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Environmental heterogeneity mediates juvenile salmonid use of thermal refuges

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Environmental heterogeneity mediates juvenile salmonid use of thermal refuges

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by

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Abstract

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by

Kimberly S. Brewitt

Thermal refuges, patches of habitat that provide physiological refuge from stressful temperatures, form increasingly important habitat in the face of rising ambient temperatures caused by large-scale habitat alteration and climate change. Thermal refuges in river ecosystems are highly heterogeneous, and habitat heterogeneity offers mobile consumers a choice of disparate habitats, allowing them to balance trade-offs between critical resources (e.g., temperature, food availability). Understanding how individuals negotiate fine-scale spatio-temporal heterogeneity in the thermal and prey resource landscape at thermal refuges may help shed light on the potential trade-offs associated with refuge use.

For this dissertation, I developed innovative methods to quantify the probability of thermal refuge use by juvenile salmonids (steelhead, *Oncorhynchus mykiss*; Chinook salmon, *O. tshawytscha)* at fine spatial and temporal scales in the Klamath River in northern California (USA), and determine how variability in the thermal and trophic landscapes at refuge sites influenced salmonid diet and thermal habitat use. In Chapter 1, I used temperature-sensitive radio tagging studies to quantify how spatio-temporal variability in abiotic (temperature, flow, time of day) and biotic (body size) variables influenced juvenile steelhead use of thermal refuges. This study represents the most in-

depth analysis of spatio-temporal variation in steelhead thermal refuge use to date. I found that mainstem temperature, time of day, and body size were the main drivers of refuge use (>80% of juvenile steelhead moved into refuges when mainstem temperatures reached 22-23°C, and all fish moved in by 25°C). In Chapter 2, I used a combination of radio tagging studies and isotopic diet analyses to quantify how spatial variability in thermal and trophic resources at thermal refuges mediate the foraging behavior and thermal habitat use of juvenile steelhead and Chinook salmon. I found that juvenile salmonids using thermal refuges obtained the majority (~75-95%) of their diet from mainstem prey sources, and that fish thermal habitat use was significantly cooler than diet-inferred fish foraging temperatures, indicating that while fish seek cooler habitat for physiological benefits, they still rely primarily on mainstem prey resources. In Chapter 3, I used lab experiments on internally tagged juvenile steelhead to determine how variation in water temperature and body size affects the amount of time it takes for Lotek temperature-sensitive radio tags to acclimate to a new ambient temperature. I found that tag temperature response time depended on the magnitude and direction (heating vs. cooling) of water temperature change and fish body size (fork length and weight); these experiments determined the sub-sampling interval for data collected in Chapters 1 and 2.

This dissertation demonstrates that fine-scale variability in the temperature and prey landscape mediate how juvenile salmonids use thermal refuges. Moreover, this research highlights the importance of habitat heterogeneity and connectivity for thermal refuges, as refuges may allow fish to more effectively exploit adjacent (and likely more abundant) non-refuge habitat (i.e. mainstem river) by providing temporary thermal respite; this could be a critical and currently under-valued benefit of maintaining refuges.

Dedicated to my scientific-minded, endlessly supportive, and loving parents,

my mother, *Leslie V. Ballard*

and in memory of my father, *Robert P. Perrin*

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Introduction

Thermal refuges, patches of habitat that provide physiological refuge from stressful temperatures, form increasingly important habitat as climate change and large-scale habitat alteration continue to drive increases in air and water temperatures (Keppel et al. 2012, Davis et al. 2013). Thermal refuges allow organisms to escape the effects of sub-lethal and lethal temperatures, and their availability and distribution can influence individual survival and physiological stress levels (Huey et al. 1989, Mathes et al. 2010), as well as impact movement and migration patterns and species distributions (Torgersen et al. 1999, Natori and Porter 2007, Monasterio et al. 2009). Thermal refuges could enable populations to persist in ecosystems that otherwise exceed thermal tolerance limits for a given species (Loarie et al. 2008, McLaughlin and Zavaleta 2012), and may be a central defining feature in the persistence of future populations at the trailing edges of a species' distribution.

In lotic ecosystems, large-scale watershed alteration (e.g., dams, irrigation, urbanization) and climate change are causing warming trends, making thermal refuges increasingly important for the survival of cold-water organisms such as Pacific salmon *(Oncorhynchus* spp.*)* (Webb et al. 2008, Ruesch et al. 2012). Temperature has a strong non-linear effect on salmonid physiological processes, and small changes in water temperature can have a large impact on metabolic and consumption rates (Jobling 1994, Myrick and Cech 2005). Fine-scale thermal and trophic heterogeneity may therefore influence how individual juvenile salmonids use thermal refuges; while temperatures remain below the critical thermal maximum for the species (estimated range 29.6-32.0°C for *O. mykiss* acclimated at temperatures ≥ 19°C; Myrick and Cech 2000, 2005), individuals may shift between thermal habitats to balance trade-offs between feeding opportunities, predation risk, and metabolic demand. For my dissertation, I focused on understanding how fine-scale spatio-temporal resource (e.g., temperature, food availability) variability influences juvenile salmonid use of thermal refuges, as this will help determine when and where refuges may be most effective in enhancing individual growth and survival, and by extension the abundance and persistence of populations.

Previous studies on juvenile salmonid use of thermal refuges have focused mainly on the effect of mean or instantaneous temperatures (Matthews et al. 1994, Ebersole et al. 2001, Breau et al. 2007), yet daily temperature fluctuations can impact salmonid growth and survival (Hokanson et al. 1977, Geist et al. 2010), and fish respond both physiologically and behaviorally to temperature variation (Baird and Krueger 2003, Beauregard 2013). In Chapter 1 of my dissertation, I aimed to assess the extent to which fine-scale thermal variability (and specifically diel temperature variation) influences juvenile steelhead use of thermal refuges. I combined temperature-sensitive radio tagging studies with simultaneous water temperature data to quantify individual movement between thermal habitats (in vs. out of refuge), and used an information theoretic-approach to determine the key environmental drivers of juvenile steelhead refuge use. All temperature variables (instantaneous mainstem temperature, diel mainstem temperature variation, and temperature differential between the tributary and mainstem) correlated positively with refuge use, and there was also a strong effect of time of day (day vs.

night) and body size on refuge use. Given impacts of watershed alteration and climate change and the growing importance of refuge habitat, the results of this chapter suggest that species persistence may depend on extremely fine-scale spatial and temporal temperature dynamics.

Variability in both thermal and trophic resources can influence fish behavior and movement, as individuals move between habitats to balance potential trade-offs in resources (Bridges 2002, Hohausova et al. 2003). In thermal refuges, juvenile salmonid 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). Competition for prey resources between individuals using refuges likely varies as a result of both fish density and naturally heterogeneous in-stream prey availability (Shearer et al. 2002, Rosenfeld et al. 2005, Hayes et al. 2007). In Chapter 2, I aimed to quantify how spatial variability in the thermal and prey resource landscapes at tributary confluences forming thermal refuges mediate the foraging behavior and habitat use of juvenile steelhead and Chinook salmon. I quantified possible mechanisms of food limitation in refuges using invertebrate drift sampling and fish density estimates, assessed juvenile salmonid diet using stable isotope analyses ($\delta^{13}C$ and $\delta^{15}N$) and a Bayesian mixing model (*MixSIAR*; Stock and Semmens 2013), and quantified thermal habitat use through radio tagging studies. I found that fish tended to hold in refuges at high densities during summer months, but both juvenile steelhead and Chinook salmon diet consisted of mostly mainstem prey (steelhead ≅ 75-95%; Chinook ≅ 53%), indicating a mismatch between their thermal and foraging habitat. These results suggest that density-dependent food limitation may be driving juvenile salmonids to forage in adjacent, sub-optimal mainstem habitat, while deriving thermal benefit by holding in refuges. This study therefore highlights the importance of both in-stream heterogeneity and habitat connectivity between coolwater tributaries and the mainstem river.

The previous two chapters document the influence of environmental variability on juvenile salmonid use of thermal refuges; in Chapter 3, I used a lab experiment to determine temperature-sensitive radio tag (Lotek MST-720T) response time to changes in ambient water temperature. Fish are poikilotherms, and change body temperature in response to changes in ambient temperature, but this response is not instantaneous and may be related to body size (Spigarelli et al. 1977, Crawshaw 1977), potentially biasing ambient temperature measurements around different size individuals. Internally implanting tags may therefore lead to error in ambient temperature estimates due to the insulatory effect of fish, which could cause a delay in tag temperature acclimation. I performed lab experiments on radio tags internally implanted in juvenile steelhead and tags outside of fish ('bare tags') to quantify how variation in the magnitude or direction (heating versus cooling) of change in water temperature and fish body size (fork length; weight; volume) affect tag response time (i.e. the amount of time it takes for tags to acclimate to a new ambient temperature). These experiments determined the sub-sampling interval for data collected in Chapters 1 and 2. Tag temperature response time depended positively on the magnitude of water temperature change and to a lesser extent on fish body size (fork length and weight), as well as on the direction of water temperature change (heating versus cooling). Tag response times were significantly shorter for bare tags than for implanted tags. While temperature-sensitive tags are useful for assessing thermal habitat use, the time lag in tag temperature acclimation represents a potential source of error for studies on internally tagged fish and should be taken into consideration, either by appropriately sub-sampling data or by explicitly modeling tag acclimation dynamics.

As rising temperatures across ecosystems exacerbate the effects of an already altered landscape, thermal refuges will form increasingly critical habitat (Vitousek 1997, Keppel et al. 2012). This dissertation adds to our understanding of how fine-scale spatio-temporal environmental variability influences juvenile salmonid use of thermal refuges by providing quantitative estimates of the effect of thermal variability on the likelihood of refuge use, and identifying a mismatch in thermal and foraging habitat for juvenile salmonids using thermal refuges. Together, these chapters underscore the importance of taking into consideration fine-scale environmental heterogeneity when considering the ecology and management of thermal refuges. As threatened salmonid populations continue to face thermally stressful in-stream temperatures, further understanding of the ecology of thermal refuges will help inform management actions for these critical habitats.

1. Chapter 1: Spatio-temporal temperature variation influences juvenile steelhead (*Oncorhynchus mykiss*) use of thermal refuges.

1.1 Abstract

Thermal refuges form potentially critical habitat for species at the limits of their thermal tolerance, especially given large-scale habitat degradation and rising temperatures across ecosystems. The Klamath River is a highly altered system where summer mainstem temperatures reach levels that are physiologically stressful to threatened Pacific salmonid populations, making thermal refuges critical for over-summer survival when temperatures near upper thermal thresholds. Small changes in water temperature can have a large effect on salmonid growth and survival, and therefore fine-scale spatio-temporal temperature variation could influence when and where refuges are important for both individual survival and population persistence. In this study, we combined monitoring of environmental variables with measures of fish temperature (a proxy for refuge use) to quantify juvenile steelhead *(Oncorhynchus mykiss)* use of thermal refuges. We used a logistic mixed effects model to determine the relative influence of instantaneous mainstem temperature and flow, sub-daily temperature variation, body size, and time of day on steelhead refuge use. Mainstem temperature was the strongest predictor of refuge use; the majority (>80%) of juvenile steelhead moved into refuges when mainstem temperatures reached 22-23°C, and all fish moved in by 25°C. Fish were more likely to use refuges with increased diel mainstem temperature variation and larger temperature differential between the mainstem and tributary. In addition, steelhead exhibited a

distinct diel behavioral shift in refuge use that varied with body size; smaller juveniles (~160 mm) were much more likely to use refuges during the night than day, whereas larger juveniles (~210 mm) exhibited a much less pronounced diel behavioral shift. Given impacts of watershed alteration and climate change and the growing importance of refuge habitat, these findings suggest that species persistence may depend on extremely fine-scale spatial and temporal temperature dynamics.

1.2 Introduction

Large-scale habitat degradation and climate change have led to species' range retractions and simultaneous increases in air and water temperatures in remaining habitat that reach or exceed thermal limits for many species (Mac Nally et al. 2009, Travis 2003, Sinervo et al. 2010). Thermal refuges, areas that provide physiological refuge from stressful temperatures, are receiving increasing attention from both ecologists and managers (Keppel et al. 2012). The availability and distribution of thermal refuges can influence individual survival and physiological stress levels (Huey et al. 1989, Mathes et al. 2010), as well as impact movement and migration patterns and species distributions (Torgersen et al. 1999, Natori and Porter 2007, Monasterio et al. 2009). Thermal refuges could allow for the persistence of populations in ecosystems that otherwise exceed thermal tolerance limits for a given species (Loarie et al. 2008, McLaughlin and Zavaleta 2012), and may be a central defining feature in the persistence of future populations at the advancing and trailing edges of a species' distribution. For example, McLaughlin and Zavaleta (2012) showed that California valley oaks *(Quercus lobata)* may experience constriction around refuges rather than a complete range shift as predicted by the current species bioclimate model under future climate warming scenarios. Similarly, thermal fronts may limit the movement of Pacific cod *(Gadus macrocephalus)* into warming arctic waters (Hollowed et al. 2013), whereas loss of the distinct "cold-pool" of bottom water under future climate conditions may facilitate predator overlap and increase predation mortality of walleye pollock *(Theragra chalcogramma)* in the Bering Sea (Zador et al. 2011, Hunsicker et al. 2013). Identifying and protecting refuges are priorities for species conservation, yet the potential importance of thermal refuges is often overlooked in climate envelope models (Pearson and Dawson 2003, Kuo and Sanford 2009).

In lotic ecosystems, large-scale watershed alteration (e.g., dams, irrigation, urbanization) and climate change are causing warming trends, making thermal refuges increasingly important for the survival of coldwater organisms such as salmon (Webb et al. 2008, Ruesch et al. 2012). Pacific salmonids are especially susceptible to changes in temperature, as habitat fragmentation has limited access to suitable habitats (Rieman et al. 2007), and elevated mean river temperatures in the Pacific Northwest are associated with increased rates of disease and reduced growth and survival of multiple salmonid populations (Farrell et al. 2008, Isaak et al. 2011). Temperature has a strong non-linear effect on salmonid physiological processes, and small changes in water temperature can have a large impact on metabolic and consumption rates (Jobling 1994, Myrick and Cech 2005). Juvenile salmonids are particularly susceptible to increases in river temperatures as they spend on average 1-3 years rearing in freshwater, depending on the species, and juvenile growth performance has been directly linked to fecundity and survival (Shapovalov and Taft 1954, Quinn 2005, Bond et al. 2008). There is therefore selective pressure for rapid growth (Satterthwaite et al. 2009), and while temperatures remain below the critical thermal maximum for the species (estimated range 29.6-32.0°C for *Oncorhynchus mykiss* acclimated at temperatures ≥ 19°C; Myrick and Cech 2000, 2005), individuals may shift between thermal habitats to balance the trade-off between feeding opportunities, predation risk, and metabolic demand. For example, Bevelhimer and Adams (1993) demonstrated that diel vertical migration allows kokanee salmon *(O. nerka)* to maximize their growth by taking advantage of thermal and trophic resource heterogeneity, obtaining food in warmer surface waters and moving to deeper, cooler habitat to digest. Determining how spatio-temporal variation in key environmental drivers affects salmonid use of thermal refuges will help determine when and where refuges may be most effective in enhancing individual growth and survival, and by extension the abundance and persistence of populations. Yet the potential importance of fine-scale spatio-temporal heterogeneity in driving thermal refuge use remains largely unexplored (McCullough et al. 2009, Torgersen et al. 2012).

Thermal refuges can be highly dynamic environments, exhibiting large fluctuations in both daily and seasonal water temperatures (Sutton et al. 2007, Dugdale et al. 2013). Previous studies on juvenile salmonid use of refuges have focused mainly on the effect of mean or instantaneous temperatures (Matthews et al. 1994, Ebersole et al. 2001, Breau et al. 2007), yet studies have shown that daily temperature fluctuations can impact salmonid growth and survival (Hokanson et al. 1977, Geist et al. 2010), and fish respond both physiologically and behaviorally to temperature variation (Baird and Krueger 2003, Beauregard 2013). Hokanson et al. (1977) found that juvenile rainbow trout held at fluctuating daily temperatures had slower growth rates relative to those held at constant temperatures with the same mean, especially when mean temperatures were above the thermal optimum for that species. In addition to daily mean and instantaneous temperatures, diel temperature variation could be an important factor in determining temperature thresholds at which thermal refuges become critical for salmonid survival (Wehrly et al. 2007).

Juvenile steelhead *(O. mykiss)* on the Klamath River in northern California provide a model system for examining the impact of thermal variability and potential impacts of future climate change on refuge use. Like many regulated rivers in the Pacific salmon's native range, the Klamath has mainstem dams that prevent fish passage to their ancestral coldwater spawning habitat; anadromous fish runs on the Klamath River are now reduced to 5% of their historical maxima, and steelhead are being considered for federal listing under the Endangered Species Act (Nehlsen et al. 1991, NRC 2008, NOAA 2009). Summer water temperatures in the Klamath can reach temperatures as high as 27°C, causing acute and chronic stress in salmonids, and making thermal refuges potentially critical habitat for over-summer survival (Sutton and Soto 2012). These elevated temperatures can lead to mass mortality events, such as the Klamath River 2002 fish kill, where between 30,000-80,000 migrating adult Chinook salmon *(O. tshawytsha)* died when low river flows and warm water temperatures induced physiological stress and exacerbated disease (Levy 2003). This event, precipitated by strong demand by irrigators for water during a drought year, is part of the on-going 'water wars,' heated conflicts pitting human needs for freshwater against ecosystem needs (Ruckelshaus et al. 2002, Poff et al. 2003). Such events are emblematic of conflicting ecological, political, and economic interests surrounding freshwater systems that will become increasingly common with global climate change.

Our objective in this study was to assess the extent to which thermal variability, and specifically diel temperature variation, influences juvenile steelhead use of thermal refuges. These analyses illustrate the potential importance of small-scale spatio-temporal heterogeneity as current watershed alteration and management increase species' risk of temperature-driven extirpation. Given ongoing and forecasted changes in temperature and flow (Mantua et al. 2010, Isaak et al. 2011), we are interested in how current thermal regimes may help us understand the effects of future watershed management and climate change on the role of thermal refuges in sustaining Pacific salmonid populations. The fine temporal scale of observations in our study allows us to gain insight into sub-daily and ontogenetic patterns of refuge use, as well as the time-scale of movements between refuges and adjacent habitats, which is a key step toward determining the specific mechanisms driving refuge use. In this study, we therefore ask: (1) How does spatial and temporal variability in temperature (e.g., diel variation, and temperature differential between the mainstem and tributary), mainstem flow, or the interaction between these variables, correlate with juvenile steelhead refuge use? (2) Are there diel or ontogenetic (body size) patterns to refuge use? And (3) what is the time-scale of movements between thermal habitats? To address these questions, we conducted field studies on juvenile steelhead at a suite of thermal refuges on the Klamath River during summer and early fall, the period of elevated water temperatures. We collected data on the spatio-temporal variation in water temperature and mainstem flow, and used temperature-sensitive radio tags to continuously track juvenile steelhead body temperatures. By correlating simultaneous water and fish temperatures, we were able to determine when individuals moved between thermal habitats. We used a logistic mixed effects model to determine the key environmental drivers of juvenile steelhead thermal refuge use across years.

1.3 Methods

1.3.1 Study system

We conducted this research on the lower Klamath River in northern California between 2010 and 2012 (Fig. 1-1). The Klamath River drains approximately 41,440 km^2 of southern Oregon and northern California (NRC 2008). 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 kilometers of native salmon habitat (Nehlsen et al. 1991). The dam also contributes to elevated water temperatures for the remaining downstream habitat (Bartholow 2005). During the summer and fall, the Klamath reservoir above Iron Gate Dam undergoes seasonal stratification, and the dam releases warm surface water rather than releasing the colder water from below the thermocline (NRC 2008). Juvenile steelhead rear in the river for 1–3 years before out-migrating to the ocean. During summer months the mainstem reaches temperatures that can be thermally inhospitable to salmonids—mean daily mainstem temperatures at the study sites ranged from 14–26°C between 2010 and 2012—and juveniles seek out thermal refuges, usually at tributary confluences (Sutton et al. 2007).

We chose four study sites below Iron Gate Dam, each located at a major cold-water tributary confluence with the Klamath River (Beaver Creek, Grider Creek, Fort

Goff Creek, and Thompson Creek), based on the presence of coolwater refuges during summer and early fall months, and the presence of juvenile steelhead and Chinook. In addition, we chose the sites based on their longitudinal distribution downstream from Iron Gate Dam, to capture spatial variation in the degree of diel mainstem water temperature fluctuation characteristic of regulated rivers (Pike et al. 2013). The number of study sites sampled varied across years (sites 1–4 in 2010; sites 1–3 in 2011; site 1 in 2012) due to access issues (landowner permission) and the number of radio tags available.

Exact definitions of 'thermal refuge' in the literature are inconsistent, including both qualitative (any area cooler than the mainstem river; see Baird and Krueger 2003) and quantitative (at least 2°C cooler than mainstem temperatures; see Torgersen et al. 2012). For the purposes of this study, we defined 'refuge' thermally, since the spatial extent of the thermal mixing zone shifts both daily and seasonally. Given that we were interested in refuge use as a distinct behavioral choice, indicating that a fish had moved into cooler thermal habitat presumably to gain some physiological benefit, we defined a refuge as any area where the water temperature was at least 3°C below mainstem temperature (Appendix A). This includes both the tributary and thermal mixing zone (i.e. where mainstem and tributary waters mix, creating an area of heterogeneous temperature; Fig. 1-2A). Given the uncertainty in fish body temperature estimates (±0.8°C) and possible undetected heterogeneity in mainstem temperatures, we defined 'mainstem habitat' as any temperature within 2°C of the mainstem, and discarded all data that were between 2 and 3°C below mainstem temperatures, since these detections were relatively uncommon and ambiguous for the purposes of the analyses.

1.3.2 Quantifying spatio-temporal patterns in water temperature

We recorded time-series of water temperature at all study sites using Hobo pendant (± 0.5°C) and PRO V2 (±0.2°C) temperature data loggers (Onset Computer Corporation, Pocasset, MA). At each study site, we deployed 2–4 data loggers in both the tributary and mainstem river at point locations approximately 10 m upstream of each confluence. In addition, we placed between 10–20 data loggers, depending on the size of the refuge, throughout the thermally mixed area at each site, to characterize the spatial heterogeneity in water temperature (Fig. 1-2). All data loggers were placed near the riverbed to minimize thermal input from direct sunlight, at depths ranging from 0.5–3 m, and water temperatures were recorded at 15-minute intervals.

We calculated hourly estimates of thermal mixing zone size at each study site by using a standard kriging algorithm in Matlab to interpolate water temperatures at 1 m resolution between all data loggers throughout the refuge. We then summed all locations $\leq 3°C$ below mainstem temperature (excluding the tributary) to estimate the total area of the thermal mixing zone. These calculations were used solely for illustration (Fig. 1-2A) and discussion purposes.

1.3.3 Monitoring fish body temperatures as a proxy for habitat use

We used temperature-sensitive radio tags to track the body temperature of juvenile steelhead at each study site between July–October 2010 (n = 102) and 2011 (n = 130), and July–August 2012 (n = 25). Fish were caught (n ≅ 20–40 per site) within 50 m of the tributary confluence (in either the tributary, thermal mixing zone, or mainstem) using a combination of angling and electro-fishing, and held in the tributary for a maximum of 12 hours before tagging. We measured fish weight and fork length, and surgically implanted the tags (Lotek's MST-720T temperature-sensor transmitter tags; 1.3 g dry weight; ±0.8°C) following a tagging protocol similar to Adams et al. (1998); tag weight never exceeded 4% of fish body weight (Zale et al. 2005). After surgery, fish were held in net-pens within tributaries for recovery for 1 hour prior to release near the area where they were caught. All tagging was done from 6:00–10:00, so as to minimize thermal stress on the fish. We used data-logging receivers (Lotek SRX_400A and SRX 600) connected to shore-based antennae at each study site to continuously log fish body temperatures at 5-second intervals for any fish within approximately 100 m of the tributary confluence. The life expectancy of the radio tags was 42 days.

We used fish body temperatures to detect mortality; when fish temperature exceeded 30°C, we assumed mortality. For fish that stayed at study sites, we performed intermittent snorkel surveys and were able to observe some of the radio tagged fish swimming in the refuge. Temperature records for these fish often showed evidence of behavior (i.e. movement between tributary and mainstem temperatures). In addition, we rafted or drove the length of the river approximately every two weeks scanning for fish that had left study sites; for the majority of fish, we were able to verify either that fish were still alive, or to confirm mortality.

1.3.4 Statistical analyses

We used time-series of tag temperatures as a measure of fish body temperature (hereafter 'fish temperature') to model individual movement between the mainstem river and refuge at each study site. To account for lags in tag temperature acclimation between detections, we sub-sampled detections at 10-minute intervals. This sub-sampling interval was based on results from lab experiments measuring tag acclimation rates, where 100% of tags in fish (n = 27; weight = 61 ± 17 g [mean ± SD]) had acclimated to a new water temperature within 10 minutes (Brewitt, Chapter 3). For each tagged fish, we correlated fish temperature with simultaneous water temperatures and assigned individual fish a *state* (1 = in refuge, 0 = in mainstem) at each time-step. Prior to statistical analyses, collinearity between candidate covariates was assessed using variance inflation factors (VIF); maximum daily mainstem temperature was highly collinear with instantaneous mainstem temperature, and was therefore excluded from the model.

We used an information-theoretic approach to determine the best-fit models for the data, and used logistic mixed effects models with a Bernoulli distribution and a logit link to model the probability of thermal refuge use across years using the *lme4* library in R (R Development Core Team 2012). The response variable was *state* (i.e. individual fish location at time *t*; 1 = in refuge or 0 = in mainstem). Individual fish, study site, and year were included in the model as random effects; this model structure is analogous to a repeated measures design, and explicitly accounts for observations being nested within the individual. To rule out the possibility that temporal autocorrelation could be affecting the model results, we used a resampling approach to test whether the probability of misclassifying two consecutive detections was outside what would be expected by chance (upper 90% confidence interval); our results indicated that temporal autocorrelation was not a concern for the models (Appendix C).

The full model included six fixed effects, which included three independent measures of temperature variation: mainstem river temperature *(T; °C),* temperature differential (*D:* mainstem temperature – tributary temperature)*,* diel mainstem temperature variation *(V),* mainstem flow (*F:* daily discharge data from Iron Gate Dam and Seiad Valley monitoring stations)*,* fish fork length *(FL; mm)*, and time of day (*L:* day = 1 or night = 0). Daylight was assigned using monthly sunrise and sunset times for the Klamath River during the study period.

In addition, we considered seven interaction terms. The first was an interaction between mainstem temperature and time of day *(TxL),* to test the hypothesis that the relationship between mainstem temperature and refuge use changes between day and night. We also included interactions between mainstem temperature and all other environmental covariates, mainstem flow *(TxF),* temperature differential *(TxD)*, and diel mainstem variation *(TxV)*, in which a positive interaction would indicate that higher mainstem flows, larger temperature differential, or larger mainstem temperature fluctuations increases the likelihood of fish using refuges at higher temperatures. In addition, we included an interaction between diel mainstem variation and flow *(VxF)*, to test the hypothesis that higher flows could potentially ameliorate the expected negative effect of mainstem variation. Finally, we included two interactions with fork length; an interaction between fork length and mainstem temperature *(FLxT)*, and between fork length and time of day *(FLxL)*, to test the hypotheses that fish size mediates refuge use in response to river temperature or time of day, respectively. For these models, we included only data collected throughout July–September each year, when mean daily mainstem temperatures exceeded 17°C, and included only individuals with at least 50 observations (n = 127; Appendix B: Table 5-1). Due to the large size of the dataset, we used data subsampled at 20-minute intervals. All variables were Z-score standardized to mean values.

We were concerned that the temperature differential parameter *(D)* could be linked to the dependent variable, since there was a positive correlation between temperature differential and the range of temperatures defined as 'refuge' habitat. To thoroughly investigate whether this parameter could represent opportunity rather than habitat preference in the model, we generated a new 'random state' operating model using the same dataset but with the response variable generated randomly from a uniform distribution, with the probability of assignment to a *refuge* or *mainstem state* weighted by the thermal range (i.e. temperature differential) available.

We used Akaike information criterion (AIC) to rank all candidate models, and used the AIC weights of each model to select the top 95% confidence set (Burnham and Anderson 2002). The goal of AIC model selection is to avoid over-parameterization by including only explanatory variables in the model. We constructed receiver operating characteristic (ROC) curves and used the area under the curve (AUC) test statistic to assess overall model fit. AUC is a commonly used test statistic for assessing model fit for logistic models, with scores ranging from 0.5, indicating model predictions are no better than random, to 1, indicating a model with perfect predictive ability (Hosmer and Lemeshow 2000). Finally, we used K-fold (K = 5) cross validation to assess how well the model performs for un-sampled groups of individuals (Hastie et al. 2009, Huff et al. 2012).

When fish did leave refuges for mainstem habitat, we calculated the duration of these events, defined as a movement from the refuge to the mainstem and back to the refuge. We note that due to the temporal integration of the fish temperature tags, this metric would not detect very short movement events (e.g., <10 minutes).

1.4 Results

1.4.1 Spatio-temporal heterogeneity in water temperature and flow

Tributary inputs created a large spatial gradient in water temperature, forming substantial thermal refuges at all study sites (Fig. 1-2A). The area of the thermal mixing zone, calculated by interpolating water temperatures between temperature loggers at each site, fluctuated in both space and time (interannual range in refuge mixing zone areas across sites = 0-4352 m^2; median = 454 m^2; see Appendix E, Fig. 8-1 for variation in area by study site). Daily fluctuations in both mainstem and tributary temperatures caused the magnitude of the temperature differential to vary across both space and time (mean temperature differential = 6.48 ± 0.59°C) (Fig. 1-2B; Appendix D: Table 7-1). In addition, daily mean and maximum mainstem temperatures varied longitudinally along the river, and diel mainstem fluctuations varied across sites and years (mean diel variation = 2.0 ± 0.54°C) (Fig. 1-2C; Appendix D: Table 7-1). The number of days when mainstem temperatures exceeded 22°C varied across years (interannual mean = 105 d). Mainstem flow also varied substantially across years (interannual range: 23.1–66.0 cms; Appendix D: Table 7-1), as well as between study sites, as tributary inputs increased mean flows substantially between upstream and downstream sites.

1.4.2 Juvenile steelhead use of thermal refuges

Fish temperatures indicate that juvenile steelhead used thermal refuges consistently across mainstem temperatures, and took advantage of the full range of the temperature differential created by the incoming tributaries (Fig. 1-3A). In addition, individuals used the mainstem thermal habitat across nearly the full range of mainstem temperatures, with 58% of individuals moving into the mainstem at least once when temperatures exceeded 24°C; all fish moved into refuges by approximately 25°C. The mean percentage of time that individuals spent in refuges varied across both sites and years (Appendix D: Table 7-2); fish exhibited enormous variation in behavior, with some individuals spending 100% of the time they were detected in either a refuge or the mainstem (36.2% and 5.5%, respectively, of individuals across years), while others moved between thermal habitats (58.2% of individuals across years). In addition, mean fish temperature across the time detected varied enormously by individual, suggesting variation in thermal tolerance and thermal habitat choice (Fig. 1-3B). There was no obvious relationship between body size and mean fish temperature ($P > 0.05$; linear regression). Mean percent mortality of tagged fish across years was 9.3% (Appendix D: Table 7-3).

Mainstem temperature, time of day, and their interactions with body size were the most important predictors of thermal refuge use. The top 95% confidence set included only the full model, and all terms except fork length were significant to 0.001 (Fig. 1-4; Appendix D: Table 7-4). Fish of all sizes (FL = 183 ± 50 mm) behaved similarly during the day, with the probability of refuge use increasing with rising mainstem temperatures (Figs. 1-5A,B; mean expected temperature at which >80% of fish entered refuges was 22.3°C). However, smaller fish (approximate first quartile FL = 160 mm) used refuges at night regardless of mainstem temperature, whereas larger fish (approximate third quartile FL = 210 mm) still used mainstem habitat at night, although less often than during the day, unless the mainstem river reached high temperatures (Fig. 1-5B). Although we treated body size as a continuous variable, for the purposes of discussion we chose size categories based on approximate first and third quartile of observed fork lengths, as these values fall near measured 2+ and 3+ age categories for Klamath juvenile steelhead (Hodge 2010).

Thermal variability, measured as diel mainstem variation and temperature differential, had a strong positive effect on the probability of refuge use. Larger diel variation increased the probability of refuge use, and the effect of diel variation was even greater at higher mainstem temperatures, as indicated by the positive interaction term (Figs. 1-4 & 1-5C,D). The mean expected mainstem temperature at which >80% of fish entered a refuge was 24.0°C at 1°C diel variation, but only 20.8°C at 4°C diel variation. The standardized coefficient for temperature differential was greater in our true model (mean = 1.152 ± 0.025) than the random state operating model (mean = 0.416 ± 0.008), indicating that this parameter is a good predictor of refuge use, beyond what would be predicted based on opportunity alone. However, the interaction term between temperature differential and mainstem temperature had a larger magnitude in the random state operating model than the true model (mean = -0.105 ± 0.008 and -0.073 ± 0.017, respectively), suggesting that this term changes primarily as a function of opportunity.

Higher mainstem flows decreased the likelihood of refuge use; the mean expected mainstem temperature at which >80% of fish entered a refuge was 21.7°C at very low mainstem flows (approximate first quartile flow = 29.7 cms) and 23.5°C at higher flows (approximate third quartile flow = 38.2 cms). There was also a negative interaction between diel variation and mainstem flow (Figs. 1-4 & 1-5C,D), suggesting that at low flows the effect of diel variation on refuge use was more pronounced.

Model evaluation results indicate that the logistic regression model predicts thermal refuge use satisfactorily (AUC = 0.67) (Hosmer and Lemeshow 2000). In addition, the small standard deviation in AUC values among replicates from the K-fold cross-validation (0.005) indicates that the model performs well for untested groups of data. Finally, the proportion of explained variance attributable to individual fish, site, and year respectively, indicates a much higher degree of variation in probability of refuge use between individual fish than between study sites or years (58.2%, 22.6%, and 19.2%, respectively).

When fish did leave refuges for mainstem habitat, the duration of movement events into the mainstem river indicates that most habitat shifts were relatively short for all individuals (median = 2.3 h) (Appendix D: Fig. 7-1). Additionally, for fish that exhibited this behavior, there was a high degree of variation in the total number of habitat shifts per individual (mean = 18 ± 29). Analyses of mean fish temperatures indicate that individuals may exhibit size-dependent preference for certain areas within a refuge. In addition, fish caught for radio tagging in the thermal mixing zone tended to be larger than those caught from within the tributary (mean = 205 ± 58.0 mm and 168 ±

33.5 mm, respectively), and maintained higher mean body temperatures across the time detected (mean = 18.3 ± 2.3°C and 16.5 ± 2.0°C, respectively).

1.5 Discussion

We found that juvenile steelhead use of thermal refuges is highly dynamic, with fish moving in and out on a near daily basis. Moreover, this dynamism is linked to fish traits (e.g., body size) and key environmental drivers, such as thermal variability, in *non-*refuge habitat. An important implication of these findings is that the existence of thermal refuges appears to allow steelhead to more effectively exploit non-refuge habitat, even as temperatures reach or exceed incipient lethal temperatures. We base these assertions on our model results, which suggest that instantaneous mainstem river temperature and sub-daily temperature variation are strong environmental drivers of juvenile steelhead use of thermal refuges. In particular, mainstem temperature was the strongest predictor of juvenile steelhead refuge use, with >80% of fish entering refuges when the mainstem reached 22°C. These results are consistent with observational studies on salmonid use of thermal refuges in both the Klamath and other systems, which found that juvenile steelhead and coho salmon moved into refuges when mainstem temperatures reached 22-23°C (Nielsen et al. 1994, Sutton et al. 2007). However, fish body temperatures indicate that some individuals were still utilizing mainstem habitat up to approximately 25°C, the temperature at which bioenergetics predictions for steelhead specific growth rate drops towards zero (Hanson et al. 1997, Beauchamp 2009). This indicates that there may be trade-offs to using refuges, and that fish may move into the mainstem to gain some other benefit, such as to forage. Studies on juvenile steelhead thermal refuge use in other systems have observed similar behavior, with fish moving into adjacent mainstem habitat even when temperatures reached 25°C (Ebersole et al. 2001, Baird and Krueger 2003).

Mainstem diel temperature variation was a strong predictor of juvenile steelhead use of thermal refuges, suggesting that the mainstem river becomes less hospitable when the magnitude of diel temperature variation increases. Fluctuating temperatures can negatively affect fish specific growth rate and mortality relative to constant thermal regimes when maximum temperatures approach incipient lethal temperatures (Hokanson et al. 1977, Meeuwig et al. 2004, Geist et al. 2010). This may be due to increases in the standard metabolic rate of fish in fluctuating versus constant temperature regimes (Beauregard 2013). Similarly, the positive interaction term in our model between mainstem temperature and diel mainstem variation indicates that the role of fluctuating temperatures is more pronounced at higher temperatures, and is likely due to the fact that the mainstem is reaching higher daily maxima, often near or exceeding upper incipient lethal temperatures for *O. mykiss* (25.6°C; Hokanson et al. 1977). While there are a number of lab studies emphasizing the importance of diel temperature variation, few studies have shown the potential importance of diel variation in influencing behavior and habitat use in the field (but see Wehrly et al. 2007 and Mather et al. 2008).

Higher mainstem flows decreased the likelihood of refuge use. This could be attributed to two possible mechanisms. One explanation is that higher flows may reduce refuge (i.e. thermal mixing zone) size. Previous Klamath River thermal refuge studies have found that higher mainstem flows can decrease refuge size (Deas et al. 2006), and thermal mixing zone sizes calculated from our temperature logger data indicate that refuge size is considerably larger at very low flows (<28 cms) (Appendix E). A second explanation is that higher flows may create more favorable mainstem habitat by increasing mainstem velocity and depth, as well as prey delivery rates, which could potentially improve mainstem habitat suitability for juvenile steelhead (Beecher et al. 1993, Hayes et al. 2007). However, this would depend on the actual flow rates and the effect on net energy intake (Urabe et al. 2010). While our data lack sufficient detail to support one of these mechanisms over the other, the negative interaction between flow and both mainstem temperature and diel mainstem variation does suggest that at higher flows, the relative impact of mainstem flow on habitat quality may increase. Future studies on the relative contributions of thermal and hydrologic variability to refuge quality should investigate whether increased flows ameliorate some of the negative effects of increased temperatures and diel variation, or if the effects of flow are linked solely to refuge area.

One particularly interesting result of our study was the distinct diel shift in refuge use and its relationship to body size; at night, smaller fish (FL ≅ 134–180 mm) utilized refuges almost exclusively, whereas larger fish (FL ≅ 190–385 mm) exhibited a similar but much less pronounced behavioral shift (Fig. 1-5A,B). The diel behavioral shift in smaller fish suggests that steelhead may be resource-limited in refuges due to density-dependent competition (Armstrong and Griffiths 2001, Sutton et al. 2007), and may move into mainstem habitat during the day to forage, but seek thermal refuge at night when the metabolic cost of remaining in warmer water rises due to limited foraging success. Fish are primarily visual foragers, and the benefits of leaving refuges to forage are likely much greater during daytime. In contrast, larger juveniles may be able to take advantage of the potential growth benefits of warmer water even at night due to their ability to maintain optimal feeding positions (Abbott and Dill 1989), and move into refuges only when temperatures near incipient lethal levels. We observed aggregations of larger juveniles holding along the margin of the refuge and mainstem, which created a fast-moving riffle area at most sites, preferred foraging habitat for steelhead. Larger juveniles maintained higher mean body temperatures than smaller juveniles, further supporting the hypothesis that larger juveniles are able to take advantage of margin habitat on the edge of refuges. Moreover, these temperatures are consistent with where fish were caught within the refuge for tagging, suggesting that within-refuge habitat preference is linked to body size. While differential predation pressure could also create the observed pattern, juvenile steelhead predators in the Klamath are visual predators (mainly birds and river otters) that prey differentially on larger juveniles, and would therefore be more likely to cause behavioral differences between different size fish during the day than night (Collis et al. 2001). Diel vertical migration as a strategy balancing foraging needs with predation pressure is a well-established ecological phenomenon (e.g., Scheuerell and Schindler 2003), but there is also evidence that animals exhibit diel migration to maximize net energy intake (Wurtsbaugh and Neverman 1988, Sims et al. 2006). Armstrong et al. (2013) found that juvenile coho salmon increased their growth potential by taking advantage of thermal and trophic resource heterogeneity in a small Alaskan stream, obtaining food in coldwater areas at night and moving to warmer habitat during the day to digest. While Klamath River thermal refuges are important habitat for juvenile steelhead both day and night, they may be especially important at night as a thermal respite when foraging opportunities are limited.

The duration of habitat shifts between refuges and the adjacent mainstem river gives insight into the extent to which thermal refuges meet the ecological requirements of steelhead, and the potential trade-off between thermal and other resources. Shifts between refuges and the mainstem river were of relatively short duration (median = 2.3 h), further suggesting that fish may be leaving to forage rather than escape habitat-associated predation pressure. The resolution of the radio tag data did not allow for detection of thermal habitat shifts shorter than ten minutes due to fish temperature acclimation rates (Chapter 3). Juvenile steelhead could therefore potentially dart into the mainstem for a short period and still maintain a cooler internal core body temperature, thereby avoiding the negative physiological effects of hotter temperatures. Both the diel pattern of refuge use and the short duration of habitat shifts suggest that maintaining connectivity between the mainstem and tributaries (a function of both higher mean flows and the physical habitat at the confluence) may be an important aspect of refuge habitat quality, as connectivity facilitates easy movement between the two habitats.

The high degree of variability in thermal refuge use between individuals (58.2% of explained variance), the large range in mean fish temperatures across individuals, and the size-dependent behavioral variation, points to a large diversity in the nature of individual interactions with the environment surrounding refuges, and suggests that there may be variation in individual thermal tolerances (Fig. 1-3B). Importantly, if there are genetic underpinnings to individual thermal tolerance, this could give insight into how populations adapt to localized changes in temperature regimes. Future studies on thermal refuges should focus on understanding what mechanisms are driving this individual variation, especially vis-à-vis relative resource availability between habitats and density-dependent effects, as well as the population level consequences of this variability. In addition, size-dependent diel behavioral shifts indicate the need to assess thermal refuge use at fine spatial and temporal scales; we propose that using temperature-sensitive tags to infer an animal's location may be an effective and innovative method to track habitat use in thermally heterogeneous environments such as thermal refuges, especially as these types of tags become smaller and more affordable (Cooke et al. 2013).

The significant effect of both measures of sub-daily temperature variation in our model support two inferences regarding management under current watershed management regimes and future climate change. First, the interaction between diel mainstem variation, mainstem temperature, and flow could enable managers to predict which tributaries may create more effective thermal refuges (i.e. higher likelihood of use), given their longitudinal location and the predictive nature of diel mainstem variation on regulated rivers (Pike et al. 2013); these inferences could inform targeted flow management and habitat restoration efforts at tributary confluences. Second, the positive relationship between temperature differential and refuge use, which could be attributed to fish gaining greater relative physiological benefit from refuges created by cooler tributaries, emphasizes the importance of maintaining good riparian habitat along stream corridors. These considerations are particularly relevant on the Klamath River, given the planned removal of the four lowest mainstem dams in 2020, which would open up over

550 km of upstream habitat for anadromous salmonids, and is projected to decrease mainstem temperatures by approximately 2–4°C in late summer and early fall months (Goodman et al. 2011, Perry et al. 2011). Klamath River mainstem temperature increased at approximately 0.5°C per decade between 1962 and 2001 (Bartholow 2005), and additional future warming will likely cause the number of days when temperatures exceed 25°C to increase. Successfully maintaining thermal refuges now may enhance survival of threatened salmonid populations until the dams are removed.

Habitat degradation and watershed alteration have led to large-scale habitat loss and elevated water temperatures. As rising temperatures across ecosystems exacerbate the effects of an already altered landscape, thermal refuges will form increasingly critical habitat. The positive relationship between diel temperature fluctuation and refuge use indicates that it is important to take sub-daily thermal variation into account when assessing habitat requirements for species nearing the limits of their thermal tolerance, as temperature variation could make the effects of increased mean temperatures even more severe than expected. Moreover, thermal refuges may allow mobile consumers to more effectively exploit adjacent (and likely more abundant) non-refuge habitat by providing temporary thermal respite; this could be a critical and currently under-valued benefit of maintaining refuges. Our study underscores the importance of taking into consideration fine-scale spatio-temporal heterogeneity in future studies of thermal refuges in other ecosystems, as species' ranges shift and contract in the face of climate change.

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1.7 Figures

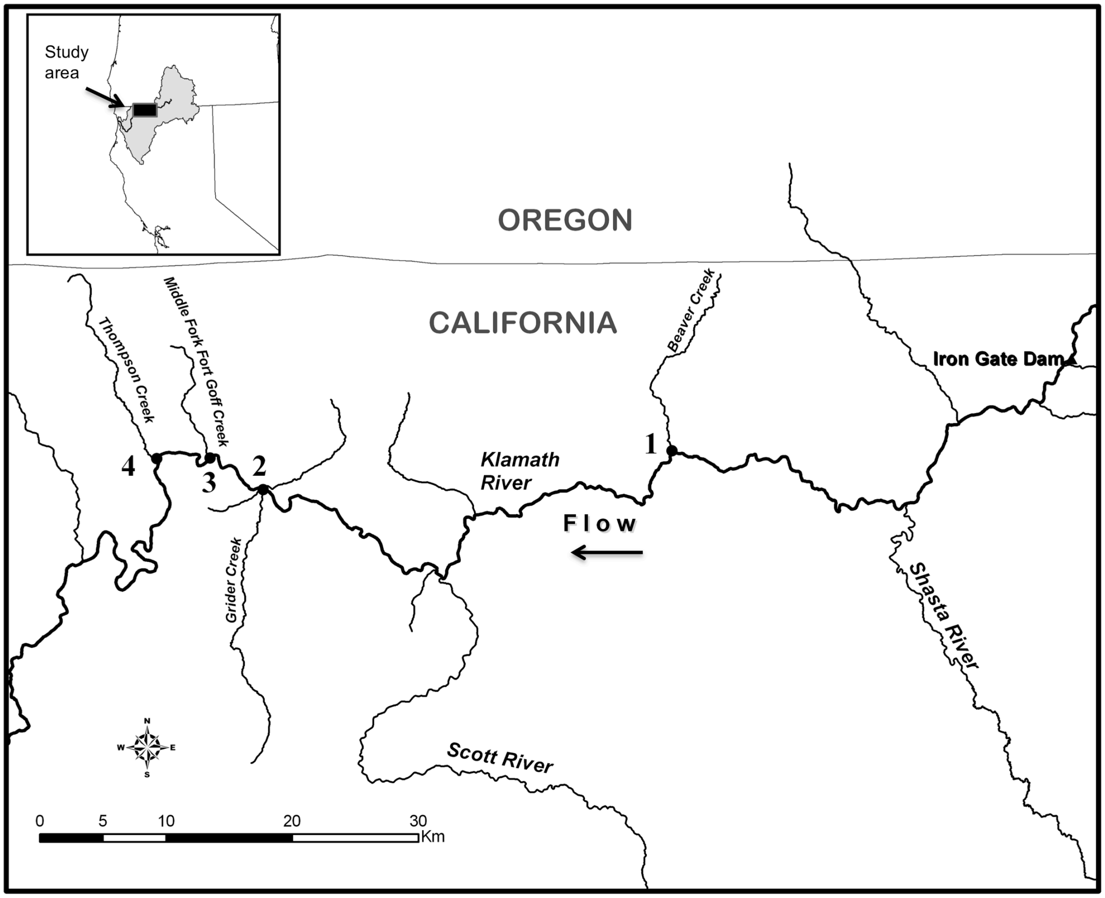

Figure 1-1. Location of study sites along the Klamath River (1 = Beaver Creek; 2 = Grider Creek; 3 = Fort Goff Creek; 4 = Thompson Creek). Inset shows location of study area within the watershed.

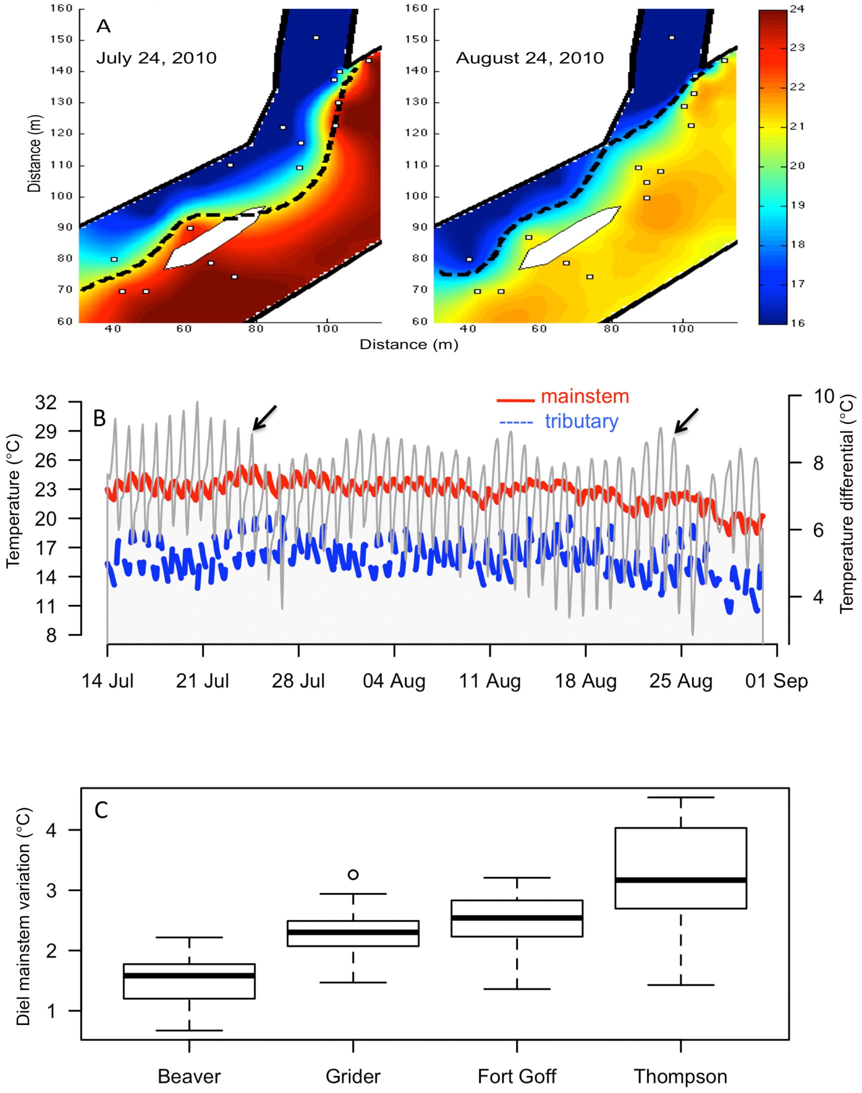

Figure 1-2. Spatio-temporal heterogeneity in water temperatures. (A) Interpolated water temperatures (ºC) at 9:00 on 24 July 2010 and 24 August 2010 at Beaver Creek (dates indicated by black arrows in (B)); white denotes land, and white squares are locations of water temperature loggers. Distance (m) on axes is measured from the lower southwestern corner of the refuge. The thermal 'mixing zone' is defined as the area where water temperatures are between mainstem and tributary temperatures; the 'refuge', defined as any area at least 3°C below mainstem temperature, is indicated by the dashed black line. (B) Tributary (dashed blue) and mainstem (solid red) water temperatures at Beaver Creek in 2010; shaded gray area shows variability in temperature differential. (C) Diel mainstem variation across study sites (upstream to downstream) in 2010.

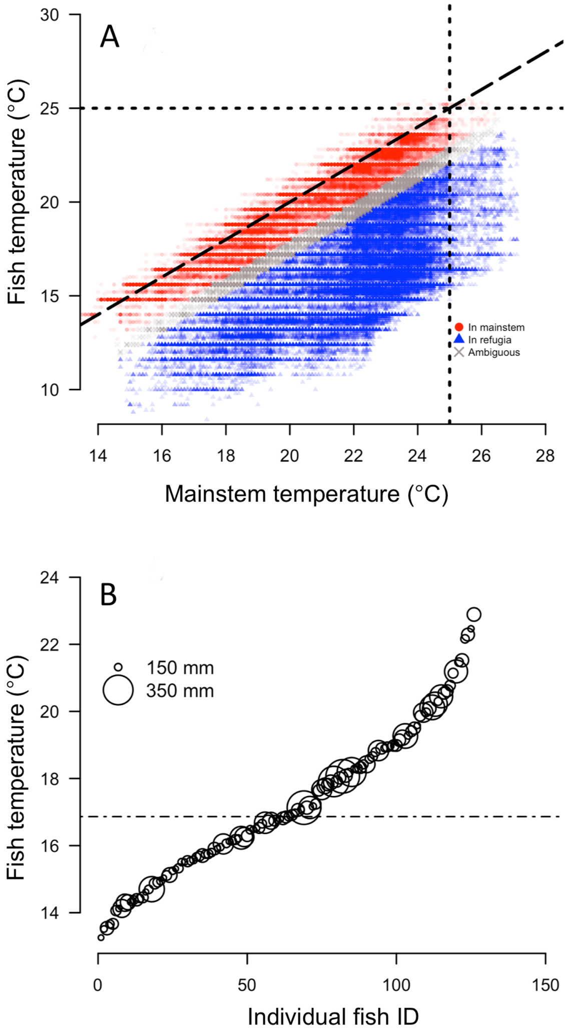

Figure 1-3. (A) Fish temperatures versus simultaneous mainstem temperatures across sites and years (total detections = 130,272). Symbols correspond to fish detections assigned to either a mainstem *state* (red circles), refuge *state* (blue triangles), or detections discarded due to ambiguity of *state* assignment (gray crosses). The dashed line is the line of equality between mainstem and fish temperature, and dotted horizontal and vertical lines at 25°C indicate temperatures at approximate bioenergetic limits for steelhead (i.e. metabolism exceeds specific consumption rate). Fish temperature detections greater than the mainstem temperature (red circles above the line of equality) are attributed to tag measurement uncertainty and undetected heterogeneity in mainstem temperatures. (B) Mean fish temperature (across time detected) per individual; circle diameter is scaled by fork length (range 134–385 mm). The dashed horizontal line denotes median body temperature (16.8°C).

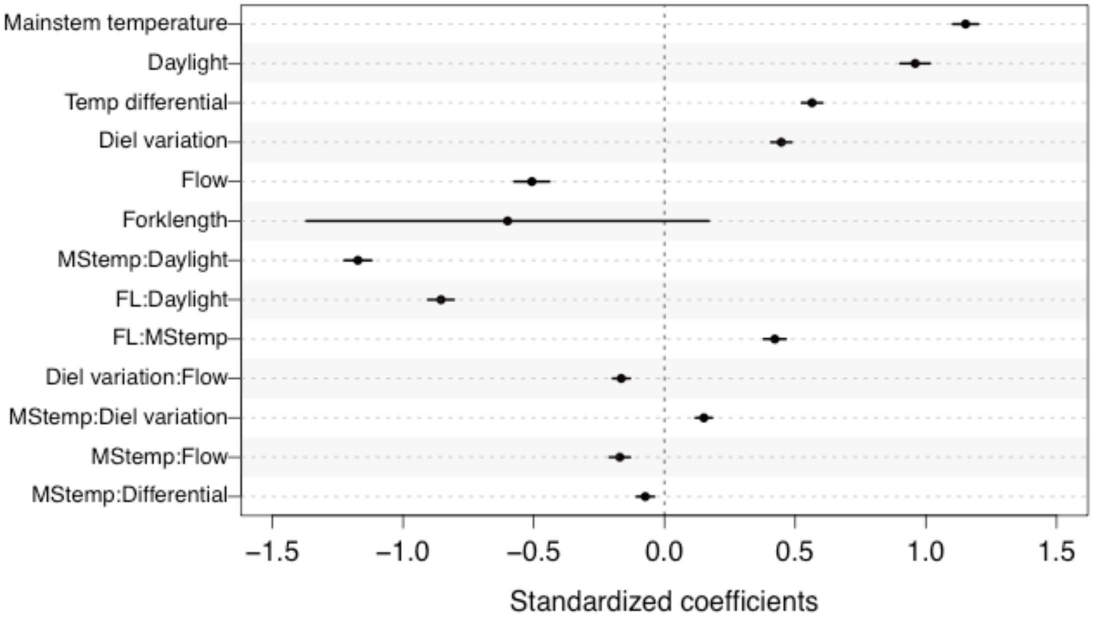

Figure 1-4. Parameter estimates for the top logistic model of the probability of an individual fish occupying refuge habitat as determined by AIC values. Error bars indicate 95% confidence intervals in parameter estimates.

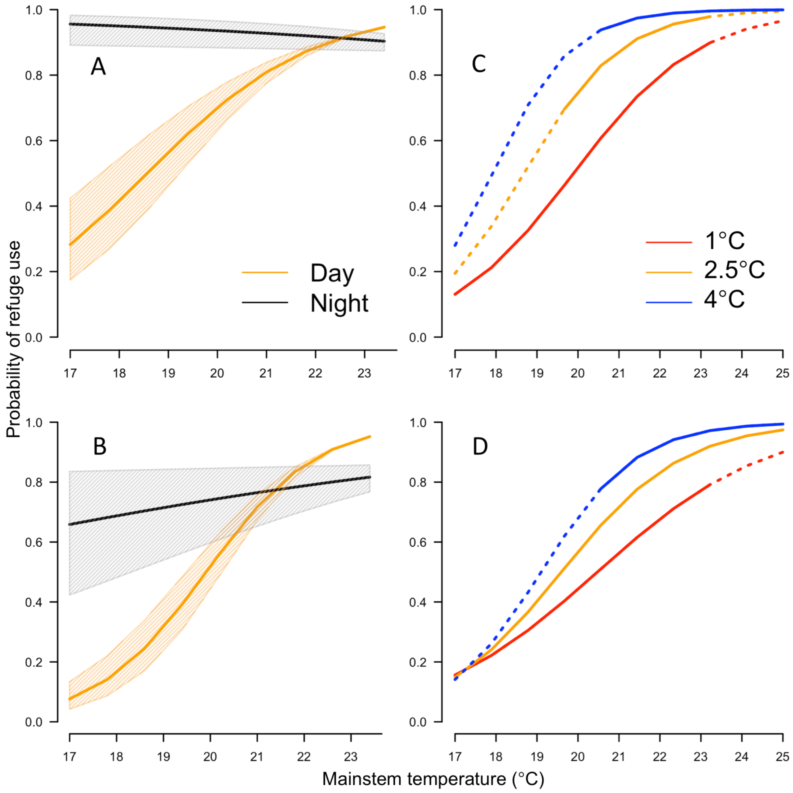

Figure 1-5. Estimated probability of juvenile steelhead use of thermal refuges as a function of mainstem temperature, and (A & B) day (orange line) and night (black line), for (A) small versus (B) large fish. Shading indicates range of ±20 mm around mean fork length for small (mean = 160 mm) and large (mean = 210 mm) juveniles. (C & D) Probability of daytime thermal refuge use at varying degrees (1°, 2.5°, 4°C) of diel mainstem variation at (C) low (29.7 cms) and (D) high (38.2 cms) mainstem flow. Dashed lines represent extrapolation beyond the range of data.

2. Chapter 2: Juvenile Pacific salmonids exploit heterogeneity in the temperature and prey landscape at thermal refuges

2.1 Abstract

Thermal refuges form increasingly important habitat in the face of rising ambient temperatures caused by large-scale habitat alteration and climate change. Habitat heterogeneity, such as that offered by thermal refuges, gives mobile consumers a choice of disparate habitats, allowing them to balance trade-offs in critical resources. Understanding how individuals negotiate heterogeneity in the thermal and prey landscape at thermal refuges may help shed light on potential costs associated with refuge use. We sought to quantify how juvenile Pacific salmonids (steelhead, *Oncorhynchus mykiss* and Chinook salmon, *O. tshawytscha*) cope with spatial heterogeneity in temperature and food abundance at tributary confluences forming thermal refuges. First, we quantified the spatial variability in temperature and trophic resources of thermal refuges with temperature loggers and invertebrate drift sampling. Second, we used temperature-sensitive radio tags and fish density surveys to quantify thermal habitat use, and stable isotopes to quantify trophic resource use. Juvenile salmonids using thermal refuges obtained the majority of their diet from mainstem prey sources. Fish thermal habitat use was significantly cooler than diet-inferred fish foraging temperatures, indicating that while individuals seek cooler habitat for physiological benefits, they still rely primarily on mainstem prey resources. We found no consistent difference in invertebrate drift delivery rates between the mainstem and tributaries. However,

consistently high densities of fish in refuges could lead to density-dependent food limitation. Our results suggest that the existence of thermal refuges allows juvenile salmonids to exploit the larger mainstem river prey base without experiencing the negative physiological cost of holding in warmer water. Furthermore, this study highlights the importance of habitat connectivity between coolwater tributaries and the mainstem river, as access to thermally sub-optimal mainstem habitat could provide an outside food source that may increase the carrying capacity of space-limited refuges.

2.2 Introduction

Thermal refuges, habitat that provides physiological refuge from stressful temperatures, form increasingly important habitat as climate change and large-scale habitat alteration continue to cause increases in air and water temperatures (Keppel et al. 2012, Davis et al. 2013). Thermal refuges allow organisms to escape the effects of sub-lethal and lethal temperatures, and have been linked to lower physiological stress levels and higher survival rates (Huey et al. 1989, Mathes et al. 2010). Given on-going climatic warming, thermal refuges have the potential to enable population persistence in otherwise thermally uninhabitable ecosystems (Loarie et al. 2008, McLaughlin and Zavaleta 2012), yet most climate envelope models largely ignore fine-scale thermal refuges (Pearson and Dawson 2003, Lawler et al. 2006, Willis and Bhagwat 2009). While thermal refuges provide small habitats with suitable temperatures, there is less understanding of the other resources that contribute to the suitability and productivity of these habitats, such as food. In fact, spatio-temporal heterogeneity in resources (e.g., prey availability, predation risk) could determine the relative costs and benefits associated with refuge use.

While refuges allow individuals to escape stressful environments (e.g., temperature, predation risk), there may be associated trade-offs. For example, predation refuges (i.e. burrows) for yellow-bellied marmots *(Marmota flaviventris)* were associated with decreased food availability (Blumstein and Pelletier 2005), and adult steelhead *(O. mykiss)* using coolwater refuges experienced increased harvest mortality (Keefer et al. 2009). 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 (Bridges 2002, Hohausova et al. 2003). Diel vertical migration in aquatic ecosystems is a well-known example of this phenomenon (Scheuerell and Schindler 2003, Sims et al. 2006, Loose and Dawidowicz 2014); juvenile sockeye salmon *(Oncorhynchus nerka)* in Alaskan lakes migrate vertically to maintain a constant light environment, thereby balancing foraging opportunities with predation risk (Scheuerell and Schindler 2003). Fish also exhibit diel horizontal migration to maximize access to food availability and digestive capacity across heterogeneous thermal landscapes (Wurtsbaugh and Neverman 1988, Bevelhimer and Adams 1993, Armstrong et al. 2013). Given the combination of widespread habitat homogenization and climatically driven habitat loss (Vitousek 1997, Thomas et al. 2006), it is increasingly imperative to understand the importance of heterogeneity in the resource landscape surrounding thermal refuges, yet these types of studies are currently lacking from the literature.

Understanding the balance between thermal challenges and trophic resources in thermal refuges is particularly important for Pacific salmonids (*Oncorhynchus* spp.), as river temperatures are increasing due to the effects of dams, channelization, irrigation, and urbanization (Poff et al. 2007, Isaak et al. 2011, Ruesch et al. 2012). Pacific salmonids are cold-water fish, and water temperature has a strong non-linear effect on salmonid metabolic and consumption rates (Jobling 1994, Myrick and Cech 2005). Salmonid growth potential is therefore strongly mediated by water temperature, and 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). In the context of stream ecosystems and cold-water fishes, "thermal refuges" refer to any cold-water habitat that allows fish to escape the sub-lethal and lethal effects of hot temperatures (Torgersen et al. 2012). These areas are often formed by incoming tributaries that create thermally heterogeneous mixing zones at their confluences. While fish gain thermal benefits from coolwater refuges, these areas may be sub-optimal in terms of competition for prey resources and disease risk due to crowding (Coutant 1987, Sutton et al. 2007, Ray et al. 2012). Studies on fish using thermal refuges in warm rivers have recorded cyclic movements out of refuges into mainstem habitat even at thermally stressful temperatures, presumably to gain some non-thermal benefit (Kaeding 1996, Ebersole et al. 2001, Belchik 2003). In this study, we examine how fish cope with spatial heterogeneity in the thermal and prey landscape surrounding thermal refuges.

Prior studies on the ecology of fish in lotic environments suggest that competition for prey resources in thermal refuges may vary as a result of both fish density and naturally heterogeneous in-stream prey availability (Shearer et al. 2002, Rosenfeld et al. 2005, Hayes et al. 2007). Within-refuge fish abundance increases with rising mainstem temperatures, often reaching high levels during hot summer months

(Sutton et al. 2007, Mather et al. 2008, Brewitt and Danner 2014). In addition, invertebrate prey densities may be higher in larger mainstem systems than in small incoming tributaries that create refuges (Leeseberg and Keeley 2014). If prey are more abundant in areas where temperatures are sub-optimal (e.g., mainstem river), fish may move to forage in warmer water but return to cooler water to digest, thereby decreasing metabolic costs (Bevelhimer and Adams 1993, Sims et al. 2006). However, these decisions may be mediated by both density-dependent interactions (Keeley 2001, Armstrong and Griffiths 2001, Imre et al. 2004) and habitat quality (Dhondt et al. 1992, Pärn et al. 2011, Huntsman and Petty 2014). We hypothesize that there may be a mismatch between the thermal and prey landscapes surrounding thermal refuges, and that fish may be leaving refuges to forage but returning to cooler water to hold.

Our goal was to quantify how juvenile Pacific salmonids (steelhead, *O. mykiss* and Chinook salmon, *O. tshawytscha*) cope with spatial heterogeneity in temperature and food abundance at tributary confluences forming thermal refuges. In the Klamath River in northern California, steelhead and Chinook salmon populations are declining, and juvenile salmonids increasingly rely on refuges for suitable thermal habitat during hot summer months (Nehlsen et al. 1991, Brewitt and Danner 2014). As more fish rely on these areas for over-summer survival, it will be important to understand possible costs to refuge use, including density-dependent food limitation. We therefore sought to quantify the spatial heterogeneity in temperature and trophic resources of thermal refuges, and then quantify thermal habitat use and trophic resource use (i.e. diet) of juvenile salmonids using refuges. We asked, 1) Are there differences in invertebrate drift delivery rates between the tributaries and larger mainstem system? 2) Are fish densities within refuges higher than in the adjacent mainstem river? 3) What proportion of juvenile steelhead and Chinook salmon diet derives from the mainstem versus tributary, and is there any seasonal, size-dependent, or species effect on fish prey source? And 4) Does fish thermal habitat use match fish diet (i.e. the water temperature where individuals are foraging)? We found that fish densities were consistently high in thermal refuges during summer months, and juvenile steelhead body temperatures indicated that fish tended to hold in the refuges. However, both juvenile steelhead and Chinook salmon diet consisted of mostly mainstem prey, indicating that while individuals seek cooler habitat for physiological benefits, they still rely primarily on mainstem prey resources.

2.3 Methods

We used a multi-faceted approach to assess the thermal and prey landscapes surrounding thermal refuges. First, we quantified the spatial variability in temperature and food availability of thermal refuges with temperature loggers and invertebrate drift sampling. Second, we used fish density surveys and temperature-sensitive radio tags to quantify thermal habitat use, and stable isotopes to quantify trophic resource use.

2.3.1 Study system

We conducted this research on the lower Klamath River in northern California in July-September 2010-2011, and July-August 2012 (Fig. 2-1). The Klamath River is 423 km long, and drains approximately 41,440 km^2 of southern Oregon and northern California (National Research Council 2008). 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 kilometers of native salmon habitat (Nehlsen et al. 1991). The dam, along with large-scale watershed alteration (e.g., irrigation), contributes to elevated water temperatures in the downstream habitat (Bartholow 2005, National Research Council 2008). During the summer and fall, the Iron Gate Reservoir undergoes seasonal stratification, and the dam releases warm surface water rather than colder water from below the thermocline (National Research Council 2004).

The Klamath River supports runs of coho salmon *(O. kisutch),* Chinook salmon *(O. tshawytscha)*, chum salmon *(O. keta)*, and steelhead *(O. mykiss).* Chinook salmon are the most abundant anadromous fish in the basin, with both fall and spring-runs, but populations are declining and are far below historic levels. Both summer and winter steelhead runs are declining and being considered for federal listing under the Endangered Species Act, and coho salmon were listed as threatened 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. Juvenile salmonid growth potential exhibits an asymmetric relationship with water temperature, with both steelhead and Chinook salmon growth potential peaking at approximately 19°C at maximum daily ration (Brett et al. 1982, Myrick and Cech 2000, Heady 2012). However, feeding rates are usually below satiation levels in the field, and actual optimal growth temperatures are therefore likely to be lower than lab experiments suggest (Richter and Kolmes 2005). During summer months the Klamath mainstem river reaches temperatures that can be thermally inhospitable to salmonids—mean daily mainstem temperatures at study sites ranged from 15-25°C between July-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 chose four study sites on the river below Iron Gate Dam, each located at a major cold-water tributary confluence (Beaver Creek, Grider Creek, Fort Goff Creek, and Thompson Creek), based on the presence of coolwater refuges during summer and early fall months, and the presence of juvenile steelhead and Chinook salmon. Hereafter, we refer to 'refuges' as the coolwater 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; see Fig. 1-2A).

2.3.2 Quantifying mainstem and tributary water temperatures

We recorded water temperatures at 15-minute intervals at all study sites from July-September 2010-2011, and at Beaver and Grider Creek study sites (where isotope sampling was performed) throughout July-August 2012. We placed 2-4 Hobo pendant data loggers (Onset Computer Corporation, Pocasset, MA; ±0.5°C) in both the tributary and mainstem river at point locations approximately 10 m upstream of each confluence. All data loggers were placed near the riverbed to minimize thermal input from direct sunlight, at depths ranging from 0.5–2 m.

2.3.3 Mainstem versus tributary drift densities

To assess invertebrate drift delivery rates as a possible mechanism of food limitation in refuges, we collected drift samples from the mainstem and tributary approximately 15 m upstream of the confluence, and upstream of where high densities of fish were observed in the tributaries (see Fig. 2-2A). We collected monthly samples

(June-August) at all four study sites in 2010, and weekly samples (July-August) at Beaver Creek (furthest upstream) in 2011. We suspended mesh drift nets (23x31 cm, 500 μm) approximately 5 cm above the substrate for 5-10 minutes, and collected water velocity measurements directly in front of the net at 60% of water column depth at both the beginning and end of the sample period to generate mean flow estimates. Samples were taken at dawn and dusk to capture temporal variation in drift, and we collected approximately two samples per thermal environment during each sampling event to capture spatial variation in drift (Appendix F: Table 9-1 & 9-2).

We collected juvenile steelhead diet samples 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). (Appendix F: Table 9-3). We attempted to catch fish for diet samples at all four study sites, but were only successful at these two sites.

To generate estimates of salmonid prey availability in the mainstem and tributary, we calculated drift delivery rates (DR; number of prey second^{-1} m^{-2}), defined as the product of drift density and water velocity:

1) $\mathrm{DR} = (n / V) * v$

where n is the number of invertebrates in a sample, V is the water volume of the sample, and v is the mean water velocity in front of the drift net. Drift delivery rate explicitly takes into account water velocity, making it a better metric than drift density for assessing how much food per unit time is reaching a one-m^2 window for fish holding in a specific thermal environment. 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 delivery rates; an effect size of zero indicates no difference. While these data were taken in 2010 and 2011, and all isotope data were collected in 2012, we are using these data simply to draw inferences about possible mechanisms of food limitation in thermal refuges.

2.3.4 Fish density in and out of refuges

We conducted snorkel surveys in August 2012 at Beaver Creek study site to assess relative fish densities in the mainstem versus refuge (mixing zone and tributary). We established nine 1x2 m^2 plots, three in the tributary (T), four in the mixing zone (MZ), and two in the mainstem (MS) (Fig. 2-2A). 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 approximately ten minutes. We conducted weekly surveys at three times of day (9:00, 12:00, 16:00), and recorded water temperature and mean juvenile steelhead and Chinook salmon densities in each plot. We calculated mean steelhead and Chinook salmon densities for all plots across the month.

2.3.5 Quantifying juvenile salmonid diet sources

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

To measure stable isotope ratios of potential fish prey sources, we collected benthic invertebrate samples from the tributary and mainstem at each site. While juvenile salmonids usually prey on invertebrates in the drift, we considered benthic invertebrates a reasonable proxy for determining the stable isotope ratios of prey sources, since we chose taxa from the benthic samples that were representative of the most common invertebrate families found in fish diets and in the drift, and that represented a range of functional groups (Appendix F). We performed invertebrate sampling twice during the two-week period prior to fish sampling in early July and late August, to capture the integration window for fish fin tissue (Heady and Moore 2012). During each sampling event, we collected samples of benthic invertebrates from both the tributary and mainstem by scrubbing the substrate at 4-7 point locations between 5-15 m above the confluence, and collecting aquatic invertebrates with a 500 μm net. We identified invertebrates to family under a stereomicroscope, and took 2-3 samples of each taxon collected (Chironomidae, Baetidae, Hydrophychidae, Simuliidae, Brachycentridae, Glossosomatidae).

We used non-destructive samples of caudal fin tissues to measure stable isotope ratios of 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, electro-fishing, and seining at both Beaver Creek (n = 53) and

Grider Creek (n = 32) in early July and late August. A small subset of the fish at Beaver Creek (n = 6) were caught in early August for radio tagging, but these fish showed no 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 ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) to investigate the contribution of mainstem versus tributary prey sources to the diet of juvenile salmonids. Prior to isotopic analyses, invertebrate samples were frozen, and all samples were subsequently dried at 15°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:

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

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 distributions 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 15th posterior sample, resulting in 6,000 independent draws for the posterior distribution. We used Gelman-Rubin diagnostics to confirm model convergence (Gelman and Rubin 1992). Sampling month was included in the model as a categorical variable, and individuals were included as a random effect. We incorporated a trophic discrimination factor of 3.2 ± 0.2 for δ^{15}N and 1.9 ± 0.5 for δ^{13}C (± 1SD) into the mixing model (McCutchan et al. 2003).

2.3.6 Fish thermal habitat versus foraging habitat

We used temperature-sensitive radio tags to track the body temperature of a subset (n = 25) of the juvenile steelhead caught at Beaver Creek in July 2012. We surgically implanted the tags (Lotek's MST-720T temperature-sensor tags; 1.3g dry weight; ±0.8°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 SRX600) to log fish body temperatures at 5-second intervals for any fish within approximately 100 m of the tributary confluence.

We used a two-part approach to assess whether juvenile steelhead thermal habitat use was similar to diet-inferred foraging water temperatures. For the subset of juvenile steelhead that were radio tagged at Beaver Creek in 2012 and also had fin clips taken for isotopic analysis, we were able to compare inferred individual diets to individual thermal habitat use calculated from radio tag data. In making this comparison, we are assuming that these individuals' behavior was similar before and after tagging, since the fish temperature represent data collected in the two weeks after fin samples were taken for isotopic diet analysis; this constraint was due to our inability to recapture radio-tagged fish post-tagging. For fish that remained at the refuge for a minimum of seven days post-tagging (n = 9), we used a mass-balance equation to translate time-series of fish temperatures into the mean proportion of time an individual spent in mainstem water (P_m). The equation we used was:

3) $$P_m = \Sigma\ ((T_f - T_t) / (T_m - T_t)) / n$$

where T_f represents fish temperature, T_t and T_m represent simultaneous tributary and mainstem water temperatures, respectively, and n is the number of observations for an individual fish.

For the larger population of un-tagged fish for which we had isotopic diet data (n = 53 at Beaver Creek; n = 32 at Grider Creek), we used a mass-balance equation to calculate the water temperature where these fish were foraging (hereafter "predicted foraging temperature"; T_{ff}) based on their diet. We used time-series of mainstem and tributary temperatures from the two 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 inferred diet proportion of each fish. The mean predicted foraging temperature for an individual fish can therefore be expressed as:

4) $$T_{ff} = \Sigma\ ((D_m * T_m) + ((1 - D_m) * T_t)) / n$$

where D_m is the inferred proportion of mainstem diet for an individual, T_t and T_m represent mainstem and tributary temperatures, and n is the number of observations for an individual fish. This allowed us to estimate the predicted distribution of fish foraging temperatures, and compare it to observed fish thermal habitat use.

To quantify thermal habitat use for the populations of fish using the thermal refuge at Beaver and Grider Creek study sites, we used data from a larger fish tagging study performed between July-September in 2010-2012 (Brewitt and Danner 2014). We calculated the fish temperature distribution for the population of fish tagged at each site during the study period 2010-2012 at Beaver Creek (n = 76) and 2010-2011 at Grider Creek (n = 40), using all fish detections sub-sampled at 5-minute intervals, and weighting all fish equally. We used a Kolmogorov-Smirnov two-sample test to test for differences between the thermal and foraging temperature distributions at each site.

2.4 Results

2.4.1 Quantifying mainstem and tributary water temperatures

Mainstem temperature profiles were similar during the two weeks prior to fish isotope sampling in early July and late August 2012, due to a cool weather front causing mainstem temperatures to drop in late August (Fig. 2-3). Mean mainstem temperatures for the two-week sampling windows in July and August 2012 were 22.5°C and 22.0°C at Beaver Creek study site, and 21.8°C and 21.6°C at Grider Creek study site, respectively.

2.4.2 Mainstem versus tributary drift densities

Invertebrate drift delivery rates were highly variable across sites, and were not consistently higher in the larger mainstem river than the tributaries. In 2010, drift delivery rates ranged from 1.5-7.9 prey second^{-1} m^{-2} in the mainstem and 1.0-8.6 prey second^{-1} m^{-2} in the tributary across the four study sites (Appendix F: Fig. 9-1). In 2011, drift delivery rates at Beaver Creek site ranged from 2.2-6.1 prey second^{-1} m^{-2} in the mainstem and 2.6-33.4 prey second^{-1} m^{-2} in the tributary. The mean (± SD) effect size between the mainstem and tributaries across all sites and years was 0.12 ± 0.95 prey m^{-2} s^{-1}; an effect size of zero indicates no difference.

2.4.3 Fish density in and out of refuges

Steelhead and Chinook salmon densities were consistently higher in the refuge than the mainstem throughout August 2012 at Beaver Creek (Fig. 2-2B,C). Mean water temperatures across survey plots and times of day in each thermal environment were 16.8°C, 20.5°C, and 22.2°C in the tributary (T), mixing zone (MZ), and mainstem (MS), respectively. Mean juvenile steelhead densities were 3.9, 3.0, and 0.7 fish per 2 m^2 in the tributary, mixing zone, and mainstem, respectively. The survey plot with the consistently highest density of steelhead (7.9 fish per 2 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 juvenile Chinook salmon densities were 3.3, 3.0, and 0.1 fish per 2 m^2 in the tributary, mixing zone, and mainstem, respectively. The survey plot with the consistently highest density of Chinook salmon (6.1 fish per 2 m^2) was located at the bottom of the mixing zone, in an area of lower velocity flow, and had a mean temperature of 20.5°C.

2.4.4 Quantifying juvenile salmonid diet sources

Stable isotope signatures were different for mainstem and tributary prey sources, spanning over five parts per mil for $\delta^{13}C$ and over ten parts per mil for $\delta^{15}N$. Juvenile steelhead and Chinook salmon isotope signatures were distributed throughout this range, indicating a high degree of variation in diet between individuals (Fig. 2-4A,B).

There were no seasonal differences in inferred juvenile steelhead or Chinook salmon diet between the two sampling periods (early July and late August). This may be because mainstem temperature profiles were similar during the two periods, due to a cool weather front causing mainstem temperatures to drop in late August (Fig. 2-3).

The posterior distributions from MixSIAR suggest that juvenile salmonids using thermal refuges obtained over 50% of their diet from mainstem prey sources (Fig. 2-5). Juvenile steelhead at both study sites obtained the majority of their prey from the mainstem river, but fish at Grider Creek had a slightly higher mean proportion mainstem diet (July = 0.98; 0.66-1.0 95% credible intervals (CI); August = 0.92; 0.14-1.0 CI) than fish at Beaver Creek (July = 0.73; 0.52-0.91 CI; August = 0.77; 0.61-0.93 CI) (Fig. 2-5A,B). Juvenile Chinook salmon at Beaver Creek had a more evenly mixed diet, with only slightly higher reliance on mainstem than tributary prey sources (July = 0.53; 0.26-0.80 CI; August = 0.51; 0.34-0.68 CI) (Fig. 2-5C).

There was a high degree of individual variation in inferred fish diet at both sites, but this variation was associated with both fish size and the location where fish were caught. Specifically, inferred diets generally grouped by the thermal environment (i.e. mainstem (MS), mixing zone (MZ), tributary (T)) where fish were caught (Fig. 2-6A,B). The mean proportion mainstem diet for juvenile steelhead caught in the mainstem (Beaver = 0.82 ± 0.13 standard deviation (SD); Grider = 0.97 ± 0.02) and mixing zone (Beaver = 0.75 ± 0.13; Grider = 0.94 ± 0.11) was much higher than 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 inferred diet from mainstem prey sources. The size distribution of steelhead within a refuge also grouped by thermal environment; tributary-caught steelhead had a smaller mean fork length (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 inferred diet obtained from mainstem sources increased with increasing steelhead body size (fork length) ($R^2 = 0.30$ and 0.23 for Beaver and Grider Creeks, respectively; linear regression), likely as a result of this size distribution across thermal environments.

Steelhead body temperatures describing thermal habitat use were significantly cooler than predicted foraging temperatures, indicating a mismatch in thermal and foraging habitat for fish using thermal refuges. Fish temperatures for the subset of radio-tagged steelhead at Beaver Creek in 2012 indicate that fish used a range of thermal habitat, with mean body temperatures ranging from 15.7°C to 22.5°C across the period sampled. 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 proportion of their diet these 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. 2-7). Moreover, we obtained a similar result for the larger population of steelhead sampled for isotopic diet analysis. There was a significant difference between the thermal and predicted foraging temperature distributions for fish at both upstream study sites (Kolmogorov-Smirnov two-sample test, $P < 0.001$ at both sites; Fig. 2-8). Fish temperatures based on temperature-sensitive radio tag data at both the Beaver Creek (mean = 17.5 ± 2.3°C) and Grider Creek (mean = 17.8 ± 2.3°C) sites were significantly cooler than predicted foraging temperatures based on the mass-balance equation for individuals at Beaver Creek (mean = 20.7 ± 1.2°C) and Grider Creek (mean = 21.0 ± 2.6°C).

2.5 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. In particular, tributary-caught steelhead and Chinook salmon derive substantial prey resources from the warmer mainstem habitat. An important implication of these findings is that the existence of thermal refuges appears to allow juvenile salmonids to exploit the larger mainstem river prey base without experiencing the negative physiological cost of holding in warmer water.

The mismatch between predicted foraging temperatures and thermal habitat use indicates that while fish seek cooler habitat for physiological benefits, they are still relying primarily on mainstem prey resources. Both the population of fish for which we performed isotopic diet analyses and the subset of radio-tagged fish for which we also had isotopic diet data apparently foraged at significantly warmer temperatures than the temperature at which they were holding. 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, yet stable isotope data suggests that they derive prey from warmer mainstem water (mean ≅ 21-22°C). Thus while cold-water tributaries perform the essential function of creating thermal refuges, tributary confluences with the mainstem river may also represent important habitat as the intersection between critical thermal and prey resources. This study thus adds to the growing appreciation that tributary junctions are hotspots of physical heterogeneity and biodiversity within river networks (Kiffney et al. 2006).

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), and isotopic diet analyses therefore imply that juvenile steelhead and Chinook salmon move out of refuges to obtain mainstem prey. This suggests that thermal refuges may be food-limited, likely due to high fish densities causing competition for prey. Drift sampling indicated that there was no consistent difference between prey delivery rate in the tributaries versus the mainstem river, and invertebrate drift densities alone are therefore unlikely to drive fish to forage outside of thermal refuges. However, fish densities were consistently high in both the tributary and mixing zone (mean fish density m^{-2} = 3.5 in August 2012) throughout the summers when the study was conducted. Drift samples were collected above the main aggregation of fish in refuges, and thus drift was likely depleted for much of the tributary habitat used by fish due to high fish densities. Within-refuge fish densities were higher than those measured in other systems; for example, Keeley and McPhail (1998) measured juvenile steelhead densities of approximately 2.8 fish m^{-2} for fish of smaller mean size (FL ≅ 70 mm) in two Canadian rivers. Moreover, steelhead territory size increases with body size

(Keeley and McPhail 1998), further evidence that fish densities in Klamath River thermal refuges were quite high. Density-dependent processes affecting refuge use have been shown in artificial stream experiments (Gregory and Griffith 1996, Armstrong and Griffiths 2001, Imre et al. 2004) as well as in the field (Rangeley and Kramer 1998). Therefore, although rates of food delivery appear to be similar between refuges and mainstem habitat, the movement of individuals into thermal refuges with rising mainstem temperatures increases fish densities, likely reducing per capita prey availability within the refuge. Density-dependent processes may therefore be driving individuals to venture into warmer water to access more prey (Fretwell and Calver 1969); these processes could set limits on the overall carrying capacity of thermal refuges.

The differences in reliance on mainstem resources between the two study sites is likely driven by the geomorphology of the confluences, and the degree to which physical habitat mediates how fish interact with the thermal and prey landscape. The majority of steelhead at the Grider Creek refuge relied almost exclusively on mainstem resources (mean proportion mainstem diet = 0.97), whereas steelhead diet at Beaver Creek was composed of a mix of mainstem and tributary resources (mean proportion mainstem diet = 0.73); these differences were largely driven by the diet of fish caught in the mainstem and mixing zone (Fig. 2-6). Beaver Creek is a large tributary (watershed area ≅ 280 km^2; J. Grunbaum U.S. Forest Service, personal communication) that creates a well-mixed fast-moving riffle zone just below the confluence where steelhead aggregate (personal observation); this geomorphology facilitates easy movement between the tributary and mainstem across a range of water temperatures. Grider Creek is a smaller tributary (watershed area ≅ 105 km^2; J. Grunbaum USFS, personal communication) that runs over a shallow rocky area before joining the Klamath River, creating a small swirling refuge pool (personal observation); the tributary is therefore less accessible to fish using the mixing zone. Tributary-caught fish showed a high degree of individual variation in diet at both sites, but the Grider Creek individuals' diets followed a bimodal distribution; some individuals (n = 7) fed mostly (~70-80%) on tributary prey, and a separate group (n = 10) fed mostly (~80-90%) on mainstem prey. At Beaver Creek, diets of tributary-caught individuals also exhibited a high variance, but spanned a gradient from ~25-75% mainstem diet, suggesting more consistent movement of individuals between the tributary and mainstem habitat, likely due to the tributary's large size and the high degree of connectivity between the Beaver Creek tributary and the mainstem (Fig. 2-5). Habitat connectivity is essential for allowing mobile consumers to integrate across a heterogeneous resource landscape, thereby maximizing growth opportunities (Armstrong et al. 2013). Tributary confluences that lack sufficient connectivity with the mainstem river likely make it more difficult for fish to move between the tributary and mixing zone to forage, and may therefore form sub-optimal refuges. Thus, while all cold-water refuges offer thermal benefits, their physical morphology may impact how fish use them.

The positive relationship between juvenile steelhead body size (fork length) and the proportion of diet comprised of mainstem prey, as well as the fact that larger fish were caught in warmer water, gives insight into the spatial structure of juvenile steelhead at tributary confluences forming thermal refuges. While mainstem prey composed the majority of steelhead diet, there was a high degree of individual variation in diet that grouped largely by the thermal environment (i.e. mainstem, mixing zone, tributary) where fish were caught. Larger juveniles (FL ~ 200 mm) caught in mainstem and mixing zone habitat were more likely to rely on mainstem resources than smaller fish (FL ~ 150 mm) caught in the tributary, who had a lower proportion of mainstem resources (but much higher variance) in their inferred diet composition. Given sufficient food availability, the mixing zone and boundary habitat is likely optimal habitat for fish growth, as mixing zone temperatures were often at the bioenergetic optima for steelhead (~18-19°C). Our results suggest that larger juveniles are likely holding dominant positions near the edge of thermal refuges where thermal and prey resources can be optimized, and displacing smaller fish, causing them to hold in cooler tributary waters. Both observational and radio-tagging studies on juvenile steelhead in Klamath River refuges have observed similar behavior, with fish (especially larger juveniles) concentrated along the edge of the refuge (Sutton et al. 2007, Brewitt and Danner 2014). However, studies on juvenile salmon using thermal refuges in other systems have observed the opposite pattern, with larger fish aggregating in cooler water than smaller fish (Ebersole et al. 2001, Breau et al. 2007). Remaining in warmer water is only beneficial if fish can access sufficient food, and the spatial structure of juvenile salmonids using thermal refuges therefore likely depends on the complex interaction between body size, water temperature, and prey availability (Hanson et al. 1997).

Juvenile Chinook salmon at Beaver Creek had a diet that was a more balanced mix of tributary and mainstem resources than steelhead. This difference likely reflects both size- and species-specific habitat selection at tributary confluences (Everest and Chapman 1972). Chinook salmon were caught in either the tributary or the lower mixing zone (~50 m below the confluence) in slower velocity areas, rather than the higher velocity riffle forming the top of the mixing zone where steelhead were found (personal observation). Chinook salmon caught in the lower mixing zone had a fairly mixed diet (mean proportion mainstem diet = 0.58), likely reflecting the mixing of mainstem and tributary resources by the time they reached the lower mixing zone. Interestingly, juvenile Chinook salmon caught in the tributary also had an evenly mixed diet (mean proportion mainstem diet = 0.47) similar to tributary-caught steelhead (0.57 at Beaver Creek), suggesting that even though these fish hold at least part-time in the tributary, they spend approximately half of their foraging time in or near the mainstem.

The size-based variation in inferred diet that we observed suggests that fish negotiate the challenge of resource variability in refuges in different ways. Balancing resource needs may be easier for larger juveniles that can establish dominant positions on the mixing zone boundary habitat, likely darting out into the mainstem to forage at short time-scales, while smaller tributary-based juveniles that still need access to mainstem resources may need to move further to gain sufficient resources. Higher fish densities in refuges may therefore disproportionately affect smaller individuals, which are more likely to suffer decreased growth rates or higher emigration rates (Keeley 2001).

Our results suggest that juvenile salmonids using thermal refuges negotiate the heterogeneous thermal and prey resource landscapes by shifting horizontally between the cooler tributary water and warmer mixing zone boundary and mainstem. While juvenile salmonids using refuges to thermoregulate likely experience lower per capita food availability than in the mainstem, they may be able to deal with this food limitation by moving periodically to the more abundant mainstem habitat to forage. Studies on the effects of food additions to density-dependent systems have linked increases in food abundance to smaller territory sizes and increases in population size (Grant et al. 1998, Imre et al. 2004). This suggests that access to the mainstem river is an important aspect of thermal refuges at tributary confluences, as access to an outside food source (i.e. through individuals moving into the mainstem to forage) may increase the carrying capacity of space-limited refuges. Furthermore, even when mainstem temperatures reach upper tolerance thresholds, the mainstem likely still provides a critical food source that sustains salmonids. Degraded river systems could reach carrying capacity at lower levels due to decreases in the total amount of suitable in-stream habitat, making density-dependence a concern for habitat restoration efforts (Achord et al. 2003). As mainstem river habitat reaches thermal tolerance thresholds for salmonids and increasing numbers of fish move into smaller areas of suitable habitat (i.e. thermal refuges), the size and distribution of refuges, as well as within-refuge density-dependent processes and mainstem prey abundance, could dictate the overall carrying capacity of the river (Ebersole et al. 2003, Petty et al. 2012, Huntsman and Petty 2014).

Our results support several important inferences for habitat management and future research on thermal refuges. Habitat homogenization and fragmentation are altering the landscape of available habitat (Vitousek 1997); our study highlights the importance of maintaining in-stream habitat connectivity, as well as the critical nature of tributary confluences as potential in-stream hotspots, as thermally sub-optimal habitat (e.g., mainstem river) may be a critical food resource for fish using refuges. These dynamics could lead to a trade-off between thermal and trophic resources if mainstem habitat becomes inaccessible due to a lack of connectivity between tributaries and the mainstem river (e.g., low stream flow) or when mainstem temperatures rise above 25°C (Brewitt and Danner 2014). Watershed alteration and lower mainstem flows have increased temperatures on the Klamath River (Bartholow 2005), and anthropogenic climate change further exacerbates these already elevated temperatures (Travis 2003, Mac Nally et al. 2009). The efficacy of habitat restoration efforts will likely depend on mechanisms of within-refuge density-dependence, and how population size responds to restoration of thermal refuge habitat quality and area (Greene and Beechie 2004). Future research on thermal refuges should focus on the effects of density-dependence, as well as how access to adjacent mainstem habitat may affect refuge carrying capacity, especially as rising river temperatures reach thermal tolerance thresholds for juvenile salmonids.

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2.7 Figures

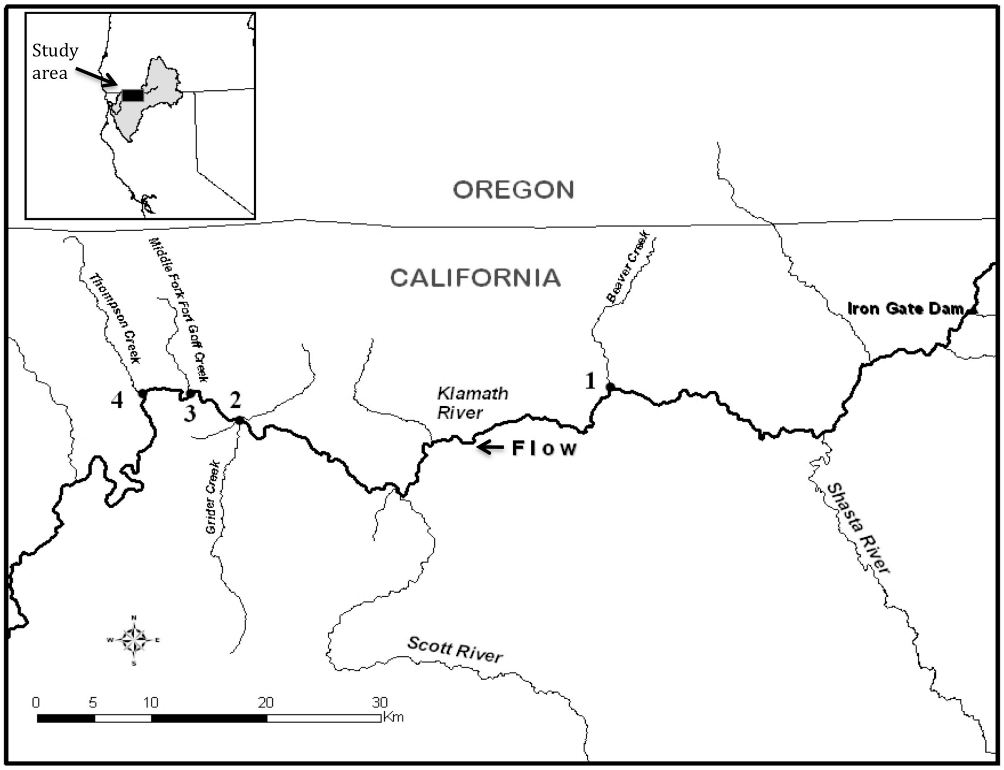

Figure 2-1. Study sites located at tributary confluences on the Klamath River. (1 = Beaver Creek; 2 = Grider Creek; 3 = Fort Goff Creek; 4 = Thompson Creek). Inset shows location of study area within the watershed.

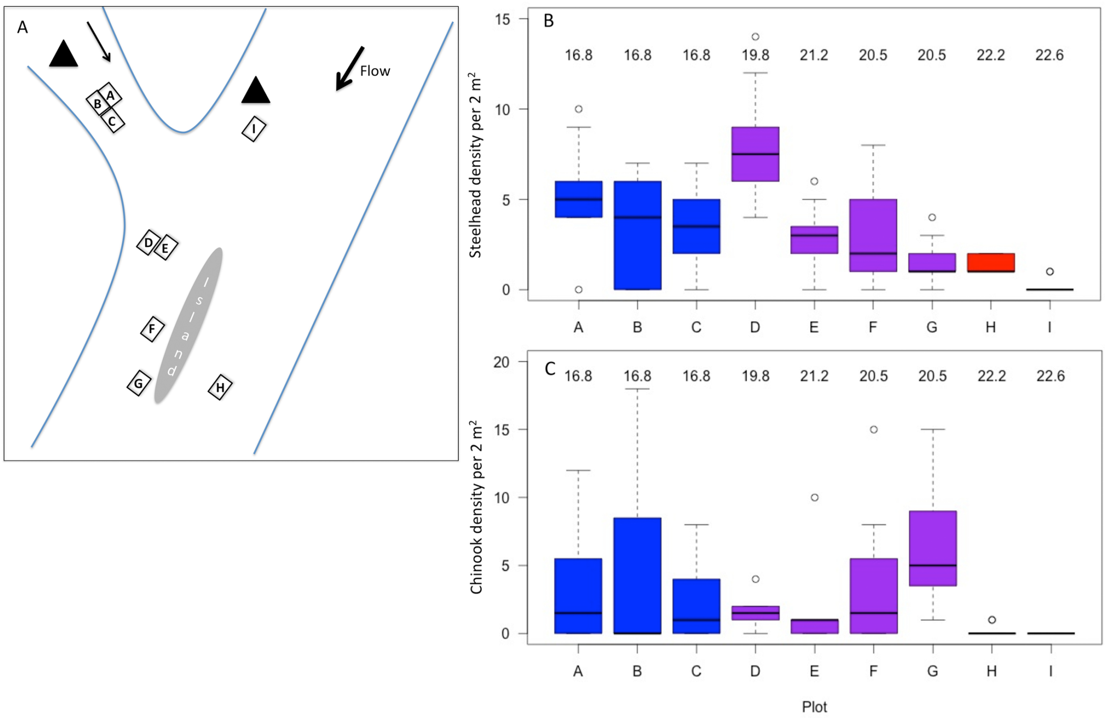

Figure 2-2. (A) Map of snorkel survey plots (open squares) and invertebrate drift sampling locations (filled triangles) at Beaver Creek confluence. (B) Densities of steelhead and (C) Chinook salmon in 1x2 m^2 plots within each thermal environment at Beaver Creek refuge in August 2012. Boxplots are coded 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.

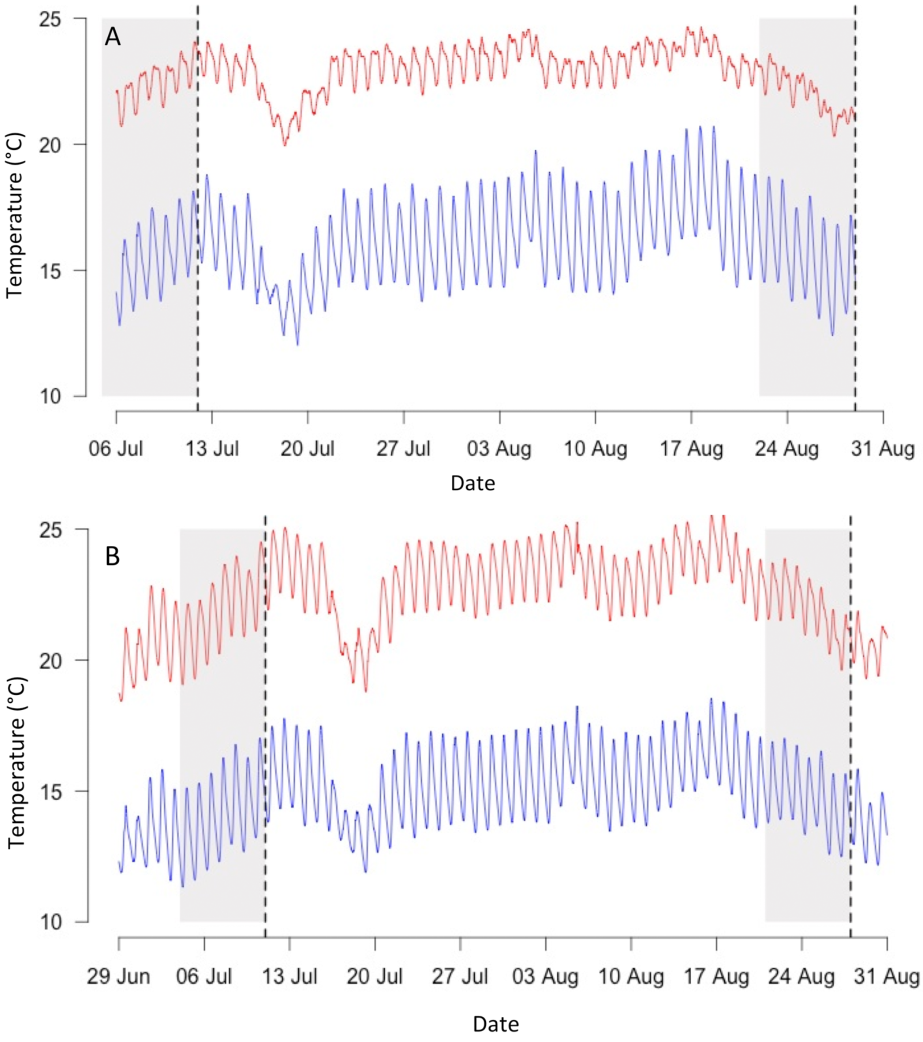

Figure 2-3. Mainstem (red) and tributary (blue) water temperatures at (A) Beaver Creek and (B) Grider Creek in 2012. Black dashed vertical lines indicate fish sampling events, and gray shaded rectangles indicate the two week period prior to fish sampling when benthic invertebrates were collected.

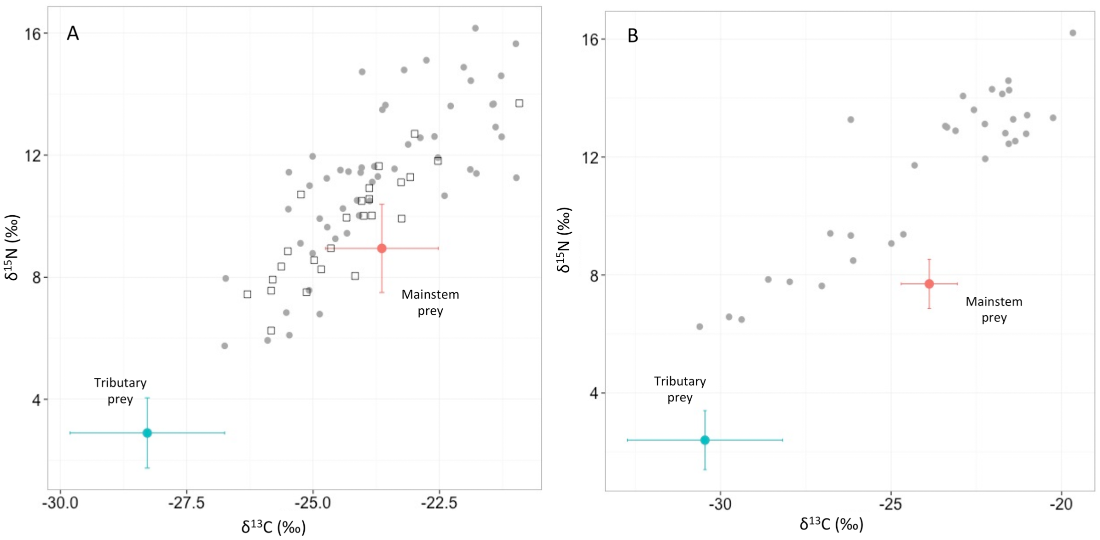

Figure 2-4. Isotope signatures of $\delta^{13}C$ and $\delta^{15}N$ for juvenile salmonids and mainstem (red) and tributary (blue) invertebrate prey sources (mean ± SD). (A) Beaver Creek steelhead (n = 53) indicated by solid gray points and Chinook salmon (n = 25) indicated by open squares. (B) Grider Creek steelhead (n = 32) indicated by solid gray points.

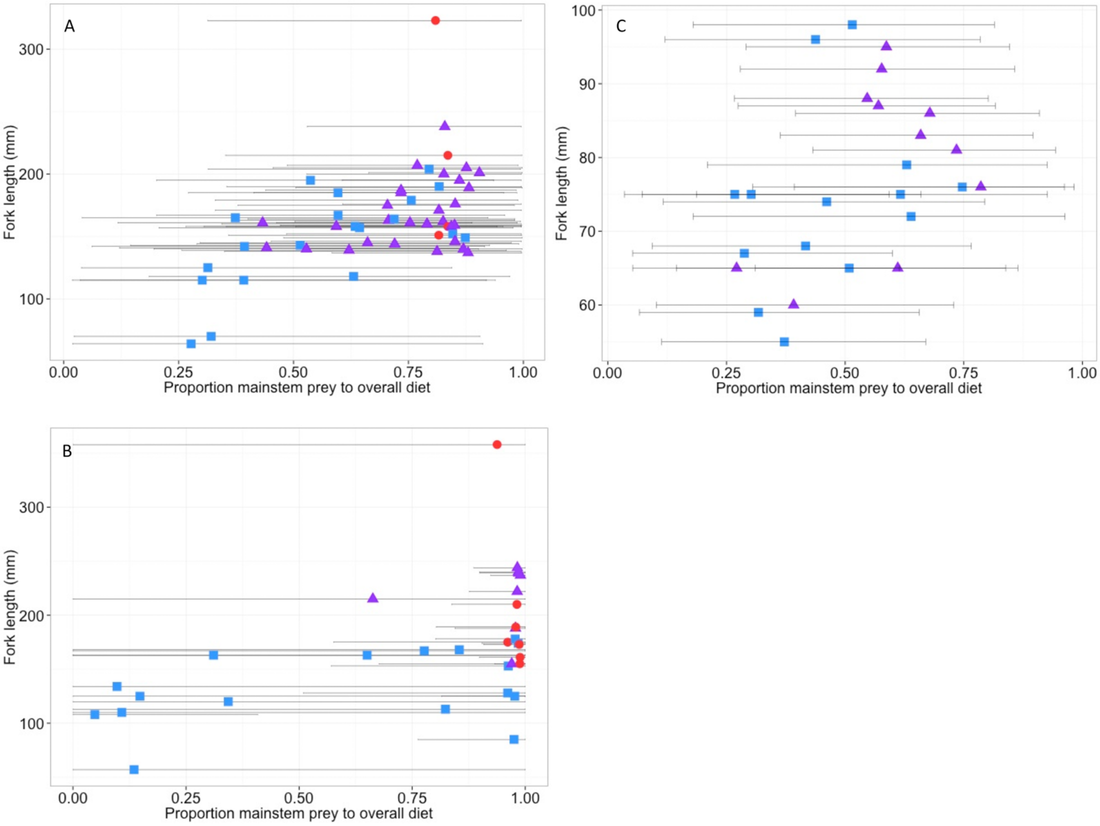

Figure 2-5. Posterior estimates from MixSIAR of the contribution of mainstem prey sources to fish diet plotted against fish body size (fork length). Mean diet estimates and 95% credible intervals (CI) for individual steelhead at (A) Beaver Creek, (B) Grider Creek, and (C) Chinook salmon at Beaver Creek. Symbols are coded by the thermal environment in which individuals were caught (tributary = blue squares; mixing zone = purple triangles; mainstem = red circles).

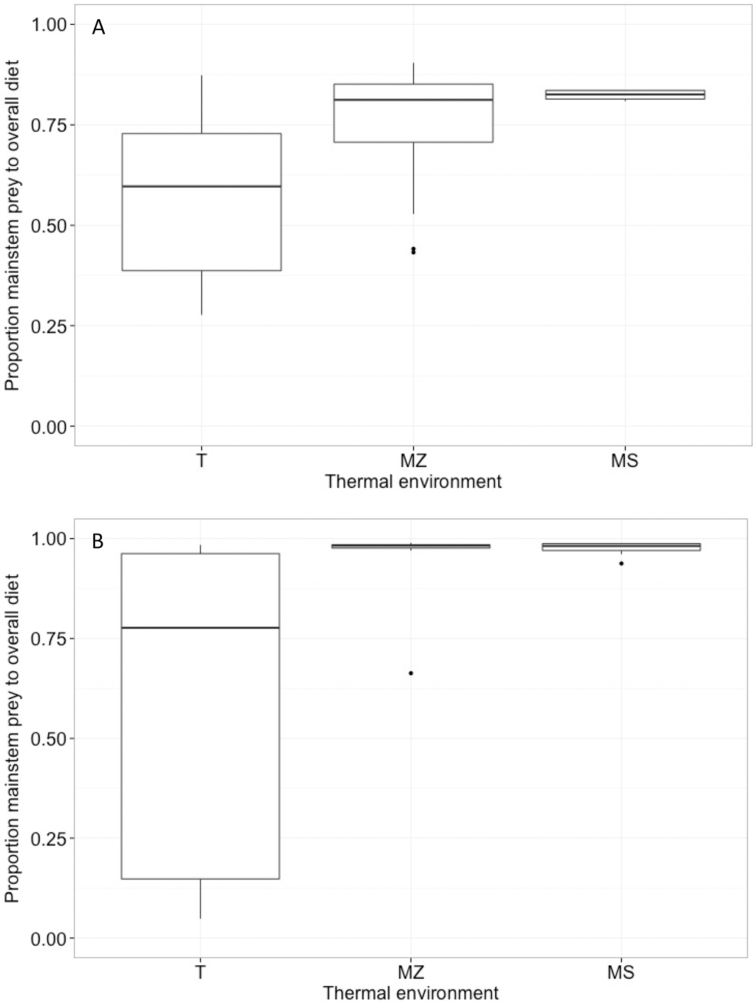

Figure 2-6. Posterior estimates from MixSIAR of the mean contribution of mainstem prey sources to juvenile steelhead diet by the thermal environment (T = tributary; MZ = mixing zone; MS = mainstem) where fish were caught at (A) Beaver Creek and (B) Grider Creek.

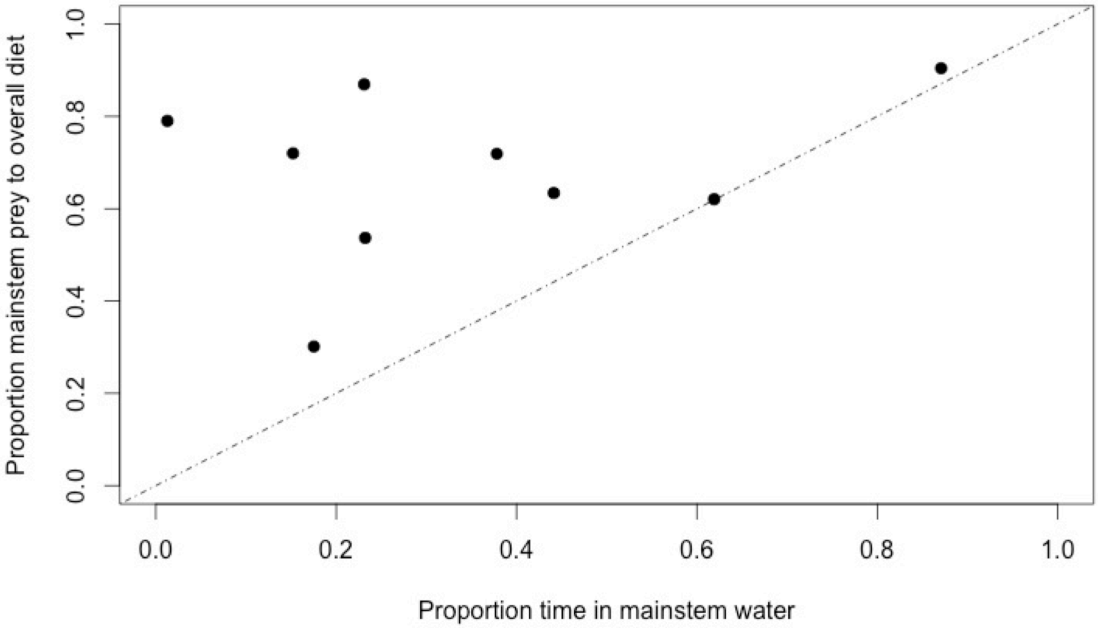

Figure 2-7. Posterior estimates of the mean contribution of mainstem prey sources to fish diet versus the proportion of time spent in mainstem water for individual juvenile steelhead radio tagged at Beaver Creek, for which there were >7 days of temperature data (n = 9).

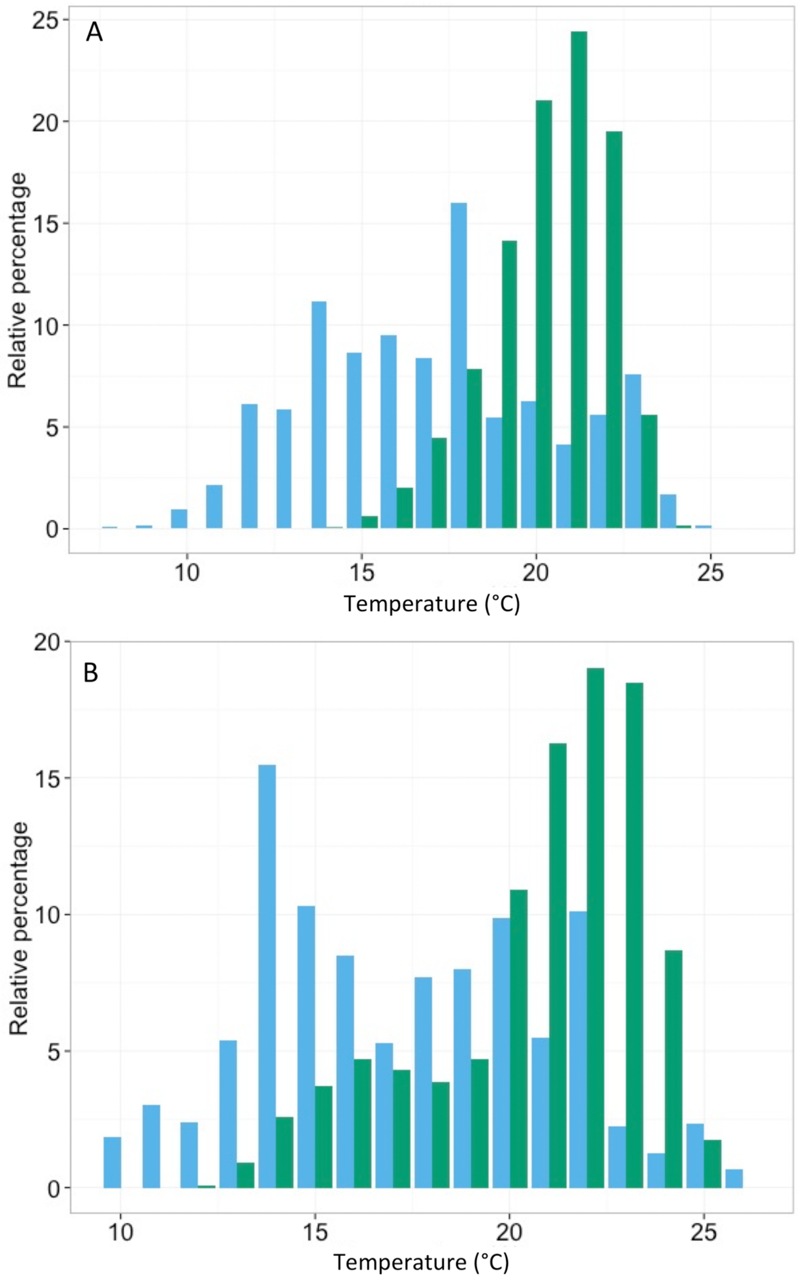

Figure 2-8. Distribution of fish body temperatures (blue) and predicted foraging temperatures (green) for steelhead sampled at (A) Beaver Creek and (B) Grider Creek in 2012. Thermal distributions are derived from temperature-sensitive radio tag data, and predicted foraging temperature distributions are derived from the mass balance equation (Eq. 4).

3. Chapter 3: Response times of implanted temperature-sensitive radio tags to ambient temperature variation: Implications for the thermal ecology of fish.

3.1 Abstract

Thermal habitat use studies are increasingly important given rising ambient temperatures caused by large-scale habitat alteration and climate change. Temperature-sensitive tags are useful for these types of studies, and these tags are often internally implanted in small ectotherms (e.g., fish) to track movement and thermal habitat preference. However, internally implanting tags may lead to error in ambient temperature estimates due to the insulatory effect of the fish, which could cause a delay in tag temperature acclimation. In studies at fine temporal scales, it may be necessary to quantify how temperature-sensitive tags respond to new ambient temperatures when an organism moves in order to accurately assess thermal habitat use. We performed lab experiments on juvenile steelhead *(Oncorhynchus mykiss)* with internally implanted Lotek temperature-sensitive radio tags ('implanted tags') to quantify how variation in the magnitude or direction (heating vs. cooling) of change in water temperature and fish body size (fork length; weight; volume) affect the amount of time it takes for tags to acclimate to a new ambient temperature ('tag response time'). We also tested radio tags exposed directly to the external environment ('bare tags') to see whether the delay in tag response time differed from implanted tags. We used generalized linear mixed effects models, and used an information-theoretic approach to determine the best-fit models for the data.

We found that implanted tag temperature response time depended positively on the magnitude of water temperature change and to a lesser extent on fish body size (fork length and weight), as well as on the direction of water temperature change. Temperature acclimation times were significantly shorter for bare tags than for implanted tags. Our results emphasize the importance of accounting for temperature-sensitive tag response times, especially as technological advances allow for the collection of data at increasingly small temporal scales. Temperature-sensitive tags are useful for assessing thermal habitat use in heterogeneous landscapes, but the time lag in tag temperature acclimation represents a potential source of error for studies on internally tagged fish and should be accounted for, either by appropriately sub-sampling data or by explicitly modeling tag acclimation dynamics.

3.2 Introduction

Studies on animal movement and habitat preference give insight into how the physical environment influences the distribution, abundance, and physiology of animals, yet observing mobile animals in aquatic systems remains one of the main challenges of studying the ecology of marine and freshwater organisms (Dell et al. 2011, Cooke et al. 2013). The advent of electronic tags with sensors that record environmental data (e.g., temperature, depth, photoperiod) has made it easier for researchers to simultaneously track both movement and habitat use. In particular, it is increasingly important to understand how organisms respond to changes in their thermal environment, given rising ambient temperatures caused by large-scale habitat alteration and climate change

(Vitousek 1997, McCarty 2001, Mantua et al. 2010). Temperature-sensitive tags are useful for thermal studies, and have been used to track thermal habitat use and behavior (Tiffan et al. 2009, Hayes et al. 2011, Huff et al. 2011, Brewitt and Danner 2014). These types of studies are especially important for coldwater fish, as critical body functions (e.g., metabolism, consumption) are mediated by water temperature (Hanson 1997). Changes in water temperature can therefore impact individual fish behavior, growth and survival (Bevelhimer and Adams 1993, Myrick and Cech 2005, Armstrong and Schindler 2013), and by extension the dynamics and persistence of populations (Benjamin et al. 2013). Temperature-sensitive tags represent an opportunity to gain a better understanding of the thermal ecology of fish and other aquatic organisms.

Advances in tag technology continue to reduce tag size and weight, and small temperature-sensitive tags are often internally implanted in fish, leading to possible error in thermal habitat studies due to the buffering effect of the fish on the tag. Internal tags are implanted either into the body cavity via surgery or into the stomach cavity via the esophagus (Adams et al. 1998), and are therefore insulated from the surrounding environment by the body of the fish. There may therefore be a delay in the tag temperature response to changing ambient temperatures. Fish are poikilotherms, and change body temperature in response to changes in ambient temperature. However, this response is not instantaneous and may be related to body size (Spigarelli et al. 1977, Crawshaw 1977), potentially biasing ambient temperature measurements around different size individuals. Studies using internally implanted temperature-sensitive tags to measure thermal habitat use need to take into account this potential fish effect on tag temperature response times.

Tag technology now allows data logging on a very fine temporal scale (e.g., 4-5 second intervals), making it necessary to quantify how temperature-sensitive tags acclimate to new ambient temperatures when an organism moves to a new thermal environment. This phenomenon may be compounded in internally implanted tags, where the fish tissue acts as a buffer. Delays in tag acclimation could dictate the minimum temporal scale of a study; if a fish moves across a thermally heterogeneous landscape, an internally implanted radio tag may record the internal fish temperature rather than the external water temperature for some period of time after the move. Companies producing temperature-sensitive tags often give no information about delays in tag response, or alternately report only how the tag responds outside of fish. As technology advances, tag response times will likely decrease, and may vary between companies and tag types, but the insulatory effect of fish will remain for internally implanted tags. To our knowledge, no studies have quantified internally implanted temperature-sensitive tags' response to changing water temperatures, yet it is important to quantify these delays in order to avoid misinterpretation of data.

The objective of this study was to quantify the delay in temperature-sensitive tag acclimation in response to environmental (i.e. water temperature) change, and to determine what variables influence this delay. This study is not meant as a comprehensive assessment of how all temperature-sensitive tags respond to changes in water temperature, but rather a demonstration of the variables that could determine tag acclimation response times. Internally implanted tags are common in freshwater studies, and many freshwater studies rely on radio tag technology, as this type of tag often works best in noisy river systems (Koehn 2000, Cooke et al. 2013). We therefore use Lotek radio tags internally implanted in juvenile steelhead *(Oncorhynchus mykiss)* as a case study to quantify the time it takes for a tag to fully acclimate to a new ambient temperature ('tag response time'). In this study, we ask: (1) Does tag response time depend on fish body size (fork length; weight; volume), or the magnitude or direction (heating vs. cooling) of change in water temperature, and (2) Does the delay in tag response differ between radio tags exposed directly to the external environment (hereafter 'bare tags'), versus those internally implanted in fish (hereafter 'implanted tags'). We performed lab experiments on radio-tagged juvenile steelhead, and found that tag response time depends on the magnitude and direction of water temperature change, as well as on fish body size (fork length and weight), and differs between bare and implanted radio tags.

3.3 Methods

We performed lab experiments to determine the response time—i.e. the amount of time it took tag temperature to reach a new ambient temperature when water temperature changed—of Lotek's MST-720T temperature-sensitive radio tags. We obtained 48 juvenile steelhead (weight range: 33-333 g) from Coleman National Fish Hatchery in Anderson, CA and performed the experiments at the National Marine Fisheries Service Lab in Santa Cruz, CA, in November 2012 – March 2013. We weighed, measured (fork length; mm), and surgically implanted radio tags (1.3 g dry weight; ±0.8°C) into the body cavity of juvenile steelhead following a tagging protocol similar to Adams et al. (1998). All fish were held in tanks for a minimum of 24 hours after tagging and prior to experimentation.

We used four aquariums as treatment tanks, and filled each tank with water ranging in temperature from 12.5°C to 22.0°C (a thermal range experienced by juvenile steelhead in California watersheds). We performed 2-6 trials on each fish; a trial consisted of moving a single fish from a starting tank to a receiving tank with a different water temperature, recording transmitted tag temperatures, and waiting for the tag temperature to fully acclimate. Tag acclimation was defined by the error rate associated with the tag (±1ºC); therefore we considered a tag to be acclimated when it was within ±1ºC of the new ambient temperature. Fish experienced changes in water temperature ranging from 1.1-7.6°C. We recorded the location (e.g. tank number) and time of each transfer between tanks. In addition, we performed similar trials using bare radio tags (n = 6) to determine if there was a delay in response time for tags not in fish.

Fish were subsequently euthanized with MS-222 (tricaine methanesulfonate), and we measured fish volume (mL) for a subset of fish (n = 31) by measuring water displacement in a cylinder. Hobo pendant temperature loggers (±0.5°C; Onset Computer Corporation, Pocasset, MA) in each tank logged water temperature each second during the experiments, and data-logging receivers (Lotek SRX 600) logged radio tag temperatures continuously at 5-second intervals throughout each experiment.

We measured radio tag acclimation times by calculating the amount of time it took for a tag to reach the new tank's water temperature (±1ºC) once it was moved. We discarded all trials in which tag temperature did not fully acclimate; of the 48 radio-tagged fish, 44 fish had sufficient data to be included in our models, with a total of 184 individual trials.

We used an information-theoretic approach to determine the best-fit models for the data, and used generalized linear mixed effects models to model radio tag response time using the *nlme* library in R (R Development Core Team 2014). We ran four separate models to test different combinations of predictor variables: the first model included all tagged fish, the second and third models included only the subset of fish for which volume was measured, and the fourth model included only the bare tag data. In all models, the response variable was response time *(t; seconds)*, and individual fish were included in the model as a random effect. This model structure is analogous to a repeated measures design, and explicitly accounts for observations being nested within the individual.

To test whether there was any effect of fish size (length, weight) on tag response time, we used a model including all tagged fish. The full model included four main effects: fish weight *(W; g),* fork length *(FL; mm),* the magnitude of change in water temperature *(dT; °C),* and the direction of change in water temperature as a two-level factor (*D;* 0 = cooling, 1 = heating). In addition, we considered four interaction terms: an interaction between the direction of water temperature change and each of the other variables, to determine whether heating or cooling temperatures had different effects on tag response time, and whether this changed with fish size *(D:W* or *D:FL)* or the magnitude of change in water temperature *(D:dT)*. In addition, we included an interaction between fish weight and fork length *(W:FL)* to determine whether fish shape had a significant effect on tag response time.

To test whether fish volume was a better predictor of tag response time than fish weight, we used two models including only the subset of fish with measured volumes (n

= 31). Prior to statistical analyses, collinearity between candidate covariates was assessed using variance inflation factors (VIF); fish volume was highly collinear with fish weight, and we therefore ran two separate models, one including fish weight and one including fish volume. One of these models was identical to the full model for all tagged fish, and in the other we replaced fish weight with fish volume. We then used the residual variation from each model to assess which model performed better.

Our final model tested whether bare tags responded differently than tags in fish, and included two main effects and one interaction term: change in water temperature *(dT),* and the direction of change in water temperature *(D)*, and an interaction between these two variables *(dT:D).*

We used Akaike information criterion (AIC) to rank all candidate models, and used the AIC weights of each model to select the top 95% confidence set of models (Burnham and Anderson 2002). The goal of AIC model selection is to avoid over-parameterization by including only explanatory variables in the model. We used the re-normalized AIC weights of all models in the top confidence set to calculate model-averaged parameter estimates and parameter weights for the predictor variables, which indicates the explanatory power of each covariate.

3.4 Results

The shape of the tag acclimation curves was similar across trials for both heating and cooling temperatures, showing an initially high rate of change in temperature following movement to a new ambient temperature, and then a slower rate of change as fish temperature approached the new ambient temperature (Fig. 3-1).

Our model results for all radio tagged fish indicate that tag response time depends on the magnitude of change in water temperature, and to a lesser extent on fish body size and the direction of water temperature change (Fig. 3-2A). None of the interaction terms were significant, and we therefore dropped these terms from the full model and re-ran all model subsets using only the main effects terms. The top 95% confidence set of models included five models (Table 3-1), and the best explanatory model for radio tag response time in fish can be expressed as:

$$(1)\qquad t = -28.66 + 33.61dT + 49.69D + 1.03FL + 0.07\mathrm{W}$$

where t is the response time (s) it takes for the tag to acclimate to the new ambient water temperature, dT is the magnitude of temperature change, D is the direction of temperature change, FL is fork length, and W is weight. The values are model-averaged parameter estimates from the top model set (Table 3-2).

Fish volume was no better at predicting tag acclimation time than fish weight. For the two models that were based on the subset of data with fish volumes, the model including fish volume gave similar results to the model including fish weight. In addition, residual variation was nearly identical for the two models (difference = 0.17), indicating that both models performed similarly.

Bare tags responded differently than radio tags in fish; tag response times for bare tags depended only slightly on the magnitude and direction of change in water temperature (Fig. 3-2B). All three sub-models were included in the top 95% confidence set (Table 3-3), and the best explanatory model for bare tag response time can be expressed as:

$$(2) \qquad t = 42.98 + 7.94dT + 22.84D$$

where t is the response time (s) it takes for the tag to acclimate to the new water temperature, dT is the magnitude of temperature change, and D is the direction of temperature change. The values are model-averaged parameter estimates from the top set of models (Table 3-4).

3.5 Discussion

Temperature is one of the most important abiotic drivers of fish physiology and behavior (Magnuson et al. 1979). As habitat degradation and climate change continue to alter the thermal regimes of freshwater and marine systems, studies on temperature-driven habitat use and behavior will be increasingly important (Coutant 1987, Keppel et al. 2012). Our results emphasize the importance of accounting for temperature-sensitive tag response times, especially as technological advances allow for the collection of increasingly fine-scale temporal data.

Response times for tags implanted in fish depended on the magnitude and direction of temperature change (Fig. 3-2A). Tags took longer to acclimate to larger changes in water temperature: approximately 5-6 minutes for a temperature change of 6-7ºC, versus 2-3 minutes for a change of 1-2ºC for juvenile salmonids of mean weight (~80 g). This delay in tag temperature acclimation should be taken into consideration (e.g., regarding sub-sampling interval) for studies on thermal microhabitat use in heterogeneous landscapes, where organisms may encounter a large array of temperatures in a short time period. For instance, fish often encounter large magnitude changes in temperature (~6-8ºC) at cold-water tributary confluences with warm rivers (Sutton and Soto 2012, Brewitt and Danner 2014). However, the definition of response time may be partially driving this pattern. Tag temperature appears to approach the asymptote (i.e. new ambient temperature) at a declining rate (Fig. 3-1). Therefore, the tag temperature variance window (±1ºC for Lotek MST-720T tags) takes up proportionally more of the acclimation period when it is smaller (i.e. smaller *dT)*, and the response time will therefore by definition be faster. Tag sensitivity may therefore dictate the minimum sampling interval of the data. Here, we used a linear model to describe tag response time. However, fitting a non-linear function (e.g., power curve) may provide a more mechanistic model, which could in turn allow researchers to determine how different tags (and tag sensitivities) might affect the temporal resolution of their data.

Both implanted and bare tags took slightly longer to acclimate to the ambient water temperature for warming temperatures than for cooling temperatures. While a few studies have reported faster cooling than heating rates in fish (Reynolds 1977), more studies have found that fish heated faster (Beitinger et al. 1977, Spigarelli et al. 1977, Weller et al. 1984), indicating probable differences between species and individuals in heating and cooling rates. Spigarelli et al. (1977) found that differential heating and cooling rate is related to fish body size and morphology; small (~10-50 g) laterally compressed alewives showed less difference between heating and cooling than larger fish. Similarly, juvenile steelhead may not display a large differential in heating vs. cooling rates. Moreover, since bare tag response times showed a similar pattern to implanted tags (i.e. faster cooling), this effect may be due to the dynamics of the tag itself. However, given physiological differences in fish heating versus cooling rates, ignoring tag acclimation times for thermal habitat preference studies at small temporal scales could skew results towards detecting warmer (or cooler) ambient temperatures than were actually experienced by the organism. It may therefore be important to assess how the direction of water temperature change affects both the fish and tags being used in a given study.

Implanted tag response times depended positively on body size (fork length and weight); larger fish acclimated more slowly to a new water temperature than smaller fish. Previous studies on changes in fish body temperature have found that body size mediates fish temperature change (Stevens and Fry 1970, Beitinger et al. 1977, Spigarelli et al. 1977), indicating that these results likely have a physiological basis. Temperature and body size mediate key physiological attributes (e.g., metabolism, consumption) in fish, and can therefore influence thermal microhabitat selection (Ebersole et al. 2001, Brewitt and Danner 2014). The fact that larger fish have higher thermal inertia suggests that they can maintain a cooler (or warmer) internal temperature for a certain period of time following a change in ambient water temperature. This time lag could enable larger individuals to exploit thermally stressful adjacent habitat for short periods without experiencing any negative physiological effects.

Electronic tags with environmental sensors are an increasingly common method for assessing habitat use in free-swimming fish, and temperature-related questions in particular are becoming more important as climate change, habitat degradation, and thermal pollution alter temperature regimes in aquatic systems (Cooke and Schreer 2003, Cooke et al. 2013). While this study focused on a particular brand and design of temperature-sensitive tag that we have applied in our research on the thermal ecology of juvenile salmonids (Brewitt and Danner 2014), these results suggest that researchers should conduct similar tag temperature acclimation experiments on implanted tags being used in studies at fine temporal scales. Temperature-sensitive tags are useful for assessing thermal habitat preference in heterogeneous landscapes, but the lag in tag temperature acclimation represents a potential source of error for all studies on internally tagged fish and should be taken into consideration, either by appropriately sub-sampling data or by explicitly modeling tag acclimation dynamics.

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3.7 Figures

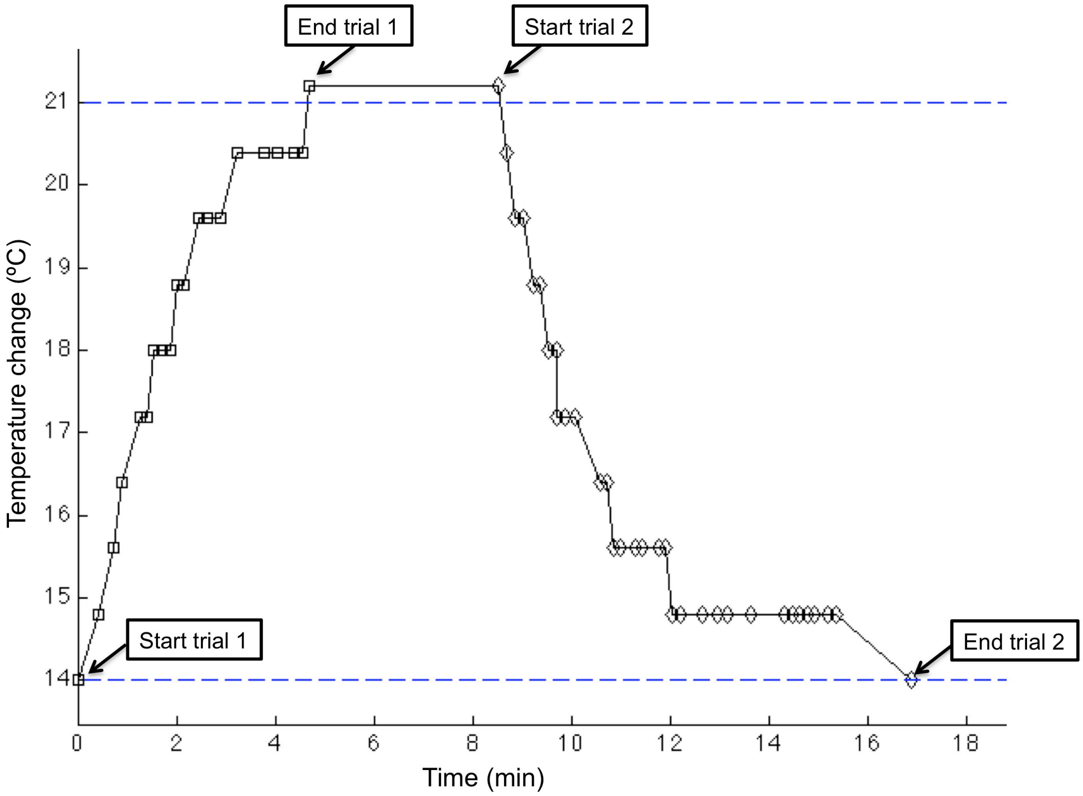

Figure 3-1. An example tag temperature acclimation curve for an internally tagged fish experiencing a 7ºC temperature change. Dashed horizontal lines represent the ambient water temperature in tank 1 (14ºC) and tank 2 (21ºC). Trial 1 (squares) shows a fish experiencing an increase in ambient temperature; the fish was moved from tank 1 to tank 2 at the start of trial 1. Trial 2 (diamonds) shows the same fish experiencing a decrease in ambient temperature; the fish was moved from tank 2 to tank 1 at the start of trial 2.

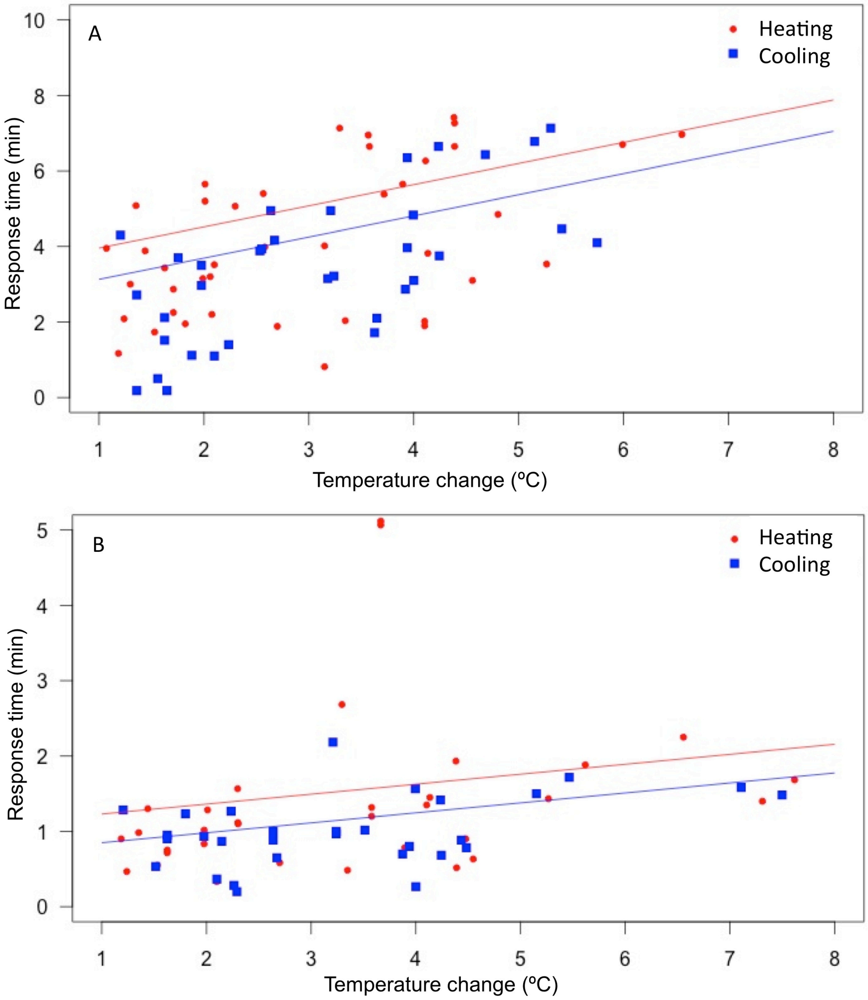

Figure 3-2. Predicted radio tag response time (min) as a function of the magnitude of change in water temperature for (A) all tagged fish and (B) bare (i.e. not in fish) tags. Raw data (points) and model fit (lines) are plotted for fish of mean fork length (~175 mm), for heating water temperature trials (red) and cooling water temperature trials (blue).

3.8 Tables

Table 3-1. Results of information-criterion comparisons for top 95% confidence set of models for radio tagged fish.

Model[a]	**df**[b]	**ΔAICc**[c]	**W_i**[d]	**ΣW_i**[e]
~ dT + D + FL	6	0.00	0.49	0.46
~ dT + D + W	6	1.60	0.22	0.67
~ dT + D + FL + W	7	1.83	0.19	0.85
~ dT + D	5	4.25	0.06	0.91
~ dT + FL	5	4.74	0.05	0.95

[a] *Variables in the model: dT, magnitude of temperature change (ºC); D, direction of temperature change; FL, fork length (mm); W, weight (g).*

[b] *Degrees of freedom.*

[c] *Change in Akaike's information criterion.*

[d] *Model (i) weight using Akaike's information criterion.*

[e] *Cumulative model (i) Akaike's information criterion weight.*

Table 3-2. Model-averaged parameter estimates, standard error, 95% confidence intervals, and relative variable importance (RVI) for radio tagged fish, for the subset of models with cumulative weighted Akaike information criterion values ≤95%. Main effects parameters: dT, magnitude of change in water temperature (ºC); D, direction of water temperature change (heating vs. cooling); FL, fork length (mm); W, weight (g).

Parameter	**Coefficient**	**Std. Error**	**Lower CI**	**Upper CI**	**RVI**
Intercept	-28.66	106.77	-238.96	181.63	n/a
dT	33.61	7.10	19.57	47.65	1.00
D	49.69	19.16	11.82	87.57	0.95
FL	1.03	0.65	-0.28	2.33	0.72
W	0.07	0.53	-0.99	1.12	0.41

Table 3-3. Results of information-criterion comparisons for top 95% confidence set of models for bare radio tags. Subscripts are the same as Table 3-1.

Model[a]	**df**[b]	**ΔAICc**[c]	$\mathbf{W_i^d}$	$\mathbf{\Sigma W_i^e}$
~ dT + D	5	0.00	0.45	0.39
~ D	4	0.95	0.28	0.63
~ dT	4	1.06	0.27	0.86

Table 3-4. Model-averaged parameter estimates, standard error, and 95% confidence intervals for bare tags (i.e. not in fish), for the subset of models with cumulative weighted Akaike information criterion values ≤95%. Main effects parameters: dT, magnitude of change in water temperature (ºC); D, direction of water temperature change (heating vs. cooling).

Parameter	**Coefficient**	**Std. Error**	**Lower CI**	**Upper CI**
Intercept	42.98	17.96	7.24	78.71
dT	7.94	3.94	0.03	15.84
D	22.84	12.42	-2.04	47.72

4. Appendix A: Methods and rationale for definition of thermal refugia (Chapter 1)

The temperature threshold for designating the area at each study site defined as a thermal refuge (i.e. any area <3°C below mainstem temperature for a given point in time) was determined through a proportional assessment of fish behavioral thermoregulation (mainstem temperature – fish body temperature) relative to the availability of water temperatures (range: mainstem – tributary temperature). The results of the proportional assessment show a bimodal distribution with a minimum near 3°C, indicating that fish were primarily in either warmer habitat (water temperatures within 2°C of the mainstem), or cooler habitat (lower than 3°C below mainstem temperature) (Fig. 4-1). To test if our definition of refuges was biasing our results, we ran the mixed effects model with different combinations of mainstem and refuge definitions; the results of all combinations were qualitatively similar to those of the final model.

4.1 Figures

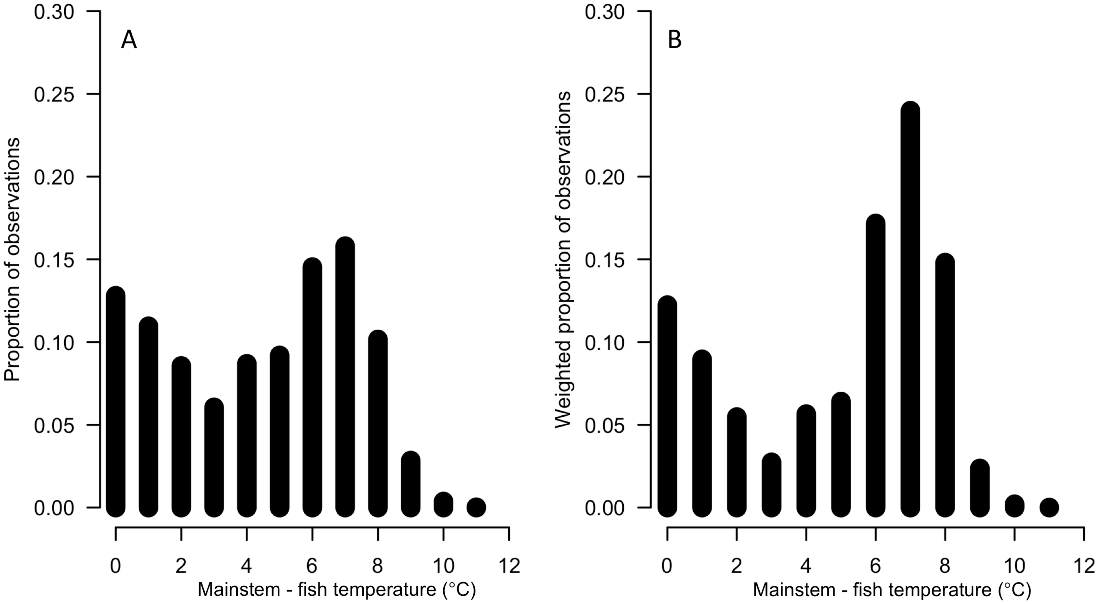

Figure 4-1. (A) The distribution of behavioral thermoregulation (mainstem – fish temperature) across individuals from 2010-2012 (total detections = 130,272) and (B) proportional assessment of thermoregulation weighted by the instantaneous range of available temperatures (range = tributary:mainstem temperature).

5. Appendix B: Supplementary study site and tagging information (Chapter 1)

5.1 Tables

Table 5-1. Summary of fish (n = 127) used in the logistic model. Total detections per fish reflect dataset subsampled at 20-minute intervals. (Origin: locations within refuge where individual was caught for tagging).

ID	Tag site	Tag date	Origin	FL (mm)	Weight (g)	Total days detected	Total detections
1	Beaver	13-Jul-2010	Tributary	135	33.2	50	3037
2	Beaver	13-Jul-2010	Tributary	177	68.5	26	853
3	Beaver	13-Jul-2010	Mixing zone	205	115	5	241
4	Beaver	9-Aug-2010	Mixing zone	149	38.9	28	907
5	Beaver	9-Aug-2010	Mixing zone	275	243	5	192
6	Beaver	9-Aug-2010	Mixing zone	163	51.1	6	140
7	Beaver	9-Aug-2010	Mixing zone	168	53.1	7	183
8	Beaver	19-Aug-2010	Tributary	172	60.3	14	396
9	Beaver	19-Aug-2010	Tributary	134	34.6	44	2621
10	Beaver	19-Aug-2010	Tributary	185	67.7	11	404
11	Beaver	19-Aug-2010	Tributary	146	38.6	4	87
12	Beaver	12-Jul-2011	Mixing zone	198	101.9	27	1370
13	Beaver	12-Jul-2011	Mixing zone	201	107.3	4	161
14	Beaver	12-Jul-2011	Mixing zone	203	99.7	6	282
15	Beaver	12-Jul-2011	Mixing zone	235	156.3	9	134
16	Beaver	12-Jul-2011	Mixing zone	181	73.9	15	894
17	Beaver	12-Jul-2011	Mixing zone	288	286.9	21	1052
18	Beaver	12-Jul-2011	Mixing zone	160	50.1	47	2280
19	Beaver	12-Jul-2011	Mixing zone	245	187.4	9	403
20	Beaver	12-Jul-2011	Mixing zone	172	61.8	18	530
21	Beaver	12-Jul-2011	Mixing zone	156	48.6	18	508
22	Beaver	12-Jul-2011	Mixing zone	180	71.8	7	246
23	Beaver	12-Jul-2011	Mixing zone	264	236.6	19	826
24	Beaver	1-Aug-2011	Tributary	136	29.9	2	77
25	Beaver	1-Aug-2011	Mixing zone	147	38.8	20	1012

26	Beaver	1-Aug-2011	Mixing zone	148	40.8	29	1327
27	Beaver	1-Aug-2011	Mixing zone	198	93.4	20	259
28	Beaver	1-Aug-2011	Mixing zone	149	35.6	27	833
29	Beaver	1-Aug-2011	Mixing zone	156	44	5	268
30	Beaver	29-Aug-2011	Mixing zone	193	79.7	3	109
31	Beaver	29-Aug-2011	Mixing zone	264	218.2	5	128
32	Beaver	1-Sep-2011	Mixing zone	224	127.6	27	1247
33	Beaver	1-Sep-2011	Tributary	159	53.7	12	94
34	Beaver	1-Sep-2011	Mixing zone	148	39.8	21	149
35	Beaver	1-Sep-2011	Mixing zone	139	33.5	27	1445
36	Beaver	1-Sep-2011	Tributary	304	291.5	24	733
37	Beaver	1-Sep-2011	Tributary	179	83.5	26	612
38	Beaver	12-Jul-2012	Mixing zone	160	54	3	65
39	Beaver	12-Jul-2012	Tributary	200	92.8	15	736
40	Beaver	12-Jul-2012	Tributary	161	53.9	20	1055
41	Beaver	12-Jul-2012	Tributary	139	33.3	49	3093
42	Beaver	12-Jul-2012	Mixing zone	187	85.7	12	542
43	Beaver	12-Jul-2012	Mixing zone	163	53	37	1851
44	Beaver	12-Jul-2012	Mixing zone	144	35.1	12	737
45	Beaver	1-Aug-2012	Mixing zone	176	61.8	24	485
46	Beaver	1-Aug-2012	Mixing zone	140	29.7	3	141
47	Beaver	1-Aug-2012	Tributary	185	76.8	7	118
48	Beaver	2-Aug-2012	Tributary	143	38.6	12	576
49	Beaver	2-Aug-2012	Tributary	164	55.4	9	335
50	FortGoff	4-Aug-2010	Mixing zone	305	115.8	31	1563
51	FortGoff	4-Aug-2010	Mixing zone	167	90.4	4	138
52	FortGoff	4-Aug-2010	Mixing zone	172	81.3	50	1396
53	FortGoff	4-Aug-2010	Mixing zone	185	49.4	51	2987
54	FortGoff	4-Aug-2010	Mixing zone	150	123.8	57	2975
55	FortGoff	4-Aug-2010	Mixing zone	290	97	7	138
56	FortGoff	4-Aug-2010	Mixing zone	159	92	6	154
57	FortGoff	4-Aug-2010	Mixing zone	160	59.2	5	224
58	FortGoff	4-Aug-2010	Mixing zone	190	83.8	41	1063
59	FortGoff	26-Aug-2010	Mixing zone	183	102.6	17	771
60	FortGoff	26-Aug-2010	Mixing zone	188	50.4	30	987

61	FortGoff	14-Jul-2011	Mixing zone	358	383.3	18	793
62	FortGoff	14-Jul-2011	Tributary	137	34.6	20	548
63	FortGoff	14-Jul-2011	Tributary	194	91.8	15	475
64	FortGoff	14-Jul-2011	Tributary	159	51	45	1989
65	FortGoff	14-Jul-2011	Tributary	169	61	5	58
66	FortGoff	3-Aug-2011	Tributary	205	122.2	46	2967
67	FortGoff	3-Aug-2011	Tributary	159	49.5	25	692
68	FortGoff	3-Aug-2011	Tributary	149	46.6	42	1841
69	FortGoff	3-Aug-2011	Tributary	134	31.1	55	2558
70	FortGoff	3-Aug-2011	Tributary	154	50.3	46	1237
71	FortGoff	3-Aug-2011	Tributary	165	56.1	51	2750
72	FortGoff	3-Aug-2011	Tributary	141	37.4	27	1264
73	FortGoff	3-Aug-2011	Tributary	140	38.3	44	1966
74	FortGoff	3-Aug-2011	Tributary	153	42.8	44	2272
75	FortGoff	3-Aug-2011	Tributary	182	84	40	1647
76	FortGoff	3-Aug-2011	Tributary	257	223.9	10	524
77	FortGoff	3-Aug-2011	Tributary	159	45.9	38	1352
78	FortGoff	3-Aug-2011	Tributary	135	34.6	38	1013
79	FortGoff	31-Aug-2011	Tributary	158	48.1	32	2182
80	FortGoff	31-Aug-2011	Tributary	142	39.1	21	176
81	FortGoff	31-Aug-2011	Tributary	160	54.1	32	1510
82	FortGoff	31-Aug-2011	Tributary	160	46.1	32	1679
83	FortGoff	31-Aug-2011	Tributary	145	38.2	24	117
84	FortGoff	31-Aug-2011	Tributary	140	37	32	795
85	FortGoff	31-Aug-2011	Tributary	174	58.7	28	275
86	FortGoff	31-Aug-2011	Tributary	179	72	3	82
87	Grider	14-Jul-2010	Mixing zone	205	176.5	29	1100
88	Grider	14-Jul-2010	Mixing zone	202	74.8	37	956
89	Grider	14-Jul-2010	Mixing zone	192	100.2	66	3609
90	Grider	14-Jul-2010	Mixing zone	189	45	24	210
91	Grider	19-Jul-2010	Mixing zone	187	46.2	3	56
92	Grider	19-Jul-2010	Mixing zone	164	81.1	6	63
93	Grider	5-Aug-2010	Mixing zone	235	89.2	3	79
94	Grider	5-Aug-2010	Mixing zone	182	69.3	11	265
95	Grider	5-Aug-2010	Mixing zone	175	81.5	8	511

96	Grider	5-Aug-2010	Mixing zone	147	87.2	20	402
97	Grider	13-Jul-2011	Mixing zone	152	44.7	3	154
98	Grider	13-Jul-2011	Mixing zone	219	140.2	2	57
99	Grider	13-Jul-2011	Mixing zone	283	288.5	43	1100
100	Grider	13-Jul-2011	Tributary	160	52.4	8	302
101	Grider	13-Jul-2011	Tributary	157	45.9	7	186
102	Grider	2-Aug-2011	Mixing zone	290	314.6	38	938
103	Grider	2-Aug-2011	Tributary	153	48.8	20	1243
104	Grider	17-Aug-2011	Tributary	170	55.4	8	358
105	Grider	30-Aug-2011	Mixing zone	385	401.7	32	1045
106	Grider	30-Aug-2011	Mixing zone	266	249.7	10	139
107	Grider	30-Aug-2011	Mixing zone	310	370.6	16	406
108	Grider	30-Aug-2011	Mixing zone	193	91.5	10	324
109	Grider	30-Aug-2011	Mixing zone	385	470.3	16	720
110	Grider	30-Aug-2011	Tributary	344	344.5	16	295
111	Grider	30-Aug-2011	Mixing zone	155	47.7	32	2153
112	Thompson	3-Aug-2010	Mixing zone	192	85.8	5	153
113	Thompson	3-Aug-2010	Mixing zone	191	76.1	3	90
114	Thompson	3-Aug-2010	Mixing zone	215	114.5	17	929
115	Thompson	3-Aug-2010	Mixing zone	238	144	39	1257
116	Thompson	3-Aug-2010	Mixing zone	301	333	8	402
117	Thompson	3-Aug-2010	Mixing zone	194	84.5	45	2732
118	Thompson	3-Aug-2010	Mixing zone	178	62.8	34	1253
119	Thompson	3-Aug-2010	Mixing zone	171	55.6	43	2442
120	Thompson	27-Aug-2010	Mixing zone	197	85.4	17	738
121	Thompson	27-Aug-2010	Mixing zone	169	55.6	33	1137
122	Thompson	27-Aug-2010	Mixing zone	215	110.1	23	69
123	Thompson	27-Aug-2010	Mixing zone	184	65.3	17	520
124	Thompson	27-Aug-2010	Mixing zone	219	117.5	33	2150
125	Thompson	27-Aug-2010	Mixing zone	181	67.3	16	1007
126	Thompson	27-Aug-2010	Mixing zone	195	79.2	32	1499
127	Thompson	27-Aug-2010	Mixing zone	221	104	20	600

5.2 Figures

Figure 5-1. View of the tributary confluence with the Klamath River at each study site. (A) Beaver Creek entering on the left, looking upstream. (B) Fort Goff Creek entering on the left, looking upstream. (C) Grider Creek entering on the left, looking downstream. (D) Thompson Creek entering on the left, looking upstream.

6. Appendix C: Methods for determining mixed effects model structure (Chapter 1)

We tested explicitly for temporal autocorrelation in the model by examining the likelihood of misclassifying the response variable *(state)*. For multiples of the time interval used in the model (20 minutes), we compared the likelihood that successive pairs of observations were misclassified to the likelihood that pairs of observations taken at random from the dataset (i.e. the null distribution) were misclassified, and asked whether these values differed (upper 90% confidence interval).

To determine the optimal random effects structure, we constructed ROC curves and compared the area under the curve (AUC) test statistic for candidate random effects structures (random intercept only, and random intercept and slope), as well as examining the histograms of the random effects residuals for normality. We constructed one model for all three years of data, since analyses of each year separately indicated that parameter values were similar across years.

7. Appendix D: Supplementary results (Chapter 1)

7.1 Tables

Table 7-1. Mean [standard deviation] in environmental variables at study sites for the time period when tagged fish were present at each site (date range reflects dataset used in the logistic model).

Year	**Study site**	**Dates**	**Mainstem temperature (ºC)**	**Temperature differential (ºC)**	**Diel mainstem variation (ºC)**	**Flow (cms)**
2010	Beaver	7/13 - 9/30	21.16 [2.38]	6.97 [1.27]	1.36 [0.38]	27.6 [3.57]
	Grider	7/13 - 9/30	21.23 [2.50]	7.42 [0.84]	2.15 [0.46]	33.5 [3.02]
	Fort Goff	8/4 - 9/30	20.40 [2.42]	6.00 [0.69]	2.07 [0.65]	33.5 [3.02]
	Thompson	8/3 - 9/30	20.93 [2.58]	7.39 [0.95]	2.61 [1.01]	33.5 [3.02]
2011	Beaver	7/13 - 9/30	21.83 [1.54]	7.12 [1.37]	1.45 [0.41]	30.2 [0.52]
	Grider	7/13 - 9/30	21.31 [1.91]	6.59 [1.28]	2.43 [0.51]	43.0 [8.43]
	Fort Goff	7/13 - 9/30	21.54 [1.87]	6.40 [0.95]	2.56 [0.51]	43.0 [8.43]
2012	Beaver	7/12 - 8/31	22.96 [0.95]	6.79 [1.20]	1.33 [0.35]	29.3 [2.18]

Table 7-2. The number of individuals tagged at each study site (N) for fish used in the logistic model, and mean (across individuals) proportion of time that fish were detected in refuges.

Year	**Study site**	**N**	**Proportion of time in refuges (%)**
2010	Beaver	11	77.6
	Grider	10	69.6
	Fort Goff	11	34.6
	Thompson	16	49.2
2011	Beaver	27	72.4
	Grider	13	62.4
	Fort Goff	27	93.6
2012	Beaver	12	86.3

Table 7-3. Mortality of tagged fish by year. Fish mortality was determined through fish body temperatures (when fish temperature exceeded 30°C, we assumed mortality). In addition, fish with fewer than 50 observations (after 20-minute subsampling of data) were considered to have insufficient data for the model.

Year	Number of tagged fish	Total fish mortality	Percent mortality	Percent of fish with insufficient data for model
2010	102	13	12.75	29.41
2011	130	4	3.08	24.62
2012	25	3	12.00	20.00

Table 7-4. Best estimate of parameter coefficients and standard error for the best-fit logistic mixed effects model. Main effects parameters: FL = fork length, T = mainstem temperature, D = temperature differential, V = diel mainstem variation, F = mainstem flow, L = time of day (day/night).

Parameter	**Coefficient**	**Std. error**	**Z-value**	**P-value**
Intercept	0.978	1.911	0.512	0.609
FL	-0.600	0.393	-1.528	0.127
T	1.152	0.025	46.513	< 0.001
D	0.565	0.020	28.128	< 0.001
V	0.447	0.020	22.295	< 0.001
F	-0.507	0.034	-14.743	< 0.001
L	0.959	0.029	33.041	< 0.001
T:L	-1.173	0.027	-43.713	< 0.001
T:V	0.151	0.017	9.116	< 0.001
T:F	-0.171	0.020	-8.525	< 0.001
T:D	-0.073	0.017	-4.251	< 0.001
V:F	-0.165	0.017	-9.731	< 0.001
FL:T	0.422	0.022	19.577	< 0.001
FL:L	-0.855	0.026	-33.473	< 0.001

7.2 Figures

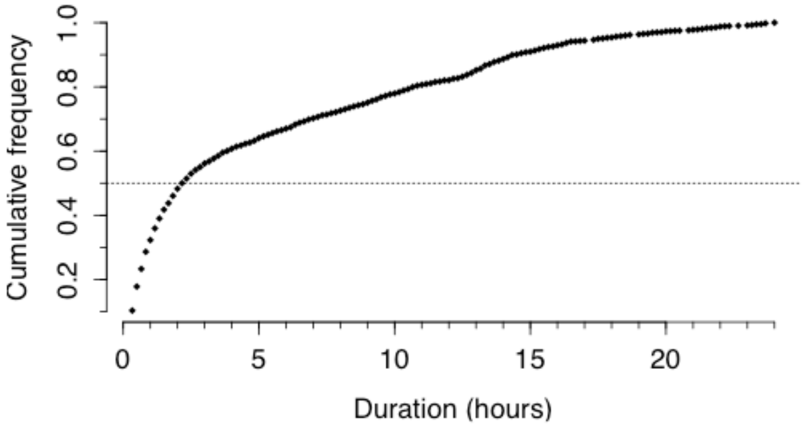

Figure 7-1. Cumulative distribution of duration of habitat shifts from refuges to mainstem across individuals. Horizontal line illustrates that 50% of habitat shifts were less than two hours.

8. Appendix E: Supplementary results for thermal mixing zone area (Chapter 1)

Thermal mixing zone size co-varied with both temperature and flow, so we were unable to de-couple the possible effects of refuge area on steelhead thermal refuge use from these other environmental variables (Tables 8-1 & 8-2). Refuge size may therefore be an important attribute determining when fish use thermal refuges, but a positive association between size and refuge use is likely a function of both the benefits of increased area and the increasing physiological cost of using adjacent mainstem habitat at low mainstem flows.

8.1 Tables

Table 8-1. Results of the logistic mixed effects model for thermal mixing zone size as a function of the four environmental variables used in the main model: T = mainstem temperature, D = temperature differential, V = diel mainstem variation, F = mainstem flow. Study site and year were included as random effects.

Parameter	**Coefficient**	**Std. error**	**T-value**
Intercept	192.023	278.245	0.690
T	9.773	2.744	3.562
V	23.669	9.478	2.497
F	-0.357	0.055	-6.458
D	110.526	4.490	24.614

Table 8-2. Results of the logistic mixed effects model for juvenile steelhead refuge use as a function of thermal mixing zone size. Individual, study site, and year were included as random effects.

Parameter	**Coefficient**	**Std. error**	**Z-value**	**P-value**
Intercept	2.202	0.812	2.711	0.007
Size	5.126	0.097	52.836	< 0.001

8.2 Figures

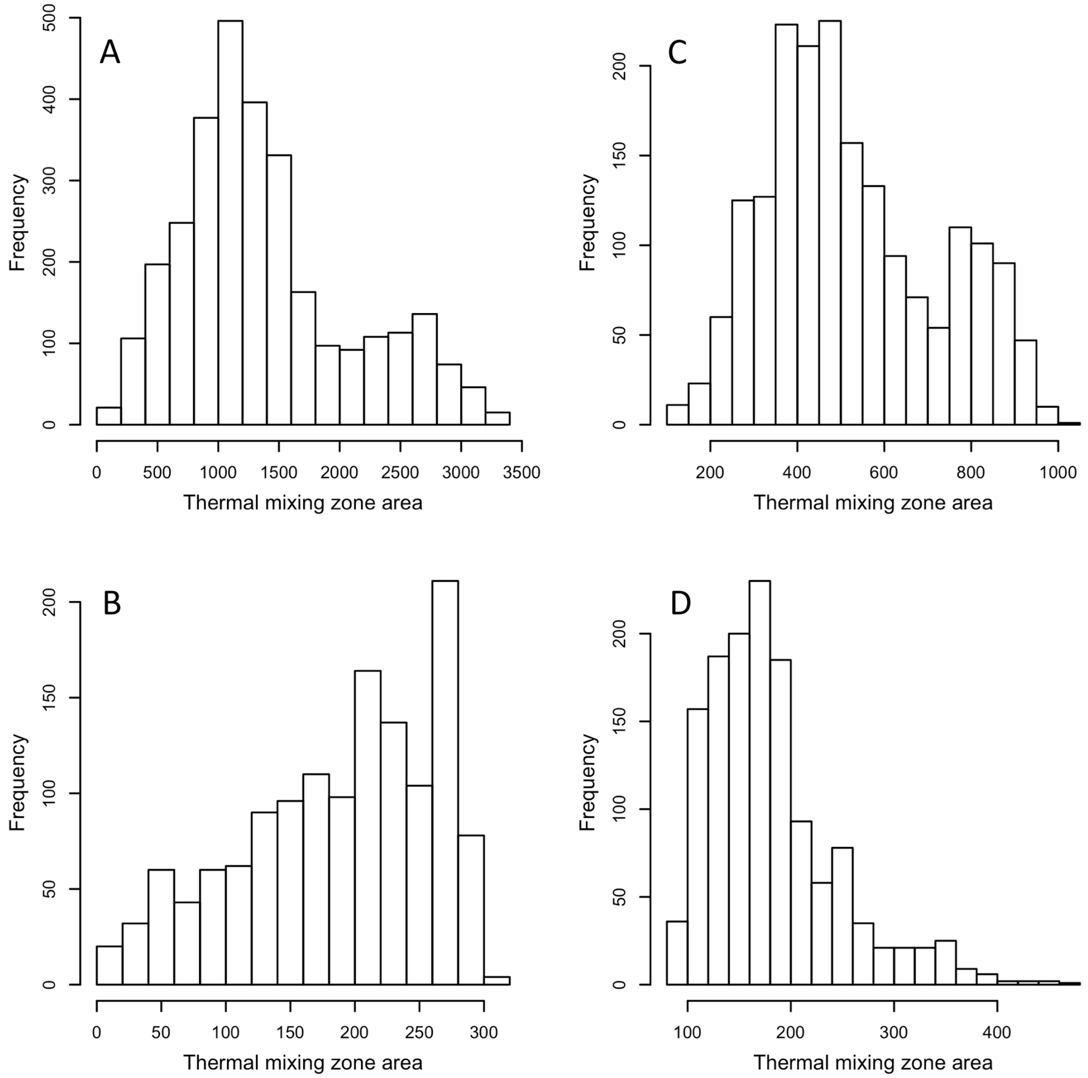

Figure 8-1. Hourly estimates of the area of the thermal mixing zone (m^2) at study sites across years when fish were tagged at (A) Beaver Creek in 2010-2012, (B) Fort Goff in 2010-2011, (C) Grider in 2010-2011, and (D) Thompson in 2010.

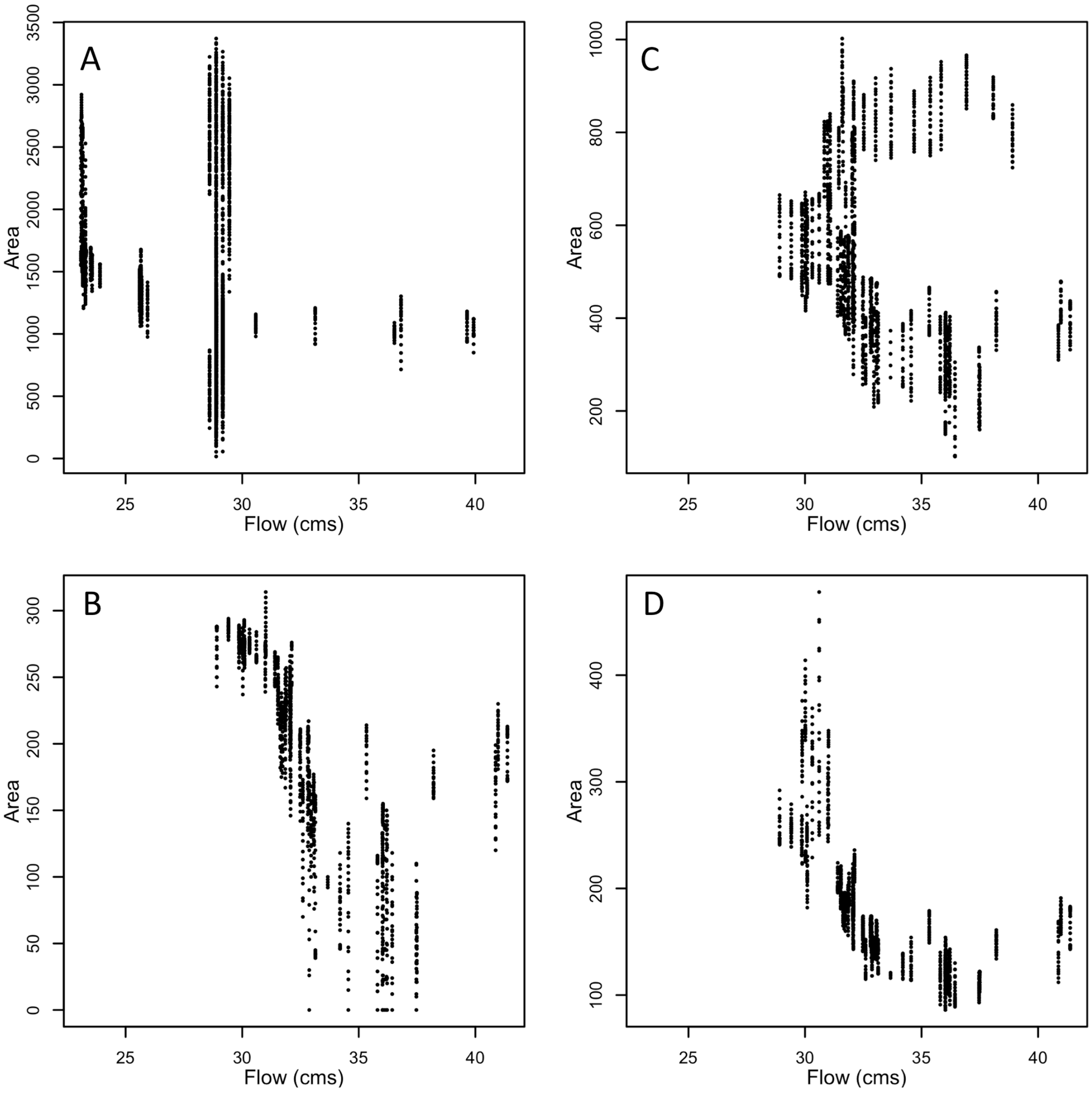

Figure 8-2. Relationship between area of the thermal mixing zone (m^2) and daily mainstem flows (cms) at each study site across years at (A) Beaver Creek in 2010-2012, (B) Fort Goff in 2010-2011, (C) Grider in 2010-2011, and (D) Thompson in 2010. Mainstem flow measurements were taken from daily discharge data from Iron Gate Dam and Seiad Valley monitoring stations.

9. Appendix F: Supplemental results for invertebrate drift and steelhead diet samples (Chapter 2)

9.1 Tables

Table 9-1. 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 9-2. 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 9-3. Sample sizes for diet samples of juvenile steelhead taken in 2010 and 2011.

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

9.2 Figures

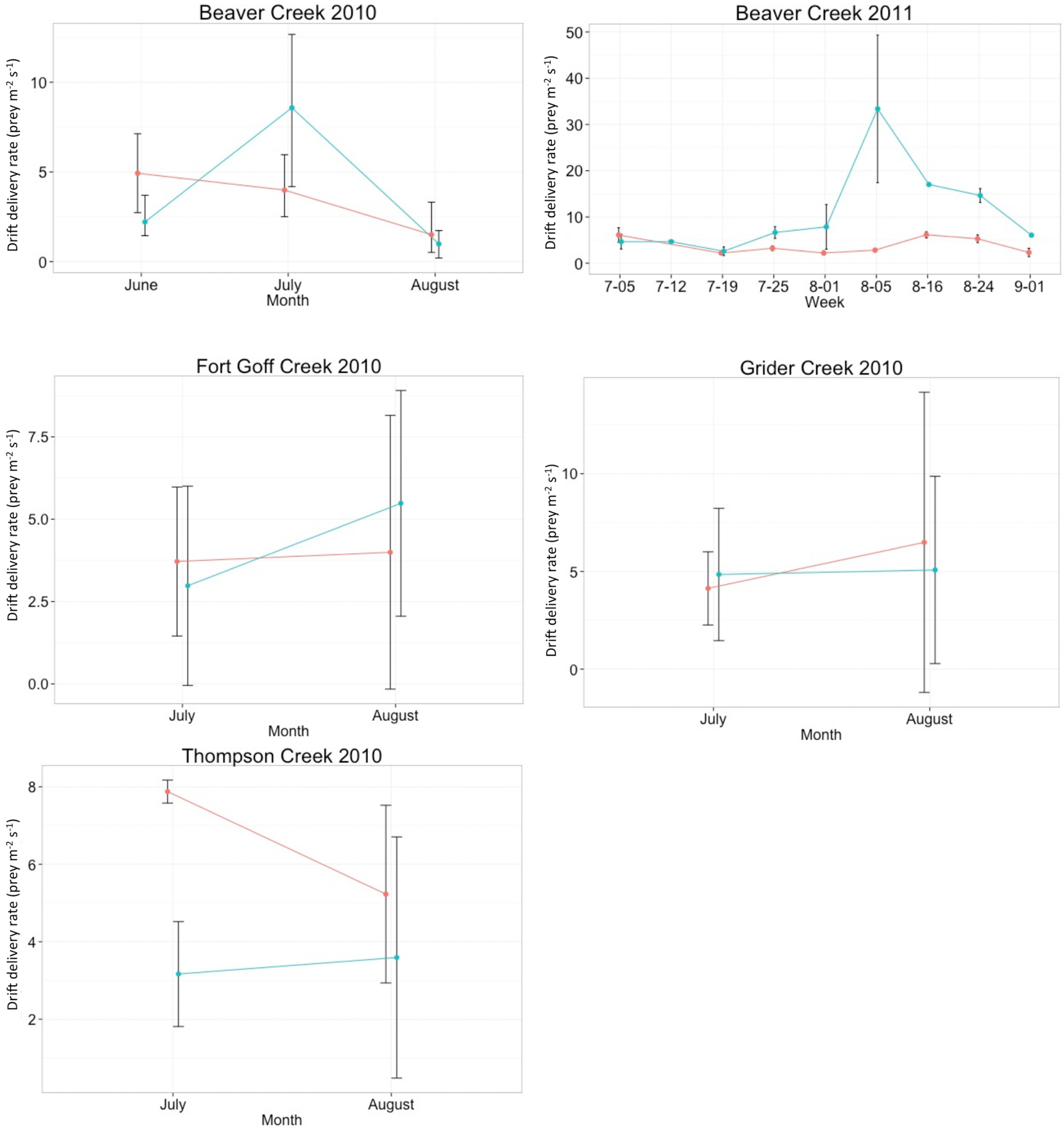

Figure 9-1. Drift delivery rates (mean ± SD) at all sampled sites in 2010 and 2011 (mainstem = red; tributary = blue).

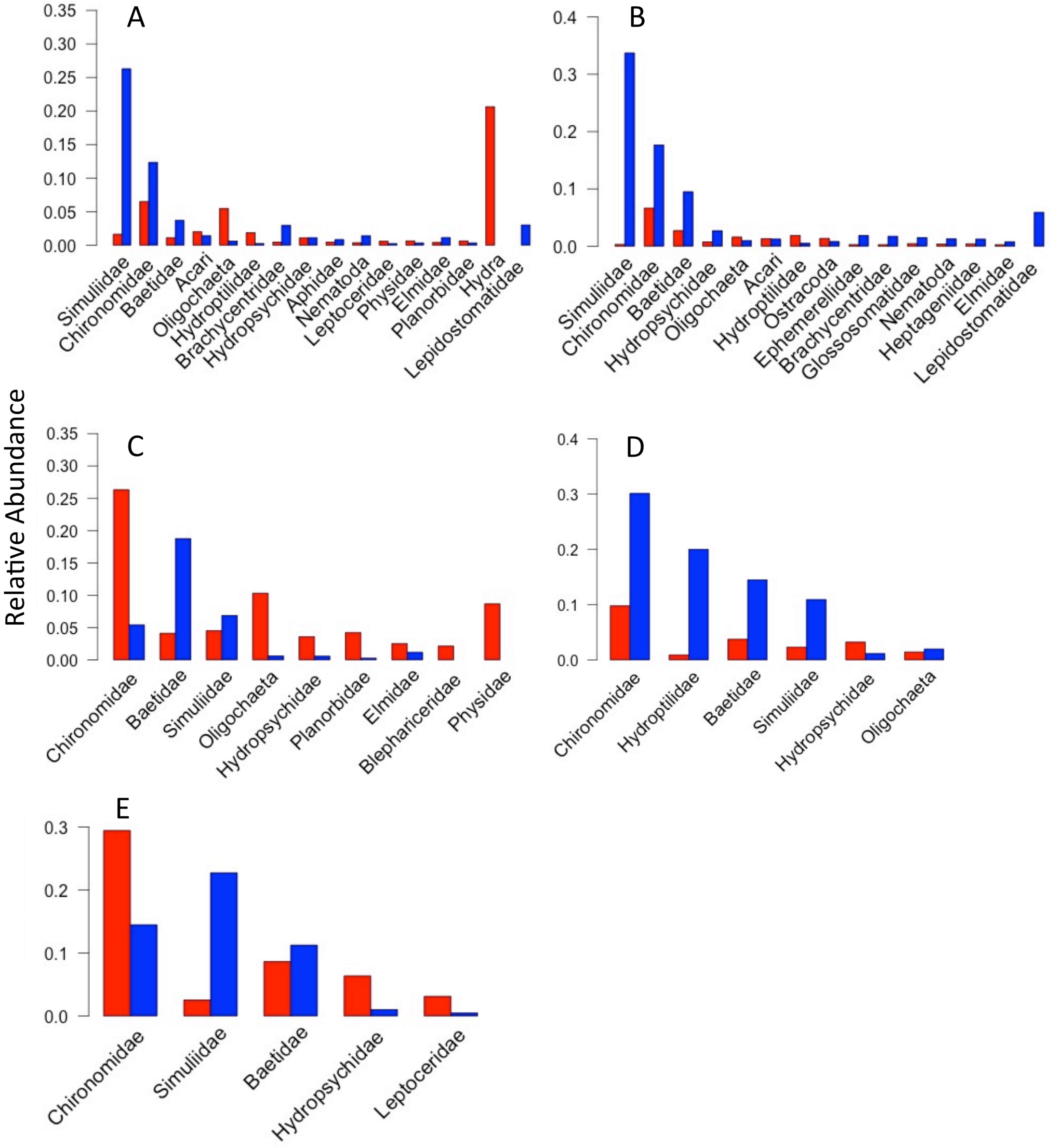

Figure 9-2. Relative abundance of invertebrate species (identified to family) in ten-minute drift samples (mainstem = red; tributary = blue). Taxa included represent most abundant represented in drift (i.e. mean abundance across samples >40 per family). (A) Beaver Creek 2010, (B) Beaver Creek 2011, (C) Grider Creek 2010, (D) Fort Goff Creek 2010, and (E) Thompson Creek 2010.

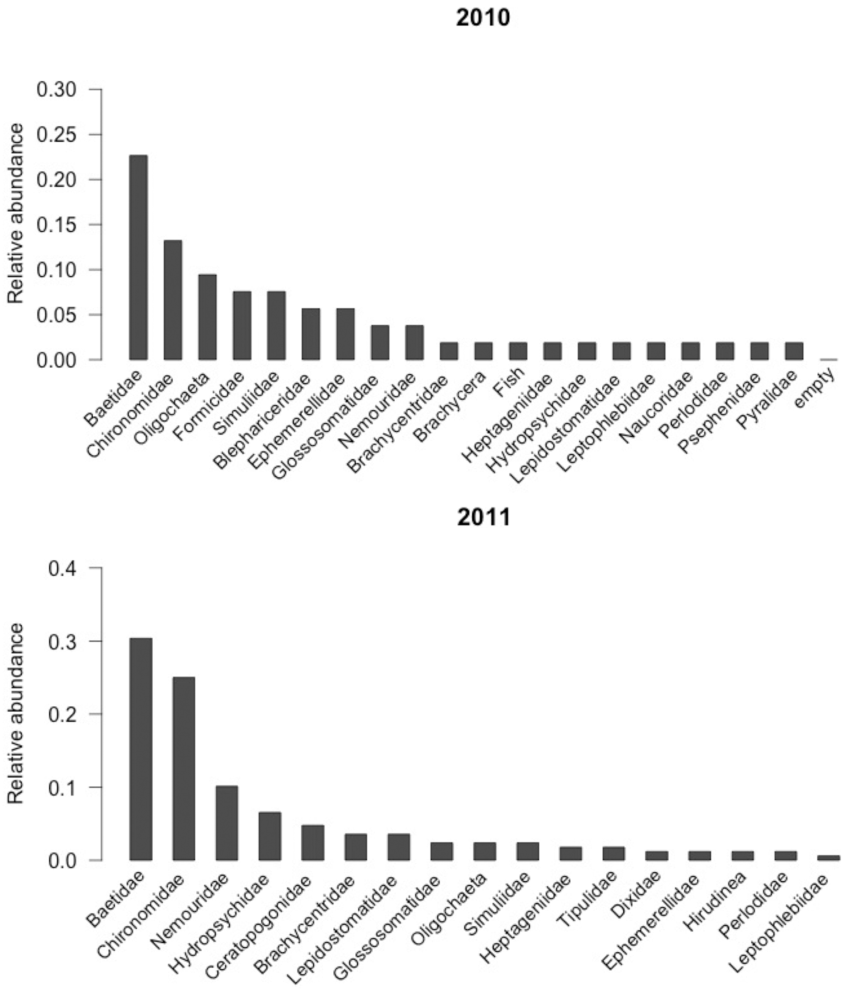

Figure 9-3. Relative abundance of invertebrate species (identified to family) in juvenile steelhead diets in 2010 and 2011.

Discussion

As rising in-stream temperatures exacerbate the effects of an already altered landscape, thermal refuges will form increasingly critical habitat for imperiled salmonids. My research demonstrates the importance of taking fine-scale thermal and trophic resource heterogeneity into account when assessing how juvenile salmonids use thermal refuges, especially as temperatures near thermal tolerance thresholds. Specifically, I demonstrated that both mainstem temperature and sub-daily thermal variation are important factors mediating when juvenile steelhead use thermal refuges (Chapter 1). Moreover, thermal refuges may allow fish to more effectively forage in adjacent mainstem river habitat by providing temporary thermal respite, and thereby decreasing the negative physiological effects of elevated water temperatures. This could be a currently under-valued benefit of maintaining thermal refuges at tributary confluences – there is almost no discussion of the invertebrate prey landscape in thermal refuges in the literature – especially as juvenile salmonids in refuges rely primarily on mainstem diet sources (Chapter 2). As studies on thermal refuges become increasingly common (Keppel et al. 2012), temperature-sensitive tags represent an opportunity for assessing thermal habitat use in heterogeneous landscapes. However, it will be important to take into account implanted tag temperature acclimation dynamics (Chapter 3), especially in studies at fine temporal scales and as tag technology allows for collection of increasingly fine-scale temporal data.

This dissertation supports several important inferences for habitat management and future research on thermal refuges. While mainstem temperature is the most important driver of thermal refuge use by juvenile steelhead, diel temperature variation could make the effects of increased mean temperatures even more severe than expected (Chapter 1), yet management of in-stream temperatures is often based on monthly mean temperatures (North Coast Regional Water Quality Control Board). It may therefore be important to include thermal refuges and fine-scale thermal dynamics in climate envelope models predicting species' response to climate change, yet currently most climate envelope models largely ignore fine-scale thermal refuges (Pearson and Dawson 2003, Lawler et al. 2006, Willis and Bhagwat 2009). In addition, while thermal refuges provide important physiological respite from stressful temperatures, maintaining access to sub-optimal mainstem habitat may provide a critical food resource and thus be important for the efficacy of thermal refuges; habitat connectivity is therefore particularly important for refuges. These dynamics could lead to a trade-off between thermal and trophic resources if mainstem habitat becomes inaccessible, due to a lack of connectivity between tributaries and the mainstem river (e.g., low stream flows), or when mainstem temperatures rise above 25°C (Brewitt and Danner 2014). Moreover, as mainstem river habitat reaches thermal tolerance thresholds for salmonids and increasing numbers of fish move into smaller areas of suitable habitat (i.e. thermal refuges), the population dynamics within refuges could dictate the overall carrying capacity of the river (Ebersole et al. 2003, Petty et al. 2012, Huntsman and Petty 2014). The efficacy of habitat restoration efforts will therefore likely depend on mechanisms of density-dependence (Chapter 2), and how population size responds to restoration of habitat quality and area (Greene and Beechie 2004).

Rivers across the U.S are experiencing warming trends, creating a thermally stressful in-stream environment for both Pacific and Atlantic salmonids (Kaushal et al. 2010, Isaak 2011), as well as for other coldwater fish (Mohseni 2003, Hari 2006). Similar trends are occurring globally (Daufresne 2004, Ficke 2007), such as in Australia where freshwater biodiversity is threatened by increasing temperatures and decreasing rainfall (Davis 2013). Coldwater refuges, whether created by incoming tributaries or other sources (e.g., groundwater), are therefore likely to be important habitat for coldwater fish in freshwater systems world-wide. While large-scale studies are important for gaining a broad understanding of the effects of rising ambient temperatures on a given species, this dissertation emphasizes the importance of taking into account smaller-scale dynamics as they could influence species persistence and distributions. Thermal refuges may be a central defining feature in the persistence of future populations at the trailing edges of a species' distribution (McLaughlin and Zavaleta 2012). This dissertation contributes to the basic understanding of the ecology of thermal refuges and demonstrates potential trade-offs and limits of these habitats.

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