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

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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.

Key words: diel temperature variation; Klamath River; *Oncorhynchus mykiss*; radio telemetry; spatio-temporal variation; steelhead; temperature; thermal refuges; thermal tolerance.

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INTRODUCTION

Large-scale habitat degradation and climate change have led to range retractions and simultaneous increases in air and water temperatures in remaining habitat that reach or exceed thermal limits for many species (Travis 2003, Mac Nally et al. 2009, 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 $\geq$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 and 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 ongoing '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.

METHODS

Study system

We conducted this research on the lower Klamath River in northern California between 2010 and 2012 (Fig. 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

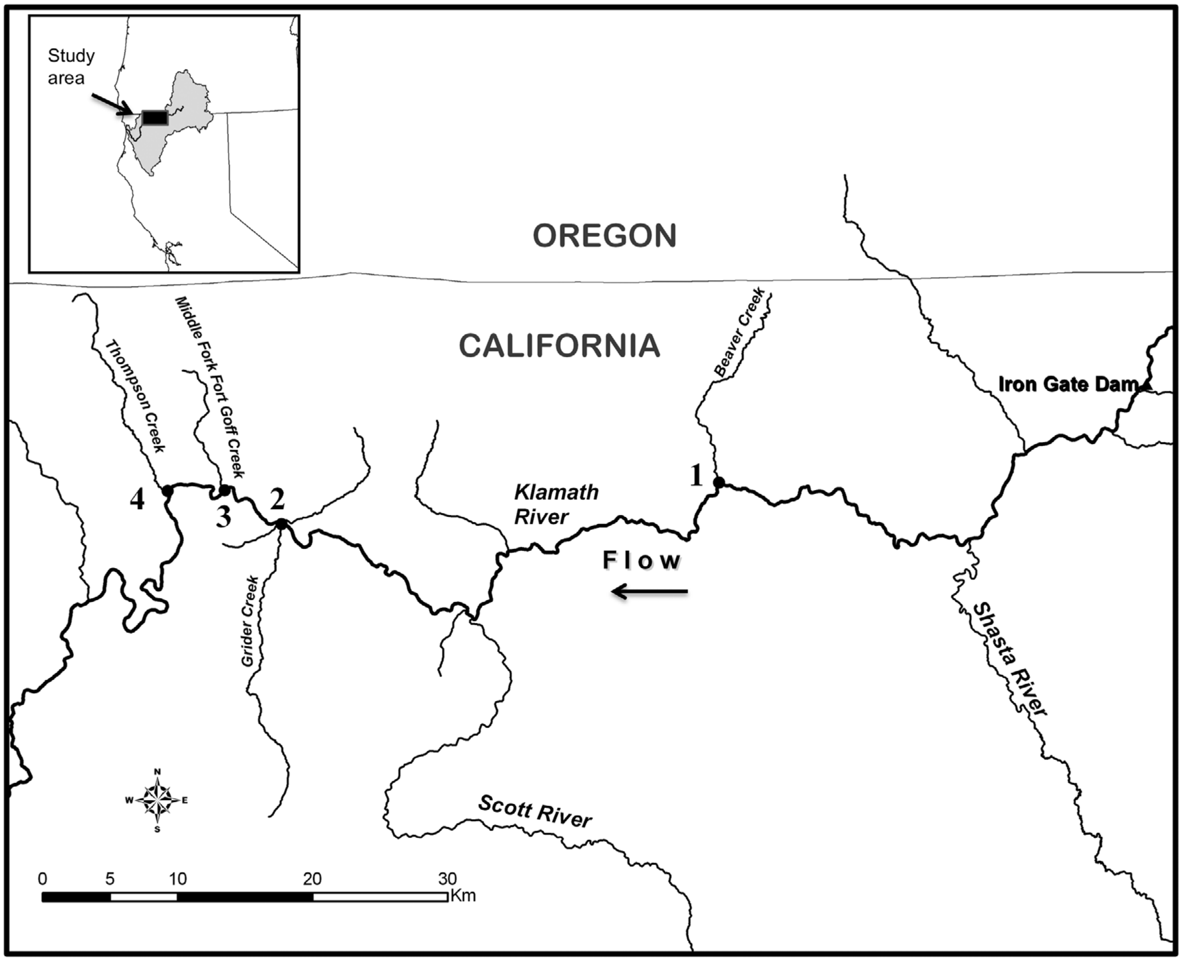

Fig. 1. Location of study sites along the Klamath River (1 = Beaver Creek; 2 = Grider Creek; 3 = Fort Goff Creek; 4 = Thompson Creek). Inset shows location of study area within the watershed.

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° to 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 coldwater 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. 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.

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, 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 and 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. 2). All data loggers were placed near the riverbed to minimize thermal input from direct sunlight, at depths ranging from 0.5 m to 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. 2A) and discussion purposes.

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 and October 2010 ($n = 102$) and 2011 ($n = 130$), and July and August 2012 ($n = 25$). Fish were caught ($n \sim 20$–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 to 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.

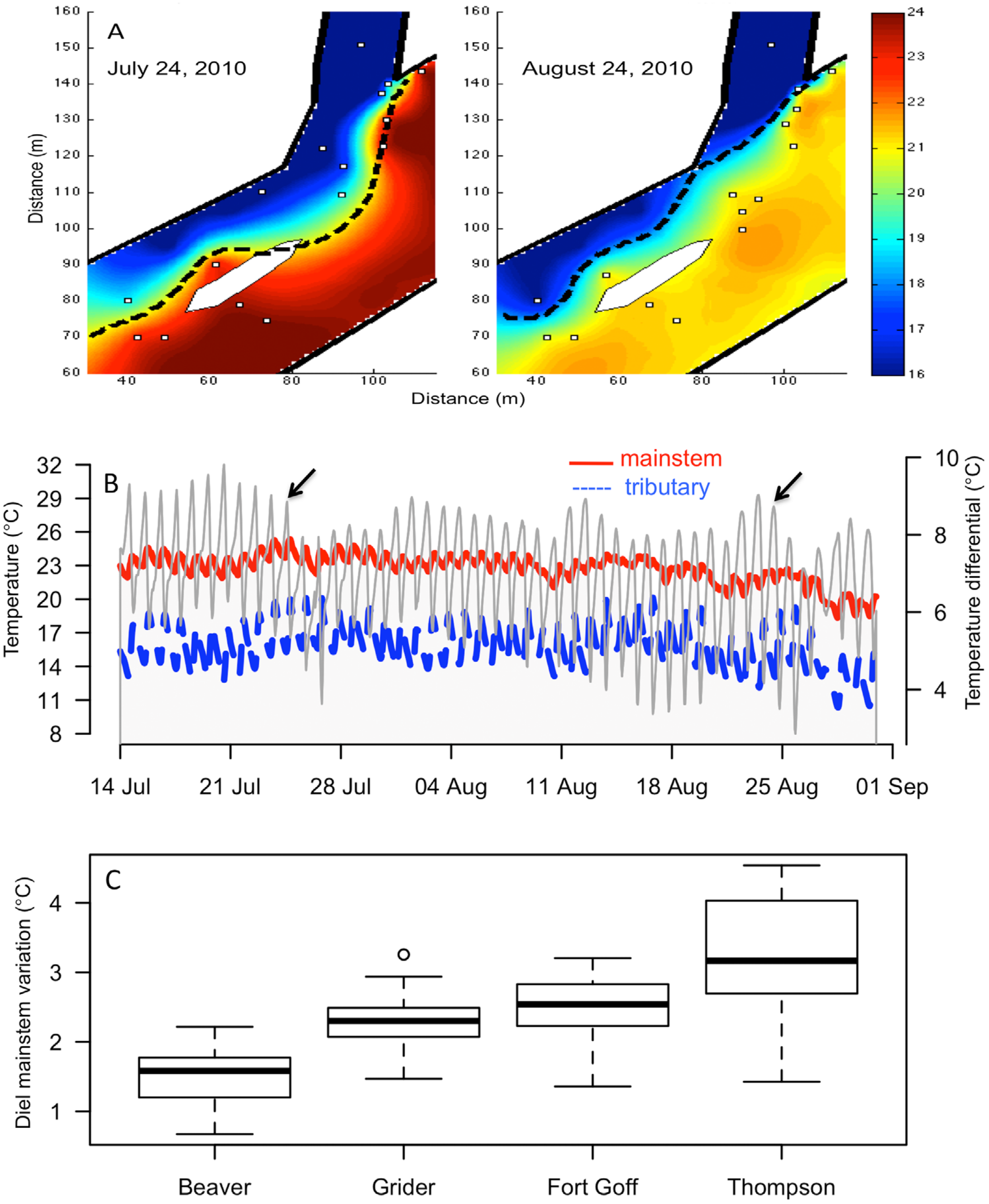

Fig. 2. Spatio-temporal heterogeneity in water temperatures. (A) Interpolated water temperatures (°C) at 9:00 on 24 July 2010 and 24 August 2010 at Beaver Creek (dates indicated by black arrows in (B)); white denotes land, and white squares are locations of water temperature loggers. Distance (m) on 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.

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 subsampling 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, unpublished data). 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 ($T \times L$), 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 ($T \times F$), temperature differential ($T \times D$), and diel mainstem variation ($T \times \mathrm{V}$), 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 ($V \times F$), 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 ($\mathrm{FL} \times T$), and between fork length and time of day ($\mathrm{FL} \times L$), 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 B1). 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 unsampled 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).

RESULTS

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. 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. E1 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 = 6.48° ± 0.59°C) (Fig. 2B; Appendix D: Table D1). In addition, daily mean and maximum mainstem temperatures varied longitudinally along the river, and diel mainstem fluctuations varied across sites and years (mean = 2.0° ± 0.54°C) (Fig. 2C; Appendix D: Table D1). 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 m^3/s; Appendix D: Table D1), as well as between study sites, as tributary inputs increased mean flows substantially between upstream and downstream sites.

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. 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 D2); 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. 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 D3).

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. 4; Appendix D: Table D4). Fish of all sizes ($FL = 183 \pm 50$ mm) behaved similarly during the day, with the probability of refuge use increasing with rising mainstem temperatures (Fig. 5A, B; mean expected temperature at which >80% of fish entered refuges was 22.3°C). However, smaller fish (approximate first

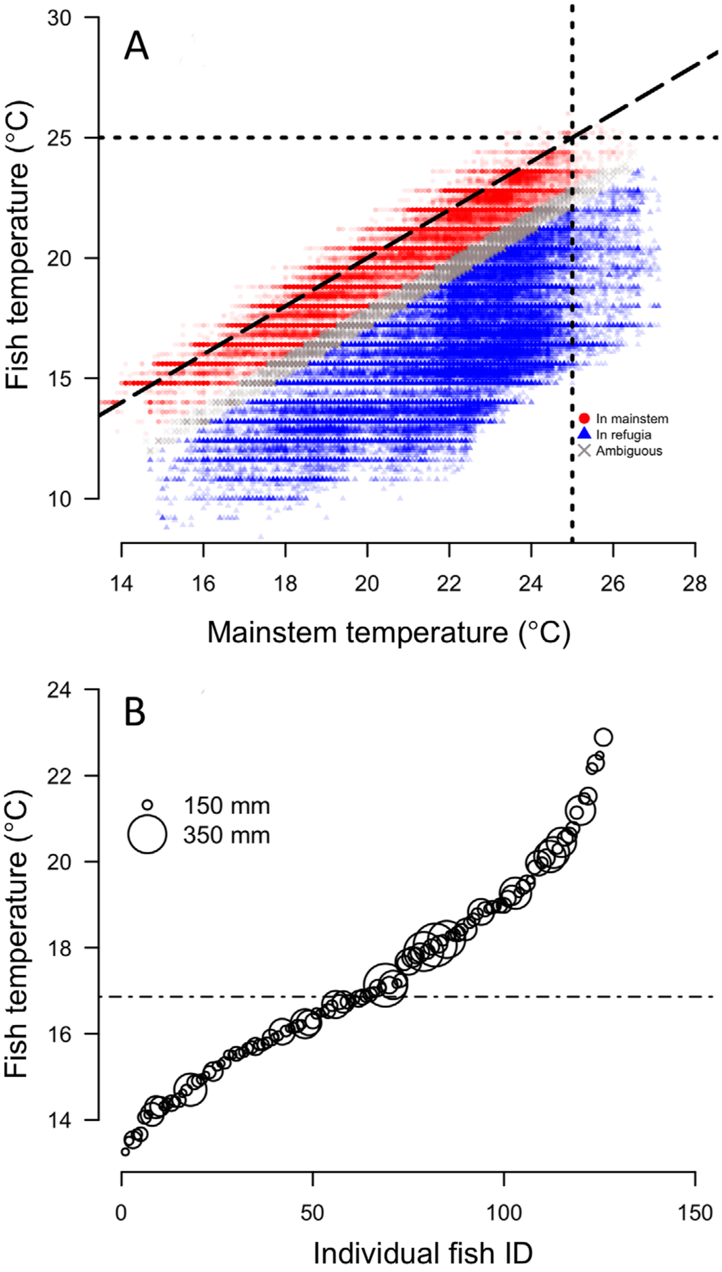

Fig. 3. (A) Fish temperatures versus simultaneous mainstem temperatures across sites and years (total detections = 130,272). Symbols correspond to fish detections assigned to either a mainstem state (red circles), refuge state (blue triangles), or detections discarded due to ambiguity of state assignment (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).

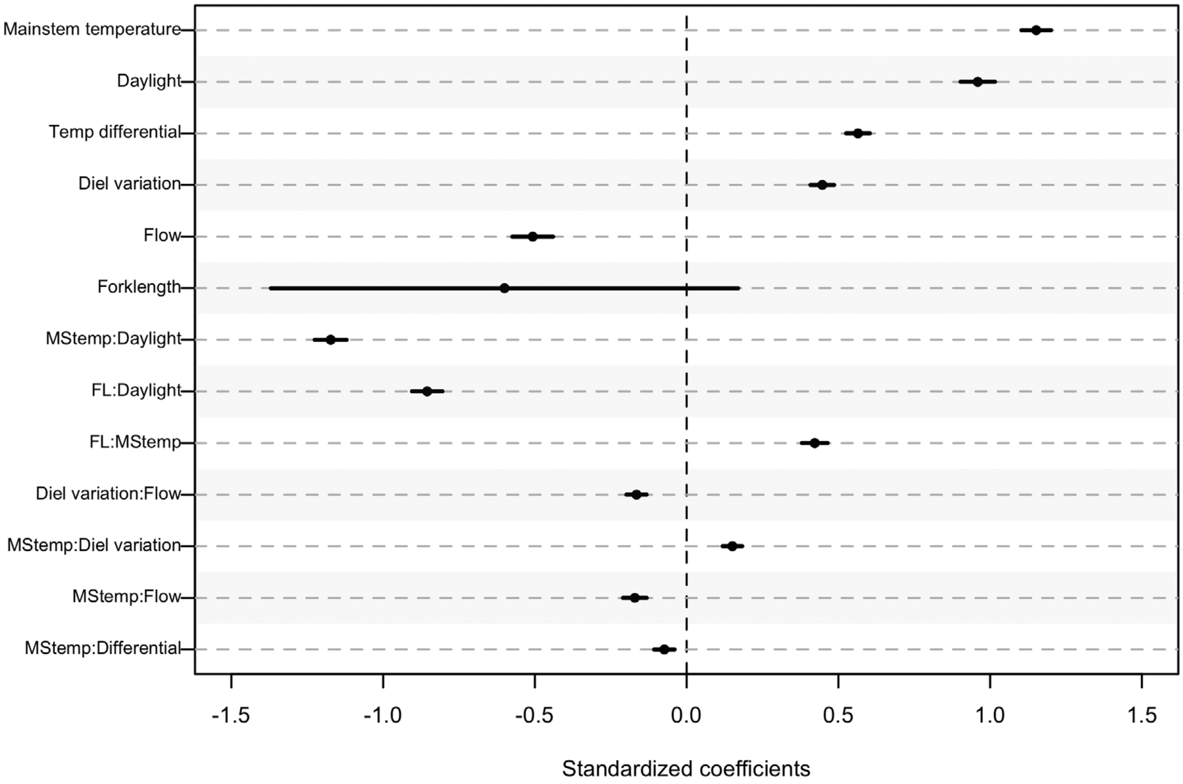

Fig. 4. Parameter estimates for the top logistic model of the probability of an individual fish occupying refuge habitat as determined by AIC values. Error bars indicate 95% confidence intervals in parameter estimates.

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. 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. 4 and 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 (1.152 ± 0.025) than the random state operating model (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 (–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 m^3/s) and 23.5°C at higher flows (approximate third quartile flow = 38.2 m^3/s). There was also a negative

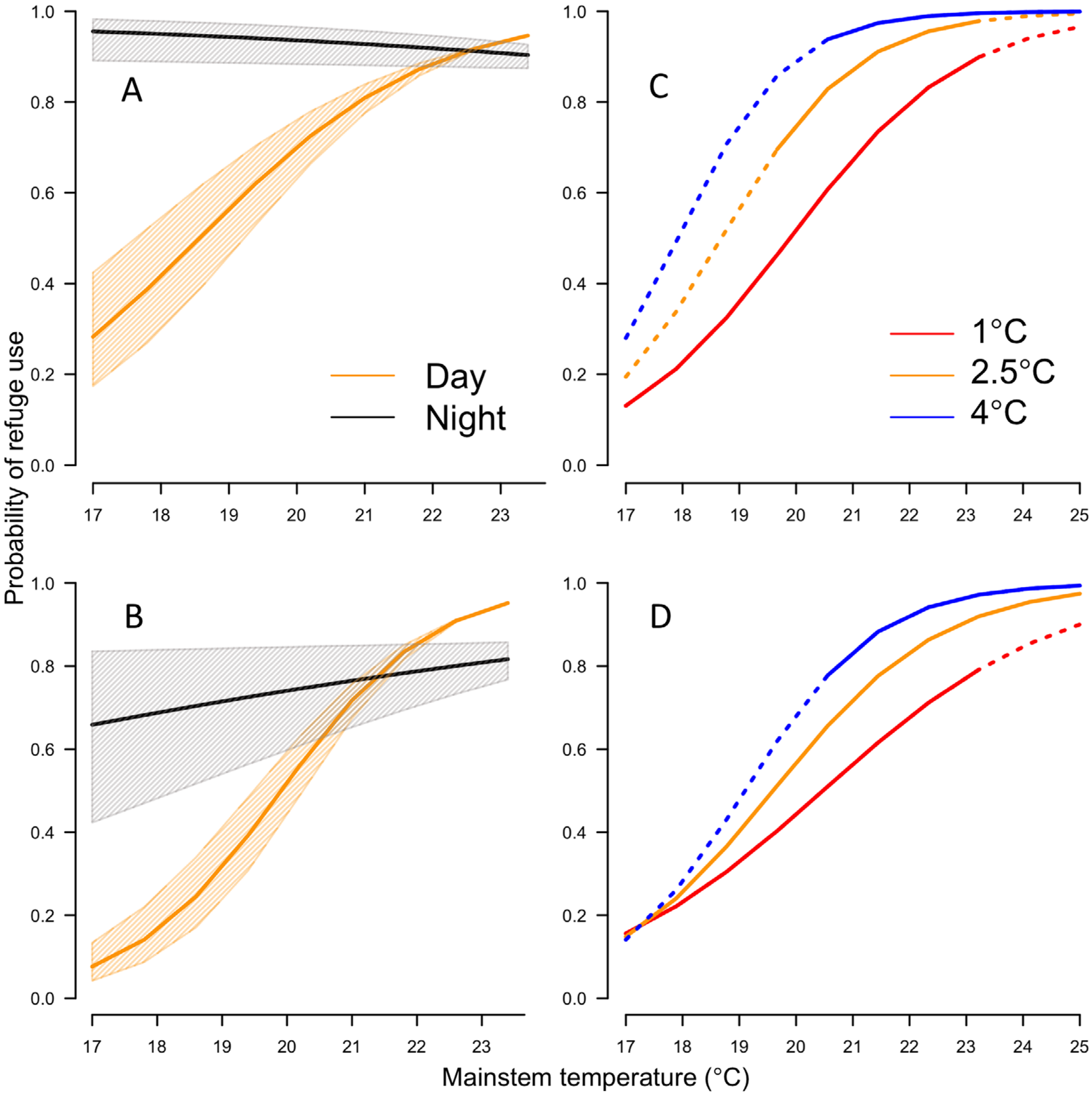

Fig. 5. Estimated probability of juvenile steelhead use of thermal refuges as a function of mainstem temperature, and (A, B) day (orange line) and night (black line), for (A) small versus (B) large fish. Shading indicates range of ±20 mm around mean fork length for small (mean = 160 mm) and large (mean = 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 m^3/s) and (D) high (38.2 m^3/s) mainstem flow. Dashed lines represent extrapolation beyond the range of data.

interaction between diel variation and mainstem flow (Figs. 4 and 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. D1). Additionally, for fish that exhibited this behavior, there was a high degree of variation in the total number of habitat shifts per individual (18 ± 29 shifts). 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 (205 ± 58.0 mm and 168 ± 33.5 mm, respectively), and maintained higher body temperatures across the time detected (18.3° ± 2.3°C and 16.5° ± 2.0°C, respectively).

DISCUSSION

We found that 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 m^3/s) (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. 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 (K. S. Brewitt, *unpublished data*). 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. 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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SUPPLEMENTAL MATERIAL

APPENDIX A

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. A1). 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.

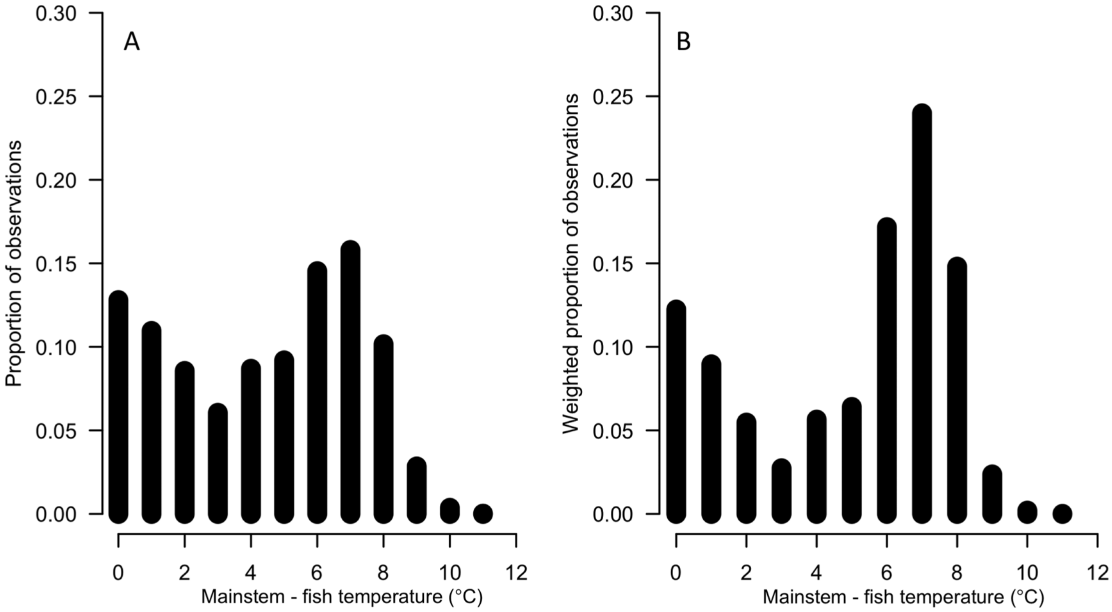

Fig. A1. (A) The distribution of behavioral thermoregulation (mainstem − fish temperature) across individuals from 2010–2012 (total detections = 130,272) and (B) proportional assessment of thermoregulation weighted by the instantaneous range of available temperatures (range = tributary:mainstem temperature).

APPENDIX B

Fig. B1. 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.

Table B1. 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

APPENDIX C

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.

APPENDIX D

Table D1. Mean [SD] 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 (°C)	Temperature differential (°C)	Diel mainstem variation (°C)	Flow (m^3/s)
2010	Beaver	13 Jul to 30 Sep	21.16 [2.38]	6.97 [1.27]	1.36 [0.38]	27.6 [3.57]
	Grider	13 Jul to 30 Sep	21.23 [2.50]	7.42 [0.84]	2.15 [0.46]	33.5 [3.02]
	Fort Goff	4 Aug to 30 Sep	20.40 [2.42]	6.00 [0.69]	2.07 [0.65]	33.5 [3.02]
	Thompson	3 Aug to 30 Sep	20.93 [2.58]	7.39 [0.95]	2.61 [1.01]	33.5 [3.02]
2011	Beaver	13 Jul to 30 Sep	21.83 [1.54]	7.12 [1.37]	1.45 [0.41]	30.2 [0.52]
	Grider	13 Jul to 30 Sep	21.31 [1.91]	6.59 [1.28]	2.43 [0.51]	43.0 [8.43]
	Fort Goff	13 Jul to 30 Sep	21.54 [1.87]	6.40 [0.95]	2.56 [0.51]	43.0 [8.43]
2012	Beaver	12 Jul to 31 Aug	22.96 [0.95]	6.79 [1.20]	1.33 [0.35]	29.3 [2.18]

Table D2. The number of individuals tagged at each study site (N) for fish used in the logistic model, and mean (across individuals) percentage of time that fish were detected in refuges.

Year	Study site	*N*	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 D3. 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 D4. 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	SE	*Z*	*P*
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

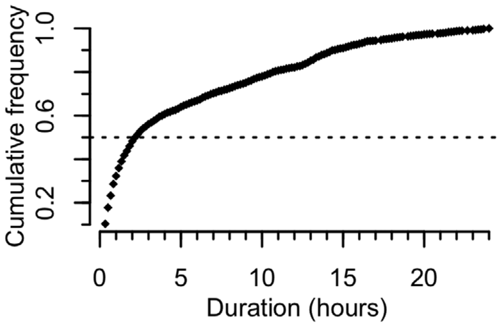

Fig. D1. Cumulative distribution of duration of habitat shifts from refuges to mainstem across individuals. Horizontal line illustrates that 50% of habitat shifts were less than two hours.

APPENDIX E

Thermal mixing zone size co-varied with both temperature and flow, so we were unable to decouple the possible effects of refuge area on steelhead thermal refuge use from these other environmental variables (Tables E1 and E2). 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.

Table E1. 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	SE	*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 E2. 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	SE	*Z*	*P*
Intercept	2.202	0.812	2.711	0.007
Size	5.126	0.097	52.836	<0.001

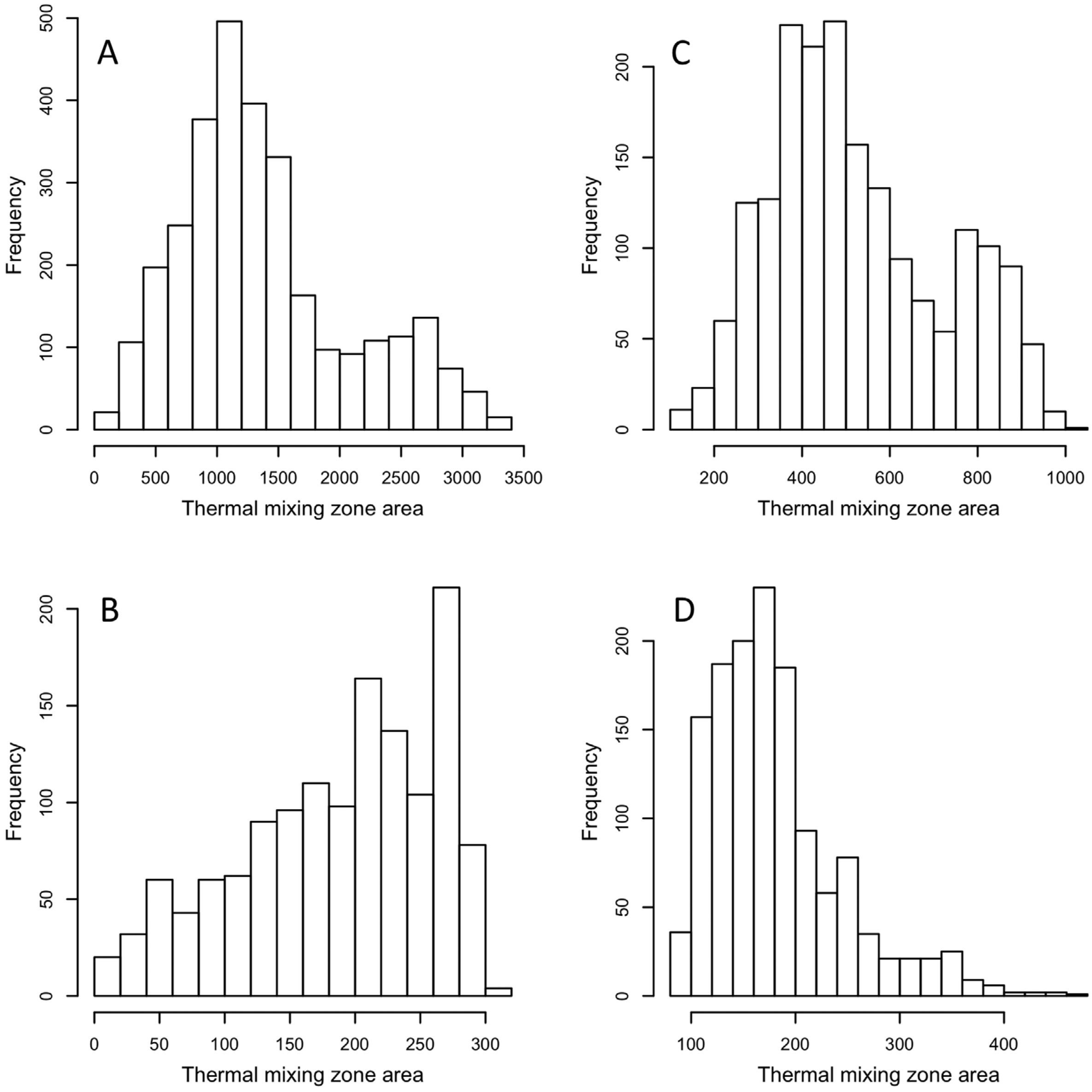

Fig. E1. Hourly estimates of the area of the thermal mixing zone (m^2) at study sites across years when fish were tagged at (A) Beaver Creek in 2010–2012, (B) Fort Goff in 2010–2011, (C) Grider in 2010–2011, and (D) Thompson in 2010.

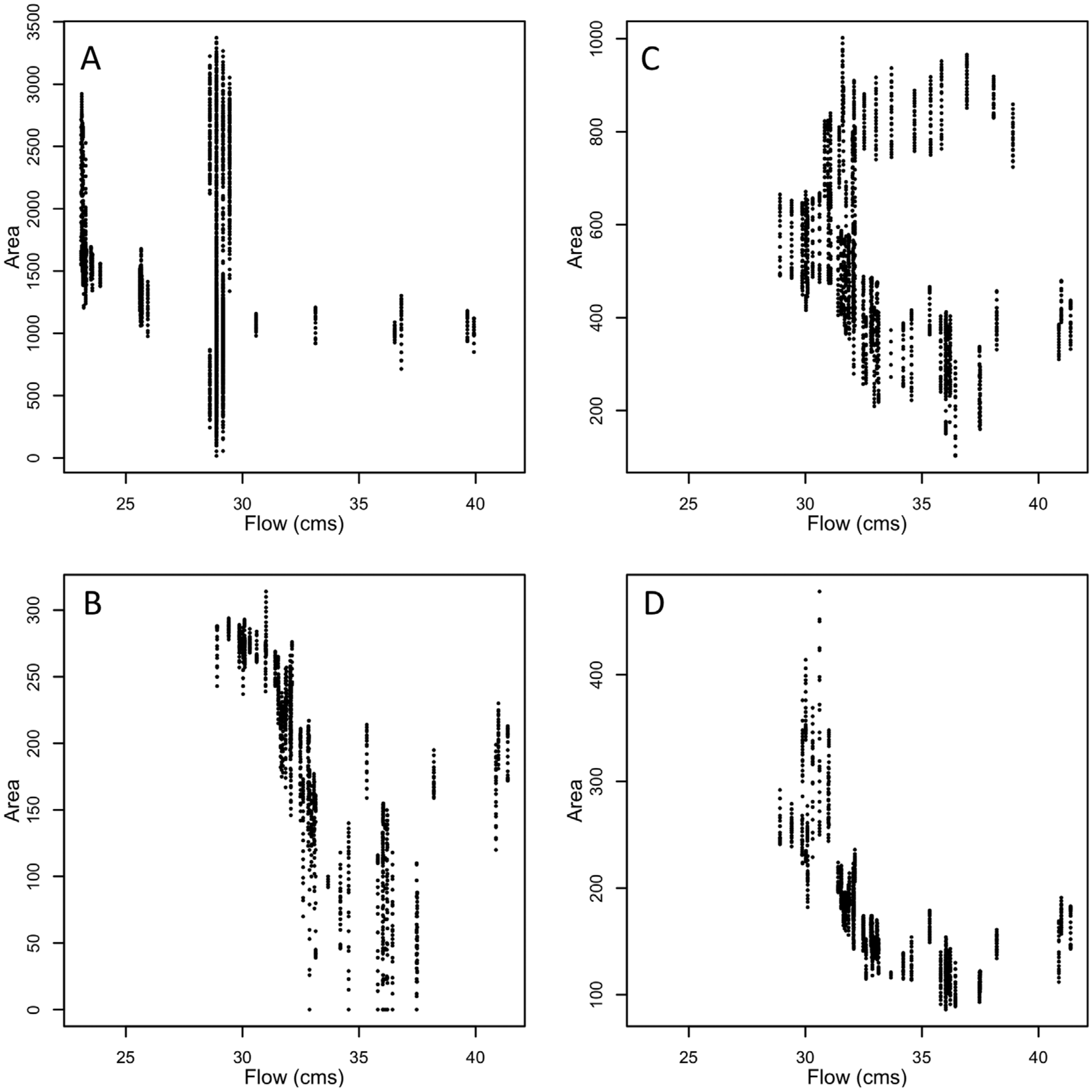

Fig. E2. Relationship between area of the thermal mixing zone (m^2) and daily mainstem flows (m^3/s) at each study site across years at (A) Beaver Creek in 2010–2012, (B) Fort Goff in 2010–2011, (C) Grider in 2010–2011, and (D) Thompson in 2010. Mainstem flow measurements were taken from daily discharge data from Iron Gate Dam and Seiad Valley monitoring stations.