.1 ± 0.9 °C) throughout July and August 2012 (Fig. A1). Mean mainstem temperatures for the 2 weeks prior to fish isotope sampling in early July and late August 2012 were 22.5 and 22.0 °C at Beaver Creek and 21.8 and 21.6 °C at Grider Creek, respectively. In contrast, mean temperatures in the cold-water refuges during these same periods ranged from 15.5 to 19.5 °C at Beaver Creek and from 13.1 to 18.8 °C at Grider Creek.

Mainstem versus tributary invertebrate energy delivery rates

Invertebrate energy delivery rates were highly variable across sites and were not consistently higher in the larger mainstem river than in the tributaries. In 2010, energy delivery rates ranged from 0.9 to 43.3 J·m⁻²·s⁻¹ in the mainstem and from 0.4 to 37.5 J·m⁻²·s⁻¹ in the tributary across the four study sites (Fig. A2).

In 2011, energy delivery rates at Beaver Creek site ranged from 13.2 to 86.7 J·m⁻²·s⁻¹ in the mainstem and from 11.6 to 828.9 J·m⁻²·s⁻¹ in the tributary. The mean (±SD) effect size between the mainstem and tributaries across all sites and years was 0.102 ± 1.18 J·m⁻²·s⁻¹; an effect size of zero indicates no difference. Thus, there was no significant difference between mainstem and tributary prey supply per unit area.

Fish density in and out of refuges

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

Quantifying juvenile salmonid diet sources

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

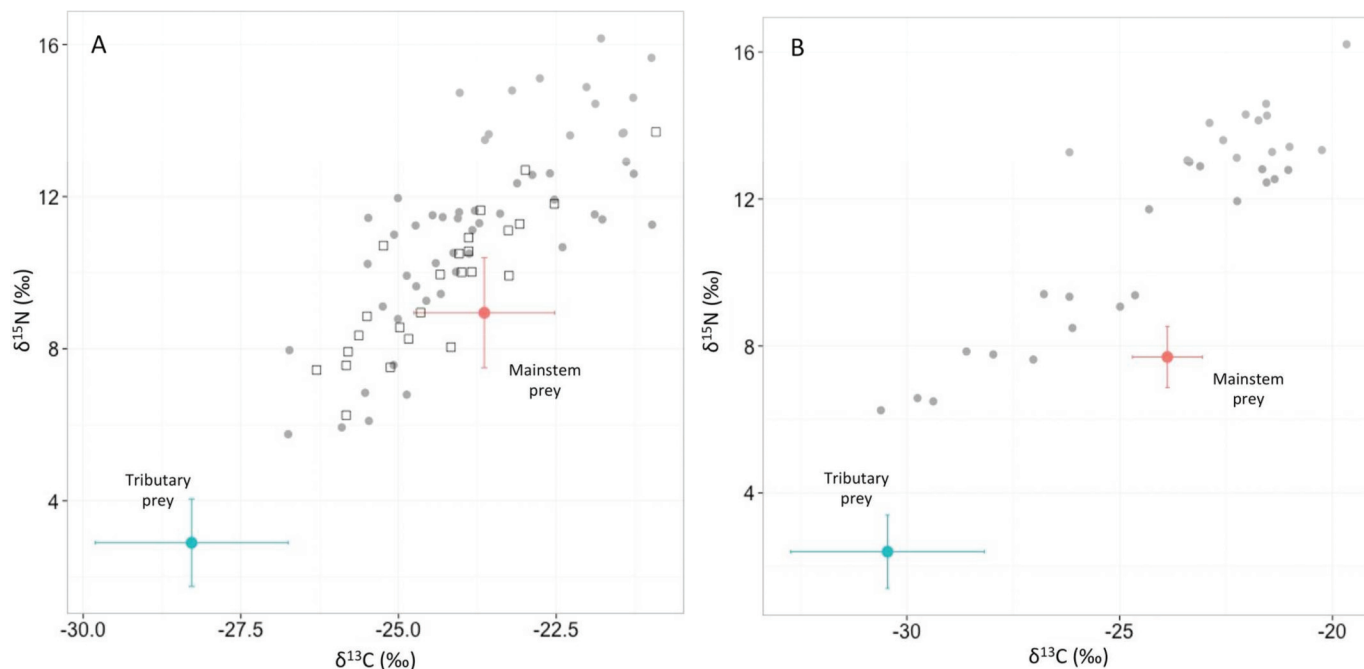
Juvenile salmonids using thermal refuges obtained over 50% of their diet from mainstem prey sources (Fig. 4). The mean (±SD) proportion of mainstem diet for juvenile steelhead caught in the mainstem (Beaver = 0.82 ± 0.13 ; Grider = 0.97 ± 0.02) and mixing zone (Beaver = 0.75 ± 0.13 ; Grider = 0.94 ± 0.11) was much higher than that for tributary-caught steelhead (Beaver = 0.57 ± 0.20 ; Grider = 0.60 ± 0.38). However, tributary-caught steelhead and Chinook salmon (0.47 ± 0.15) still obtained approximately half their diet from mainstem prey sources (Figs. 4a, 4b).

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

Fish thermal habitat versus foraging habitat

Steelhead body temperatures describing thermal habitat use were significantly cooler (~ 3.5 °C) than diet-inferred steelhead foraging temperatures, indicating a mismatch in thermal and foraging habitat for fish using thermal refuges. Fish temperatures for the nine radio-tagged steelhead at Beaver Creek indicate that fish used a range of thermal habitat (range in mean body temperature across sample period: 15.7–22.5 °C). Based on these body temperatures and river temperatures over the same time period, the mean (±SD) proportion of time individuals spent in mainstem water (0.22 ± 0.23) was relatively low, whereas the mean (±SD) proportion of prey these same individuals obtained from mainstem sources (0.72 ± 0.15) was higher than would be expected if fish were feeding in the area in which they were holding based on body temperatures (Fig. 5).

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



In the population-level analysis comparing thermal habitat use and diet-inferred foraging temperatures, fish body temperatures at both Beaver Creek (mean \pm SD = 17.5 ± 2.3 °C) and Grider Creek (17.8 ± 2.3 °C) were significantly cooler than diet-inferred foraging temperatures at Beaver Creek (20.7 ± 1.2 °C) and Grider Creek (21.0 ± 2.6 °C) (Kolmogorov–Smirnov two-sample test, $p < 0.001$ at both sites; Fig. 6).

Discussion

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

Our results suggest that juvenile salmonids using cool-water refuges shift laterally between the refuges and the mainstem river to access mainstem prey. While we did not measure fish movement directly, steelhead body temperatures used as a proxy for location indicate that fish move in and out of thermal refuges (Brewitt and Danner 2014). The high proportion of mainstem prey in isotope-inferred fish diets therefore implies that juvenile steelhead and Chinook salmon move out of refuges to obtain mainstem prey. There is evidence of fish in other systems using diel vertical or horizontal migration as a strategy to maximize access to food availability and metabolic efficiency across heterogeneous thermal landscapes (Wurtsbaugh and Neversman 1988; Bevelhimer and Adams 1993; Armstrong et al. 2013). For example, Armstrong and

Schindler (2013) found that juvenile coho salmon forage in colder water but shift to warmer water to digest, thereby increasing digestive capacity; conversely, Sims et al (2006) found that a benthic shark (*Scyliorhinus canicula*) lowered daily metabolic costs by hunting in warmer water and shifting to cooler water to digest.

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

Steelhead size appears to mediate how individuals negotiate the variability in temperature and prey availability in refuges, as indicated by size-based variation in the isotope-inferred diet of steelhead. Larger juvenile steelhead (FL ~ 200 mm) caught in the mainstem and mixing zone were more likely to rely on mainstem prey (~ 75 – 95 % mean proportion of mainstem prey) than smaller steelhead (FL ~ 150 mm) caught in the tributary, who had a lower proportion (~ 60 % mean proportion of mainstem prey) of mainstem prey in their isotope-inferred diet composition (see Fig. 4).

Fig. 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 intervals (CIs) are shown for individual steelhead at (A) Beaver Creek and (B) Grider Creek and Chinook salmon at (C) Beaver Creek. Symbols are coded by the thermal environment in which individuals were caught (tributary, squares; mixing zone, triangles; mainstem, circles).

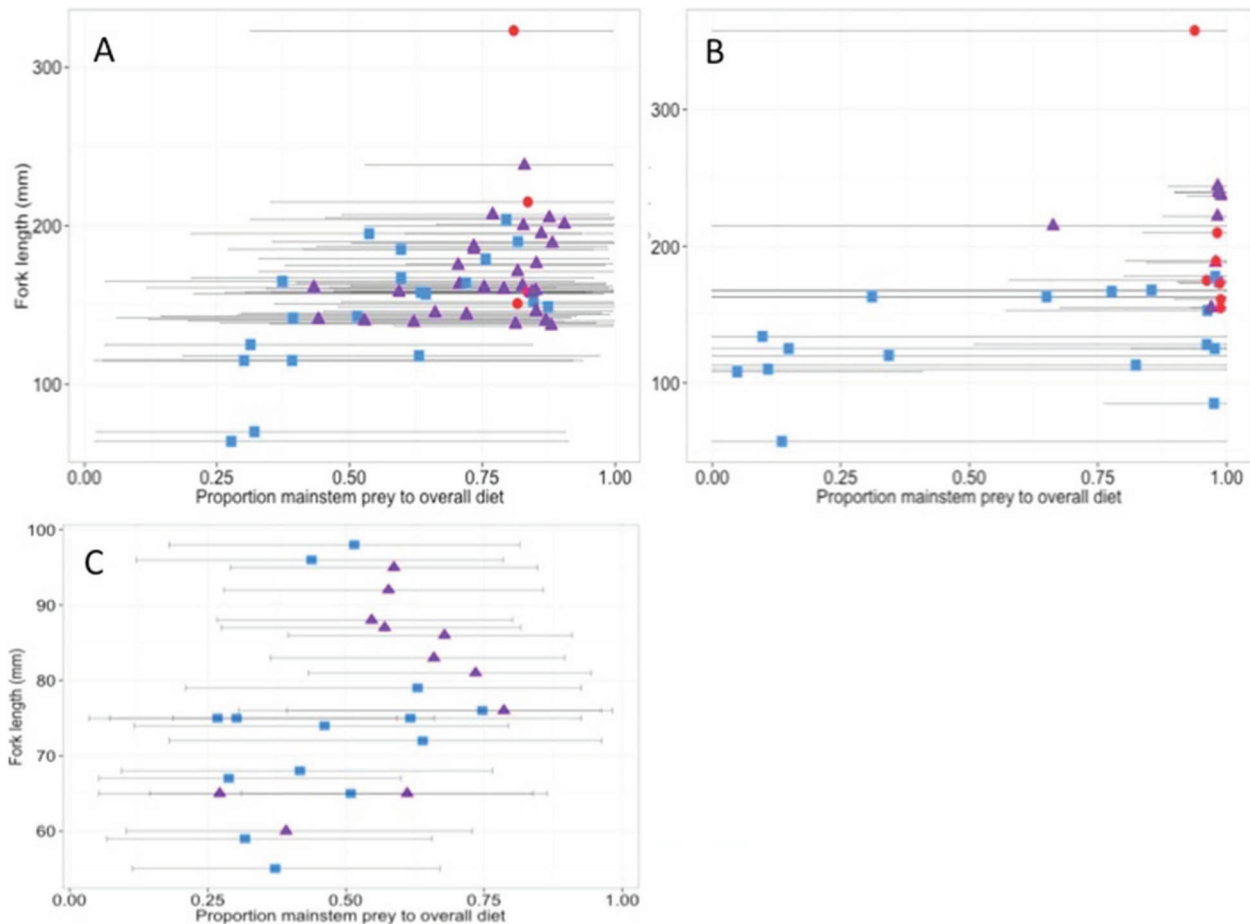
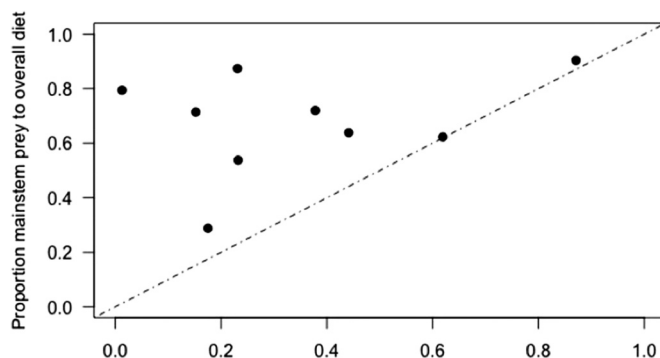


Fig. 5. Posterior estimates of the mean contribution of mainstem prey sources to fish diet versus the proportion of time spent in mainstem water for individual radio-tagged juvenile steelhead at Beaver Creek, for which there were >7 days of temperature data ($n = 9$).

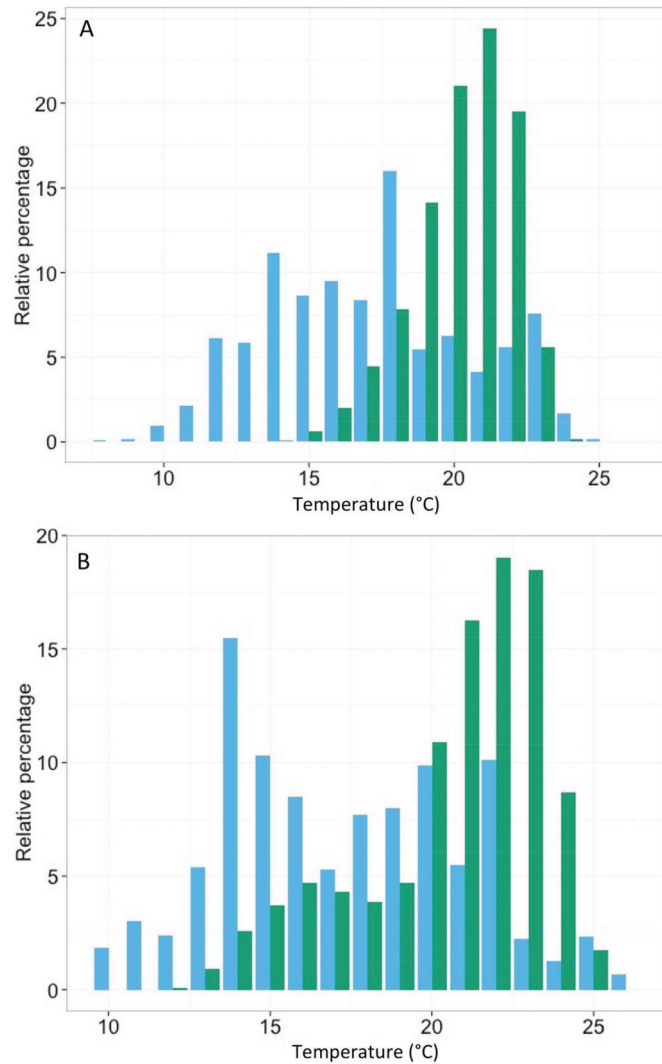


Given sufficient food availability, the mixing zone and boundary habitat (i.e., boundary between the refuge and mainstem river) is likely optimal habitat for fish growth; mixing zone temperatures were often at the bioenergetic optima for steelhead (~ 18 – 19 °C) (Brett et al. 1982; Myrick and Cech 2000; Heady 2012). Larger juveniles may be holding dominant positions near the edge of thermal refuges where temperature and prey availability can be opti-

mized, while smaller fish hold in cooler tributary waters. Both observational and radio-tagging studies on juvenile steelhead in Klamath River refuges report that fish (especially larger juveniles) were concentrated along the edge of refuges (Sutton et al. 2007; Brewitt and Danner 2014). An alternate hypothesis is that larger individuals are able to make longer feeding forays into the mainstem river before incurring thermal stress, since it takes longer for the body temperature of larger fish to equilibrate to ambient temperatures (Pepino et al. 2015). Balancing resource needs may therefore be easier for larger juvenile steelhead that can establish dominant positions on the mixing zone boundary habitat and likely forage in the mainstem river at longer time scales, while smaller tributary-based juveniles that still need access to mainstem prey would need to move further to gain sufficient resources.

Juvenile steelhead and Chinook salmon did not always hold in the coldest areas of the refuge, and both species tended to cluster in areas that met their species-specific habitat requirements (Everest and Chapman 1972; Quinn 2005). Chinook salmon were caught in either the tributary or the lower mixing zone (~ 50 m below the confluence) in slower-velocity areas rather than in the higher-velocity riffle forming the top of the mixing zone, where larger steelhead were found. Juvenile Chinook salmon also exhibited size-based variation in holding position within the refuge; Chinook salmon caught in the lower mixing zone (~ 20.5 °C) were larger (mean FL = 88.3 mm) than those caught in the tributary (~ 16.8 °C) (mean FL = 83.3 mm). The preference of larger Chinook

Fig. 6. Distribution of fish body temperatures (light grey; blue in colour version) and diet-inferred foraging temperatures (dark grey; green in colour version) 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). [Colour online.]



salmon for warmer water suggests that, as with steelhead, larger individuals may be holding dominant positions nearer the boundary of thermal refuges, where temperature and prey availability can be optimized; given sufficient prey availability, Chinook salmon growth potential is optimal at ~19 °C (Brett et al. 1982; McCullough 1999). Maintaining habitat heterogeneity in thermal refuges could provide the necessary temperature and prey resources for different species and size classes of juveniles present and facilitate coexistence due to differential habitat selection and foraging strategies.

Our results emphasize that not only are thermal refuges at tributary junctions important for fish seeking thermal respite, but the efficacy of refuges may also depend on food web dynamics in both tributary and mainstem habitats. Even if the mainstem river is apparently too hot for fish to reside in, it still provides key food resources for fish in thermal refuges. While the scope of this study was limited to the Klamath River, the isotopic values for fish at both study sites make a compelling case that fish using thermal refuges are foraging in the mainstem; moreover,

the population-level analysis of steelhead shows a clear mismatch between diet-inferred foraging temperatures and thermal habitat use (Fig. 6), indicating that fish are disproportionately foraging in the mainstem river. Rivers along the west coast are experiencing warming trends similar to the Klamath River (Knowles and Cayan 2002; Payne et al. 2004; Bartholow 2005; Isaak et al. 2012), making thermal refuges essential habitat for the over-summer survival of cold-water fish such as stream-rearing salmonids. As mainstem temperatures rise, any density-dependent food limitation in refuges will likely be exacerbated by higher in-refuge fish densities (Ebersole et al. 2001; Belchik 2003; Sutton et al. 2007; Brewitt and Danner 2014), as well as by low tributary flows that could cut off connectivity from the mainstem river (Van Kirk and Naman 2009). While cold-water tributaries are also susceptible to climate-induced warming, proper riparian shading can maintain cooler temperatures and protect thermal refuges formed by tributary junctions with the mainstem river (Boughton et al. 2012). Management that targets the maintenance of robust thermal refuges at tributary junctions with the mainstem river will provide essential habitat for juvenile salmonids seeking to exploit a heterogeneous thermal and prey landscape.

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Appendix

Appendix Tables and Figures appear on the following pages.

Table A1. Sample sizes for invertebrate drift samples in 2010.

Site	Sample month	Thermal environment	<i>n</i>
Beaver Creek	August	MS	4
	August	T	3
	July	MS	6
	July	T	6
	June	MS	6
	June	T	6
Fort Goff Creek	August	MS	4
	August	T	3
	July	MS	4
	July	T	4
Grider Creek	August	MS	4
	August	T	4
	July	MS	4
	July	T	4
Thompson Creek	August	MS	4
	August	T	4
	July	MS	2
	July	T	4

Note: Samples were collected monthly at each site; thermal environment indicates samples taken from either the mainstem river (MS) or tributary (T), and *n* indicates number of samples taken.

Table A2. Size (*n*) and date of invertebrate drift samples taken at the Beaver Creek site in 2011.

Week	Thermal environment	<i>n</i>
5 July	MS	2
5 July	T	2
12 July	T	2
19 July	MS	1
19 July	T	2
25 July	MS	2
25 July	T	2
1 Aug.	MS	2
1 Aug.	T	2
5 Aug.	MS	2
5 Aug.	T	2
16 Aug.	MS	2
16 Aug.	T	2
24 Aug.	MS	2
24 Aug.	T	2
1 Sept.	MS	2
1 Sept.	T	2

Note: 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.

Table A3. Size (*n*) and date of juvenile steelhead trout diet samples taken in 2010 and 2011.

Year	Date	<i>n</i>
2010		
Beaver Creek	29 June	2
Beaver Creek	27 July	10
Fort Goff Creek	4 Aug.	10
2011		
Fort Goff Creek	14 Oct.	12

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

Mainstem sample	Tributary sample
Chironomidae	Chironomidae
Baetidae	Baetidae
Hydrophychidae	Hydrophychidae
Simuliidae	Simuliidae
Brachycentridae	Glossosomatidae
	Perlidae

Fig. 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 2-week period prior to fish sampling when benthic invertebrates were collected. [Colour online.]

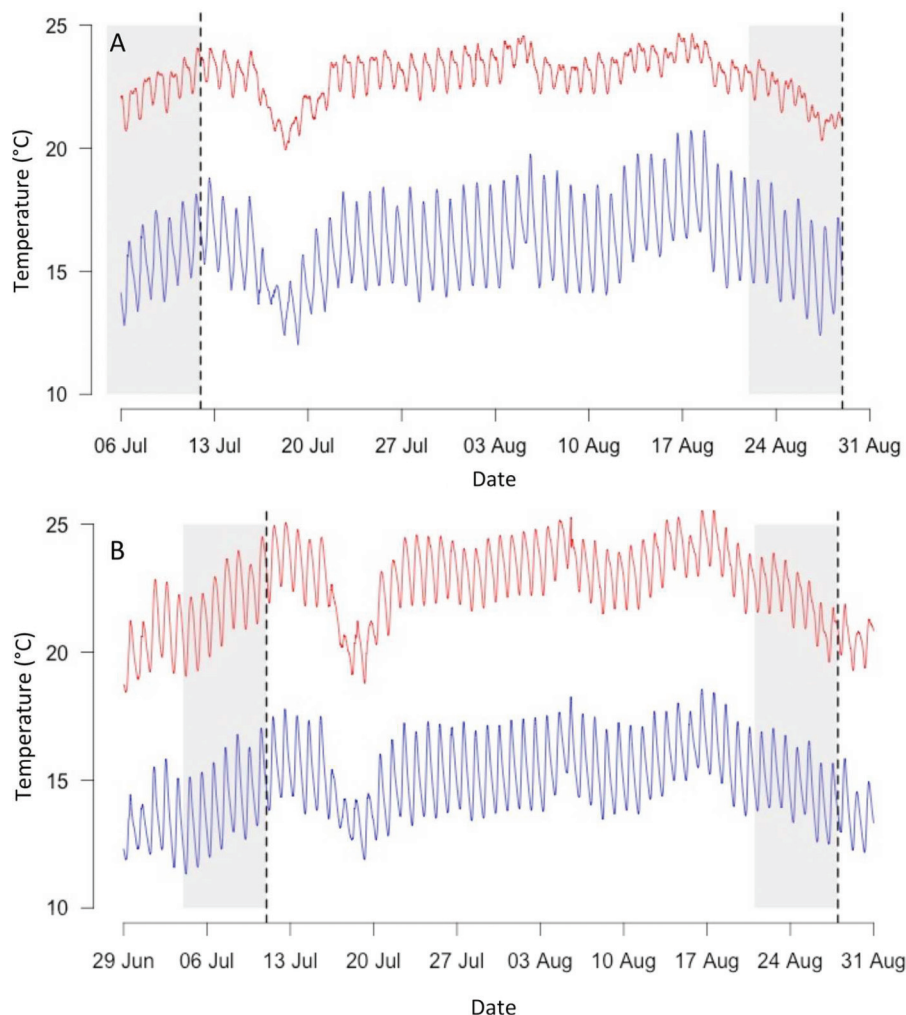


Fig. A2. Invertebrate energy delivery rates (mean \pm standard deviation) at all sampled sites in 2010 and 2011 (mainstem = red; tributary = blue). [Colour online.]

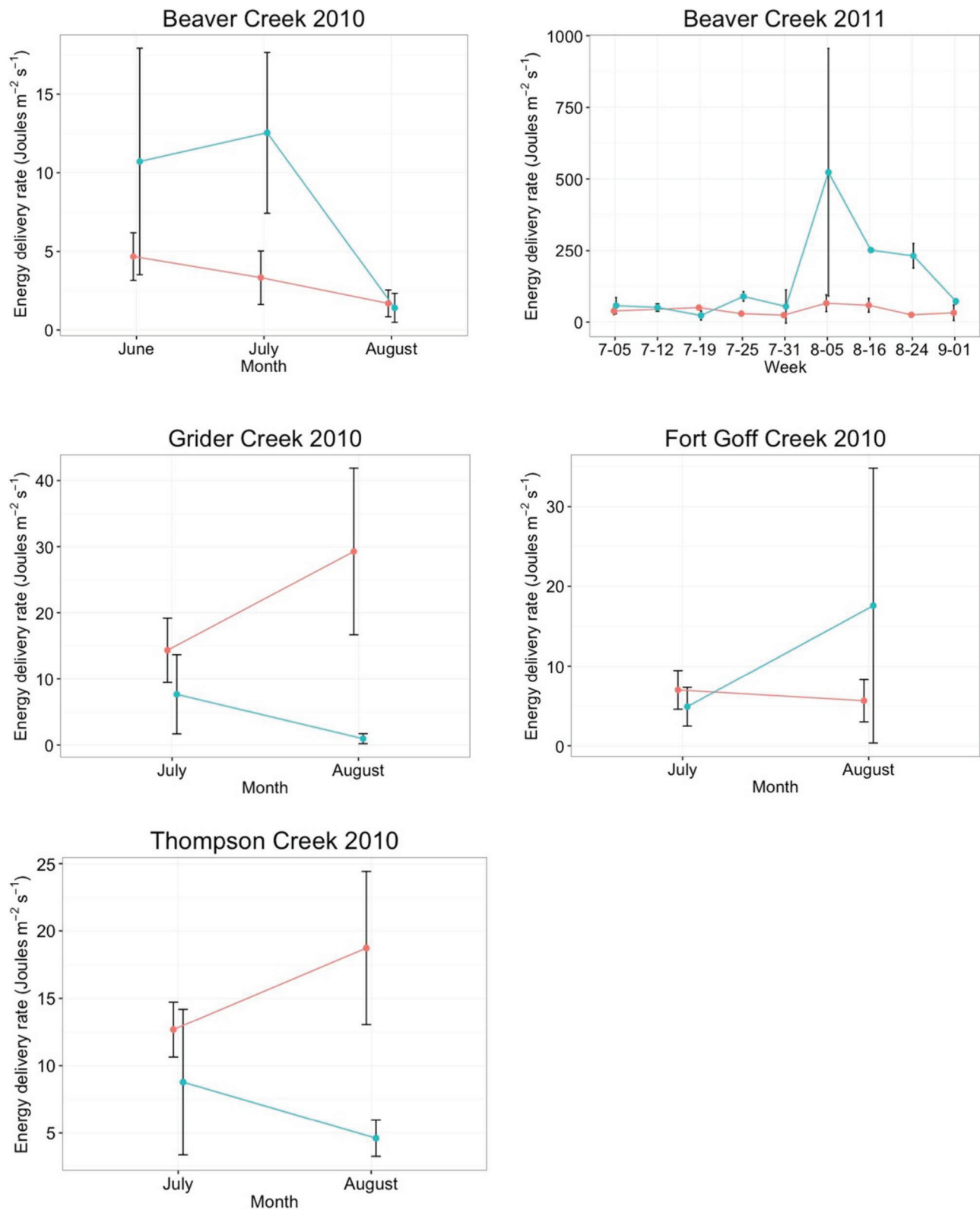


Fig. A3. Relative abundance of invertebrate species (identified to family) in 10 min drift samples (mainstem, red; tributary, blue). Taxa included represent those most abundant in drift (mean abundance across samples >40 per family). [Colour online.]

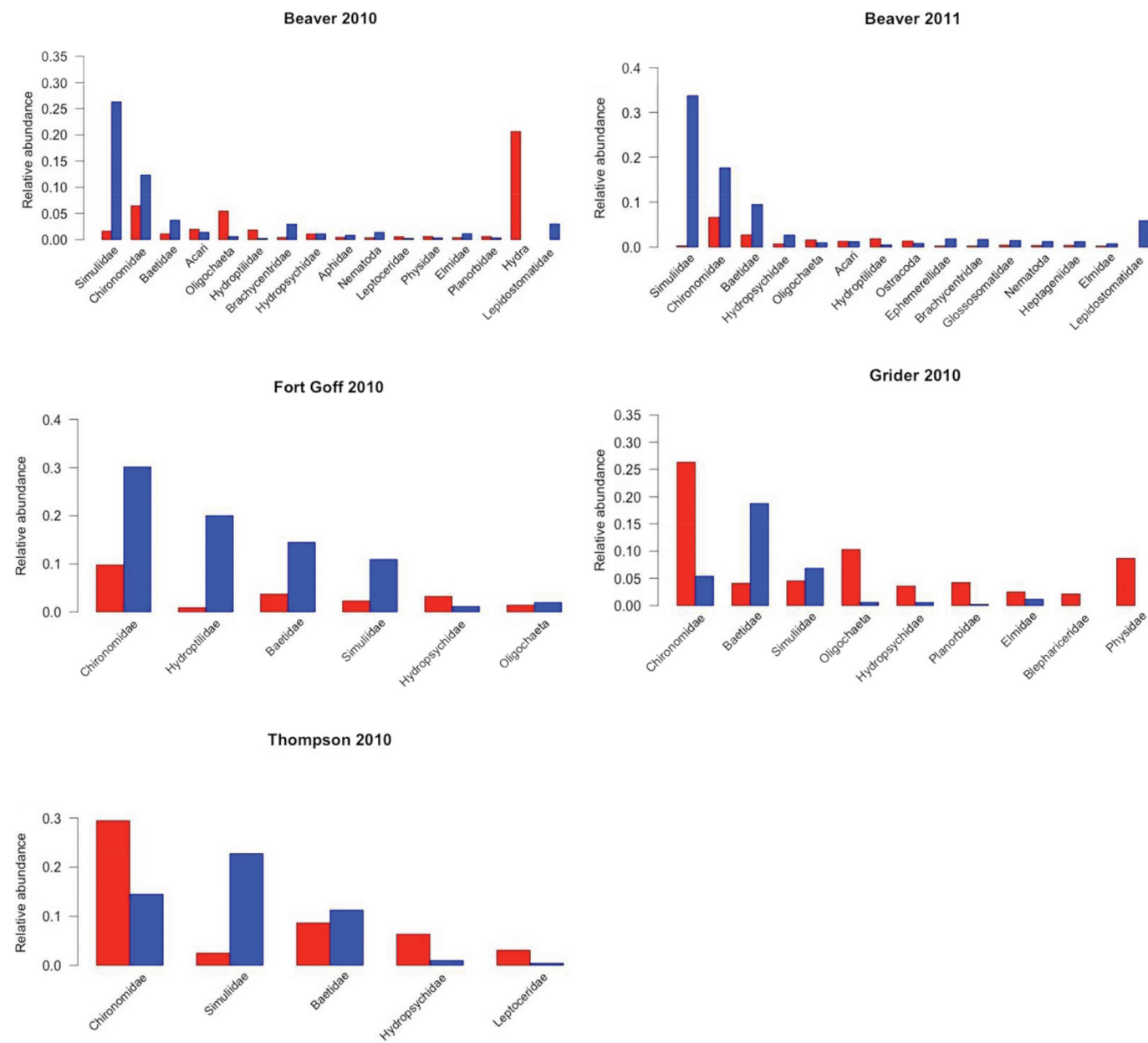


Fig. A4. Relative abundance of invertebrate species (identified to family) in juvenile steelhead diets in 2010 and 2011.

