Life history, distribution, and impact of nonnative smallmouth bass *(Micropterus dolomieu*) at range boundaries in the Columbia River Basin

Erika S. Rubenson

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Reading Committee:
Julian Olden, Chair
David Beauchamp
Lauren Buckley

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Abstract

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Erika S. Rubenson

Chair of the Supervisory Committee:
Julian Olden, Professor
School of Aquatic and Fishery Sciences

Riverine distributions of nonnative smallmouth bass (*Micropterous dolomieu*) are changing in response to climate change, with smallmouth bass upstream extent increasing as temperatures warm to within its thermal tolerances. Although smallmouth bass continue to shift its distribution into new upstream habitat, little is known about the mechanisms that drive or limit distribution changes, its current distributions in the Columbia River Basin, or the potential impacts expanding smallmouth bass may have on juvenile salmonids, each of which I address in this dissertation. Like most ectotherms, each life history stage (i.e., egg, juvenile, adult) of smallmouth bass has unique thermal optimums and limits that may disproportionately affect successful establishment of new self-sustaining populations. In my first two chapters, I examined the effects of increasingly colder temperatures on spawning patterns, reproductive success, and recruitment across a broad (>60 km) spatial extent in the North Fork John Day River. In my third chapter, I assessed the inter-annual differences in maximum upstream distribution, seasonal changes in local abundances, and seasonal movement patterns of adult bass to assess the role of adult dispersal in range dynamics. Using these data on reproduction, recruitment, and adult movement patterns, I found key behaviors and physiological constraints that limit or drive range expansion and present management strategies that may be useful to managers tasked with preventing future range expansion of smallmouth bass. In my fourth chapter, I developed a species distribution model of smallmouth bass for the Columbia River Basin using a combination of historical distribution data and environmental DNA (eDNA) distribution data collected at predicted range boundaries. Here, I found that smallmouth bass is widely distributed and predicted to overlap with 3-62% of critical salmonid spawning and rearing habitat. Under a moderate climate change scenario, I found that smallmouth bass is predicted to increase its distribution by over two-thirds, highlighting management challenges throughout the Columbia River Basin. Finally, in my fifth chapter I examine potential competition and predation dynamics in a food web that includes smallmouth bass and juvenile Chinook salmon (*Oncorhynchus tshawytscha*). Here I found little evidence for a strong predation or competition threat to juvenile Chinook salmon, suggesting that although smallmouth bass is likely to increase its overlap with native salmonids, its greatest impact to salmonids appears to occur in mainstem habitats. Taken together, the five chapters of this dissertation advance the understanding of the physiology, behavior, distribution, and impact of nonnative smallmouth bass in the Columbia River Basin, which will directly contribute to the management of streams home to myriad threatened and endangered native species.

Chapter 1. Spatiotemporal spawning patterns of smallmouth bass Micropterus dolomieu at its upstream invasion edge

Authors: E.S. Rubenson, J.D. Olden

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1A. Abstract

Climate change and land-use practices are causing widespread warming of streams, forcing resident species to adapt or migrate. For instance, in the John Day River, Oregon (Columbia River Basin), rising temperatures are facilitating the range expansion of Smallmouth Bass into critical salmon rearing habitat. Integral to understanding and ultimately predicting upstream range expansion of Smallmouth Bass is to examine its reproductive ecology at range boundaries. Our research seeks to address this knowledge gap by exploring potential temperature-mediated effects on Smallmouth Bass reproduction at the leading edge of its nonnative riverine distribution in the Pacific Northwest. We used continuous snorkel surveys to characterize its upstream extent in the North Fork John Day River, observed spawning patterns and measured adult nest-guarding male size, fecundity, brood development, habitat attributes and nest success over two years (2014, 2015). We revealed a pattern of asynchronous and protracted spawn timing across the leading invasion edge, >90% nest success and few changes in reproductive attributes (e.g., fecundity, brood development) as the thermal regime became increasingly colder. Instead we found increased selectivity of nest substrata and decreased guarding requirements in upstream habitats. These results suggest that reproductive success does not limit the upstream range expansion of Smallmouth Bass and highlight potential ecological benefits that may offset the energetic demands associated with dispersing upstream. Overall, our study enhances the current understanding of how reproduction influences range expansion of nonnative Smallmouth Bass populations in streams, enabling us to better guide managers tasked with minimizing the spread of this nonnative species in the future.

1B. Keywords

Invasive species, secondary spread, dispersal, headwater

1C. Introduction

Temperature is a key ecological attribute in freshwater ecosystems, influencing productivity, water quality, habitat availability, and the metabolism, abundance and distribution of ectothermic organisms (Magnuson et al. 1979; Caissie 2006). In recent years, rising air temperatures, changing hydrologic regimes and increasing land-use change have acted synergistically to cause widespread stream warming (Kaushal et al. 2010; Carpenter et al. 2011). Elevated stream temperature is now one of the most pervasive water quality issues in the Pacific Northwest (PNW) region of the United States, and ongoing climate change and loss of riparian vegetation is predicted to exacerbate this problem (Isaak et al. 2012; Wu et al. 2012; Arismendi et al. 2013). Warming temperatures threaten native coldwater fish species, including Pacific salmon, both in potential loss of thermally suitable habitat (Wenger et al. 2011; Ruesch et al. 2012) as well as through facilitating range expansion of nonnative, warmwater predators such as Smallmouth Bass *Micropterus dolomieu* (Rahel and Olden 2008; Lawrence et al. 2014). Smallmouth Bass has both direct predation impacts on salmonids as well as indirect non-consumptive impacts such as through effects on behavior, growth and stress (e.g., Carey et al. 2011; Kuehne et al. 2012).

Smallmouth Bass is a warmwater sportfish native to central and parts of eastern United States that has been routinely stocked outside its native range, both throughout North America and globally (Carey et al. 2011; Loppnow et al. 2013). In the PNW, Smallmouth Bass has been purposefully introduced since the early 20th century, beginning in 1923 with a couple hundred fish in the Willamette River, OR and several thousand fish in the Yakima River, WA in 1925 (Lampman 1946). Intentional stocking by state natural resource agencies continued throughout the middle and late parts of the century, and in recent decades has generally slowed. However, illegal transplants by the public and secondary spread of individuals continues to contribute to a potentially growing distribution throughout the PNW (Carey et al. 2011).

Smallmouth Bass has recently been found in cooler headwater habitats that contain endangered subyearling Pacific salmon (Lawrence et al. 2012), heightening concerns about its distributional potential and ecological impacts. Despite emerging evidence that Smallmouth Bass range is expanding upstream, little is known about the critical mechanisms that may promote or inhibit this expansion in the PNW. As a known predator and competitor with proven negative implications for native species (Fritts and Pearsons 2006; Tabor 2007; Sanderson et al. 2009; Kuehne et al. 2012), it is essential to have a detailed understanding of these critical drivers to better forecast future range expansion and guide management and education efforts.

The ability to successfully reproduce and recruit in novel habitats is perhaps the most fundamental determinant of range expansion by invasive species (Ibáñez et al. 2014). Smallmouth Bass reproductive success is contingent on a number of factors that include temperature-mediated processes such as spawn timing (Ridgway et al. 1991), parental care

(Ridgway and Friesen 1992), offspring development rates (Shuter et al. 1980) and brood survival (Lukas and Orth 1995). Despite a wealth of research on these processes in native-range lakes (e.g., Raffetto et al. 1990; Rejwan et al. 1997; Wiegmann et al. 1997; Scott et al. 1997; Phelps et al. 2008; Steinhart and Lunn 2011), surprisingly little is known for nonnative populations in headwater rivers that are subjected to thermal regimes defined by short growing seasons, variable summer growth temperatures and long winters. Such environmental conditions in its native range can cause Smallmouth Bass to exhibit significant variation in reproductive strategies (Dunlop et al. 2005), and current models that predict reproductive success often perform poorly outside of the native populations for which they were developed (Dunlop and Shuter 2006). These differences necessitate regional assessments (and ultimately specific parameterization of models) to better inform managers tasked with minimizing Smallmouth Bass spread and impacts.

Our research seeks to inform the mechanisms of Smallmouth Bass range expansion by exploring potential temperature-mediated effects on reproduction at its upstream leading edge of invasion. Rivers draining the Cascade Range of western North America are characterized by a steep temperature gradient over a relatively small spatial scale, providing a powerful opportunity to examine the direct effects of temperature on spawn timing, adult size, fecundity and overall reproductive success. We first define the upstream leading edge of Smallmouth Bass spawning distribution in the John Day River Basin, then characterize reproductive patterns, and finally assess nest success along the continuous spatial extent over two years. We specifically explore whether reproduction in the leading edge limits the successful establishment of new upstream populations. Suitable temperatures for Smallmouth Bass spawning have been documented to be 12-25°C, and average daily water temperature has been shown to be the single most important variable to predict spawn timing in streams (Graham and Orth 1986). Brood development is also directly related to water temperature (Shuter et al. 1980), with broods developing more slowly in cooler temperatures thus increasing nest-guarding requirements for males (Ridgway and Friesen 1992). We therefore hypothesized that the gradient of decreasing upstream temperatures would result in 1) a decrease in size and, in turn, a decrease in fecundity of reproductive adults due to relatively shorter growing seasons, 2) slight delays to spawning initiation in response to later warming, 3) slower brood development rates and 4) declines in nest success as thermal regimes become colder.

1D. Methods

In 2014 and 2015, we conducted snorkel surveys of Smallmouth Bass distribution and spawning behavior in the North Fork John Day River (NFJDR), a Columbia River tributary. The NFJDR originates in the Blue Mountains (Cascade Range) of northeastern Oregon. It is the third longest free-flowing (undammed) river in the contiguous United States, snowmelt fed and supports one of the last remaining wild-only Chinook Salmon *Oncorhynchus tshawytscha* Evolutionary Significant Units (Fig. 1.1). Guided by previous snorkel surveys conducted by Lawrence et al. (2012) that defined the upstream leading edge of Smallmouth Bass, our survey extent included 65-kms (river kilometers (RKM) 53-117) in 2014 and 70-kms (RKM 53-123) in 2015. This represented the spatially-variable upstream leading edge of adult Smallmouth Bass distribution and spawning activity. The surveys began at the initiation of spawning and continued throughout the summer until spawning activity ceased (10 June – 26 August 2014 and 27 May – 29 July 2015).

Thermal regime.—Water temperatures were monitored using an array of temperature loggers (n=15) (HOBO pendant, Onset Electronics, Onset, MA, U.S.A.) recording at 1-hour intervals from 28 May 2014 – 5 October 2015. Water temperatures were assigned to individual nests using a linear extrapolation of recorded temperatures as a function of river kilometer (R^2 values for all models ranged from 0.958 to 0.992, and all $P < 0.001$). Because nest initiation most often occurs as temperatures rise above 15°C (Carlander 1977; Shuter et al. 1980), we used degree days > 15°C to describe the thermal history of the adult fish prior to spawning. Spawning degree days were calculated by summing average daily temperatures for all days that exceeded the 15°C threshold beginning each spring.

Fish distribution and nest surveys.—In 2014, spatially continuous snorkel surveys were conducted in a manner to allow for a comprehensive spatial assessment of spawning as well as detailed temporal monitoring of nest progression. Snorkel surveys were continued 5-km beyond the most upstream Smallmouth Bass observation, regularly checking further upstream for seasonal shifts in distribution. The resultant 65-km survey extent was delineated into channel-units (i.e. riffle, glide-like riffle, glide, glide-like pool, pool) according to changes in current velocity and mean and maximum channel depth profiles following Lawrence et al. (2012). Every channel-unit was sampled, excluding shallow riffles <0.25 m deep. Two snorkelers on opposite shorelines simultaneously surveyed each channel-unit in an upstream direction. Snorkelers reported the number and size class (<10cm, 10-20cm, 20-30cm, >30cm) of all observed Smallmouth Bass in each channel-unit to an above-water data recorder and geo-referenced the exact location of individual nests. In 2015, glide-like pool habitats were subsampled at 5-km intervals and the survey extent increased to RKM 123 to match the observed increase in distribution.

Nest attributes in 2014 were characterized by a consistent observer who measured length and width of swept area (cm), length and width of area in which eggs were present (cm), water depth (m), distance from shore (m) and dominant and subdominant substratum (%) of each nest. Substratum was represented as a composite score determined by multiplying the field-measured substrata data (i.e. percentage of dominant and subdominant substratum) by a score (7 = bedrock, 6 = boulder, 5 = cobble, 4 = gravel, 3 = sand, 2 = silt, 1 = organic matter) and then summing across substratum types (following Lawrence et al. 2012). Nest substratum compositions were then contrasted with available habitat (channel-unit habitat surveys completed in 2009 and 2010 by Lawrence et al. 2012, 2014) to determine substratum selectivity relative to habitat availability across the survey extent. Total body length (cm) of nest-guarding males (n = 183) was visually estimated for occupied nests. Visual size estimates were verified by a subsample of adult males captured (n = 21), with an average difference between actual and estimated total length of 0.71 cm (SD = 2.5). Five scales were also removed from behind the pectoral fin of every captured fish and placed directly on wax paper and stored in coin envelopes for aging. In the laboratory, impressions of the scales were made on acetate slides and viewed under a dissecting microscope at 48x magnification. Scale annuli were then counted to determine age (DeVries and Frie 1996).

Nest progression was monitored via visits to each nest at 3-6 day intervals, averaging 4 visits per nest. Date, time and stage of development were annotated at each visit. Nest development was divided into 4 stages: eggs, hatched embryos, swim-up and metamorphosis following Ridgway and Friesen (1992). Nests were considered successful if swim-up fry were produced (Ridgway and Friesen 1992). Due to the extensive nature of the survey, a small number of nests were discovered after the initiation of egg hatching. If nests were discovered mid-development cycle, the date of egg deposition was back calculated using surrounding nest development rates in comparison to the stage when the new nest was discovered. If no other nests were discovered in near proximity, the egg deposition date was back calculated using recorded water temperature and the development equations published in Shuter et al. (1980). In a few cases, nests were checked 1-2 days after swim-up occurred. In these rare cases, however, the swim-ups had not yet dispersed and the channel-unit nest density was sufficiently low to confidently assign the group of swim-ups to the marked nest location underneath. We also characterized and monitored nests that were swept and guarded by a male but never received eggs from a female.

In 2014, eggs were collected from 59 nests across the survey extent using a turkey baster (pointed plastic tube with squeeze bulb attached). A 2 x 2-cm gridded quadrat was placed over each nest, and eggs were collected from 10 random cells within the quadrat. The total number of eggs was then estimated using an area-density calculation (validated by counting all eggs from one nest). From the eggs collected, 10 eggs were blot dried and weighed to the nearest 0.1 g in the field and then frozen for lab processing. In the lab, mean radius of 5 eggs and their oil droplets were measured from each nest using a digital dissection microscope, with average radius of egg and oil droplet calculated from the 5 measurements (Gingerich and Suski 2011).

Statistical analysis.—Univariate statistical approaches were conducted in R version 3.2 (R Development Core Team 2015). Piecewise (or segmented) linear regression was used to assess differences in spawn timing along the longitudinal gradient using the *segmented* library in R. This package was designed to fit regression models with broken-line relationships (Muggeo 2008). Given apparent non-linearity in some relationships, we used segmented linear regression to quantify an abrupt change in the response variable, identifying a specific breakpoint or threshold beyond which the slope of the relationship significantly changes.

1E. Results

Spatiotemporal spawning patterns and brood development

The upstream extent of Smallmouth Bass adults was highly concordant with the leading edge of spawning activity in both years. Adult abundance and upstream extent consistently increased from June to August – similar to patterns observed by Lawrence et al. (2012). In early June of both years, the leading edge of adults were concentrated below RKM 66, but as temperatures warmed above 15°C, a portion of the adult population moved upstream, demonstrating a seasonal shift in upstream distribution. Upstream extent peaked in August when adults were recorded up to RKM 110 (2014) and RKM 117 (2015).

A total of 271 nests were discovered in 2014 over the 65-km river stretch representing the leading edge of Smallmouth Bass. Of those, 75% (n = 202) received eggs and 25% (n = 69) did not. Spawning was first initiated on 10 June 2014 (downstream of RKM 66), corresponding with water temperatures increasing above 15°C. With time, nest density increased in downstream habitats and nest activity simultaneously expanded upstream (Fig. 1.2a). Successive nest initiation progressed continuously through 16 July 2014, at which time the most upstream nest was spawned at RKM 90. No Smallmouth Bass were observed above RKM 90 until August. In August, adult distributions expanded into upstream units and 4 additional nests were observed 10-20 km beyond the previous most upstream nest, extending the upstream limit of reproductive behavior to RKM 110. Spatiotemporal patterns in 2015 were similar to 2014, except that spawning was initiated two weeks earlier (27 May 2015) in response to earlier warming, and the upstream progression of nest activity occurred more rapidly (Fig. 1.3). In total, 144 nests were found in the subsampled habitat, 67% (n = 96) of which received eggs. In comparison, 107 nests were recorded in the same subsampled habitats in 2014.

In both years, discharge decreased and average daily water temperatures steadily increased beginning in mid-May. Average daily temperatures peaked in July and began decreasing in mid-August. On average, a 3°C temperature differential existed between the most downstream (RKM 53) and most upstream (RKM 117) unit. Average daily temperatures in 2015, however, were approximately 2-4°C warmer from 29 May to 7 July across the entire survey extent (Fig. 1.4), likely in response to considerably lower discharge relative to historical conditions. Regression analysis revealed a significant association between spawning degree days (> 15°C) and the date of nest initiation ($R^2 = 0.567$, $P < 0.001$ for 2014; $R^2 = 0.248$, $P < 0.001$ for 2015) (Fig. 1.5a, b). The decrease in explained variability in 2015 is likely due to average daily water temperatures rapidly rising above 15°C at the beginning of spawning season, resulting in faster accumulation of degree days (and therefore greater dispersion of points) relative to 2014. Evidence for potential non-linear relationships in 2014 prompted a piecewise regression to be conducted; this analysis revealed a significant change in slope occurring at RKM 73 ($R^2 = 0.784$, $P = 0.049$) demonstrating that spawning was increasingly delayed in time upstream of RKM 73 in 2014. This threshold response demarcates a shift in reproductive patterns and suggests a secondary mechanism in addition to temperature that may influence Smallmouth Bass spawning at the leading edge of its upstream distribution.

Brood development rates varied considerably along the longitudinal extent and between years. On average, broods in 2014 were fully developed (eggs to swim-up) in 14 days (range: 7-26 days; SD = 4.357; n = 184) and 9 days (range: 5-14 days; SD = 1.849; n = 89) in 2015. Contrary to our hypothesis, a significant negative linear trend was detected in 2014 indicating that nests in more upstream reaches had broods that developed faster than nests in downstream reaches ($R = -0.634$, $P < 0.001$). In 2015, when warmer water conditions existed through the river, the same declining trend was not observed, but instead the entire survey extent showed rapid brood development with no significant differences between downstream and upstream segments.

Characteristics of reproductive adults

Estimated sizes of adult nest-guarding males in 2014 (n = 183) ranged from 180-345 mm TL (mean = 253 mm; SD = 30) and measured sizes (n = 21) ranged from 203-325 mm TL (mean = 247 mm; SD = 32). Ages ranged from 2-6, representing 9%, 62%, 5%, 19% and 5% of spawners respectively. Similar adult sizes were observed in 2015 (results not shown). Although it is often reported that larger males tend to nest earlier (Ridgway et al. 1991), we found no significant correlations between male size and spawn timing in the NFJDR. The maximum size of nesting males did, however, decline from 345 mm to 280 mm and average size declined from 258 mm (SD = 31) to 237 mm (SD = 22) in upstream segments, indicating a tendency for the largest males to nest downstream (Fig. 1.6a).

Reproductive females were rarely seen spawning; therefore we report patterns in egg size and quantity. Across the spatial extent, egg volume averaged 5.517 mm^3 (SD = 0.856) and egg weight averaged 0.007 g (SD = 0.002). Egg counts ranged from 1597-8850 (mean = 4526; SD = 2289). No trend, however, was observed for egg counts or egg size over the spatiotemporal extent of the survey (Fig. 1.6b), suggesting a consistent body size distribution of females.

Habitat characteristics of nests

The majority of Smallmouth Bass nests were constructed in either glide-like pool (43%) or glide habitat (36%) at an average depth of 0.57 m (SD = 0.34) and a distance from shore of 1.4 m (SD = 1.7). The remaining nests were in shallow portions of pools or in protected shoreline habitat adjacent to higher flow units. Nests were primarily located in habitats containing cobble and gravel substrate (mean substrata score = 4.3; SD = 1.0). The predominant substrate in the NFJDR is cobble (55%) and boulder (36%) (Lawrence et al. 2012, 2014). There were no significant longitudinal changes in substrate availability across the survey extent, with the exception of bedrock. Bedrock, however, was less than 15% of any channel reach and represented less than 2% of overall available substrate. Although habitat and nest characteristics showed similarity across the spatial extent, a slight shift in habitat selection did occur in the upstream habitats (Fig. 1.6c). In downstream reaches, a wide variety of substrata including vegetation, organic matter and sand, were used to construct nests indicated by a large variance in nest substrata scores (min = 1; max = 5.6). By contrast, nest construction was more confined to cobble and gravel (min = 3.3; max = 5) in upstream reaches.

Nest success

Overall nest success rates were high in the NFJDR. Of the 202 total nests that received eggs in 2014 (Fig. 1.6d), 192 were confirmed as either successful (achieving swim-up stage) (n = 184) or failed (no swim-ups produced) (n = 8). Conservatively, we assumed the remaining 10 nests also failed (total failed n = 18), resulting in a 91% nest success rate. Nest success rates were similar (93%; n = 89) in 2015, with all nests verified as either successful or failed. In contrast to our hypothesis that increasingly cooler thermal regimes in upstream habitat would lead to an increase in nest failure, 94% (n = 17) of the failed nests in 2014 and 86% (n = 6) of the failed nests in 2015 were located in more downstream reaches of the survey extent.

1F. Discussion

Our study represents the first broad-scale survey of riverine Smallmouth Bass spawning in its nonnative range. We determined the leading edge of distribution in the NFJDR using snorkel surveys and compared reproductive attributes along the steep thermal gradient. The leading edge of Smallmouth Bass distribution changed seasonally, with spatial extent and number of individuals in upstream channel-units increasing throughout the summer months. In concert with distributional expansion, we revealed a striking pattern of asynchronous and protracted spawn timing across the spatial extent. Broadly, Smallmouth Bass capitalize on a constantly expanding thermal regime as temperatures warm over the spawning season.

Temperature alone, however, did not consistently explain spawn timing or nest success. We suggest that temperature defines reproduction at a local scale, but that the seasonal change in adult distribution may play a more important role in shaping spawning dynamics in the extreme leading edge. For instance, in 2014 we revealed a significant spatial threshold at RKM 73 that delineated a pronounced shift in spawn timing relative to growing degree days. In the core distribution of spawning (downstream of RKM 73), we found that accumulation of degree days > 15°C reasonably predicted spawn timing, whereas spawn timing in the upstream edge was substantially delayed relative to degree days > 15°C. It is revealing that this pattern was not observed in 2015. One explanation for the observed delay in spawn timing in 2014 and not 2015 may involve an interaction between adult seasonal movement patterns (unpublished data) and the timing and magnitude of spring high-flow events. Discharge (measured at RKM 24, USGS gauge station 14046000) in late June 2014 averaged 2-3 times higher than June 2015 (mean daily discharge of 23.1 m^3/s versus 8.4 m^3/s). The high discharge in 2014 may have delayed spawning adults that reside downstream during winter months from dispersing upstream until after the temperatures were above 15°C, delaying spawning initiation. By contrast, we hypothesize that when discharge was considerably lower in 2015, that upstream movement was not restricted, thus allowing adults to reach upstream habitat in time to spawn as temperatures reached a daily average of 15°C. Additional investigation is warranted to determine the overwinter location of adult Smallmouth Bass and track seasonal movement patterns relative to flow and temperature.

We hypothesize that Smallmouth Bass may accrue reproductive benefits at the extreme upstream edge of their distribution. First, we found that although habitat availability was similar across our entire survey extent, upstream nests were less likely to be placed in suboptimal substrata, suggesting a decrease in competition for nesting locations. Second, when delayed upstream spawning was more pronounced in 2014, eggs from nests in upstream reaches experienced higher temperatures during incubation than nests further downstream (18-21°C versus 12-15°C in downstream nests). This resulted in faster brood development rates and ultimately demanded less time and energetic investment by nest-guarding males. Decreased guarding requirement by adult male Smallmouth Bass has been linked to increases in nest success (Lukas and Orth 1995), and may explain the high rate of upstream nest success. By contrast, temperatures in 2015 were conducive to rapid brood development across the survey extent, contributing to high nest success across all habitats.

Taken together our results indicate that reproductive adults in upstream reaches may experience lower competition for spawning habitats and decreased guarding requirements, which together could outweigh the energetic demands associated with dispersing upstream. It is important to note, though, that the largest males spawned in downstream reaches, suggesting that habitat in downstream reaches may be preferred. If downstream spawning habitat is limited, then smaller fish may be being outcompeted and therefore forced upstream to spawn. Although the mechanism responsible for fish using habitat in upstream reaches to spawn is still unclear, those fish that did attempt to reproduce upstream demonstrated high spawning success. This suggests that, on the whole, reproduction is not a primary limiting factor in defining the leading upstream edge of Smallmouth Bass invasion in coldwater streams.

Despite potential ecological benefits for upstream nests, the delay in spawn timing also resulted in a shorter first-summer growing season. Considerable research suggests that young-of-the-year Smallmouth Bass must achieve a size sufficiently large to survive a winter starvation period (Shuter et al. 1980) in order to recruit into the population. Excessive delays in spawning could result in insufficient growing degree days for overwinter survival. Furthermore, the lower average summer temperatures in upstream habitats are likely to reduce growth potential for young-of-the-year (Lawrence et al. 2015), likely impacting follow-on year recruitment rates. For instance, although the most upstream nests (> RKM 90) were successful in rearing eggs to swim-up fry, the late date and short growing season produced small sized young-of-the-year (23-36 mm as measured 27 Sep 2014) that are unlikely to survive a winter starvation period (Shuter et al. 1980). In support of this, there were no age-1 Smallmouth Bass discovered above RKM 90 in 2015 (unpublished data). In this case, recruitment, not reproduction, limited successful establishment in upstream habitat. Future research is needed to explore whether or not recruitment largely limits Smallmouth Bass range expansion in the PNW.

Contrary to the expectation of progressive changes to reproductive patterns with declining upstream temperature, we found a slight decline in maximum size of reproductive males in upstream habitat, but no change in fecundity and consistently high (>90%) nest success rates across the survey extent. Although nest success rates >90% have been documented in other systems, more typical are rates of approximately 50% (Cooke et al. 2003). Common reasons for nest abandonment are angling (Kieffer et al. 1995), stochastic environmental events (Lukas and

Orth 1995), rapid temperature fluctuations (Cooke et al. 2003), low prey availability or high predator abundance (Steinhart et al. 2004). In 2014 and 2015, NFJDR had only moderate angling pressure and no notable flood events or rapid temperature changes. We did observe potential predators near nests during monitoring surveys, but very few attempts were made by predators to consume the eggs even if nests were left unguarded for an extended period (results not published). The potential predators most commonly noted were conspecifics and typically in low numbers if present at all. We also observed low numbers of Northern Pikeminnow *Ptychocheilus oregonensis*, Redside Shiner *Richardsonius balteatus*, Speckled Dace *Rhinichthys osculus* and Bridgelip Sucker *Catostomus columbianus*. In short, the angling pressure, flow regime and resident community of predators in the headwaters of NFJDR are highly conducive to successful reproduction.

1G. Conclusion

We demonstrated that reproductive success in cool headwater streams is likely not limiting future range expansion potential of Smallmouth Bass in the PNW. The spatiotemporal spawning patterns along the range boundary created both ecological costs and benefits dependent on when spawning occurred relative to summer growing conditions, but these trade-offs did not affect overall nest success rates. Indeed, Smallmouth Bass had high nest success relative to native populations and fecundity did not vary along the longitudinal gradient. Instead, delayed spawn timing in upstream habitats benefitted from warmer temperatures during brood development and decreasing time guarding. These delays, however, also afforded a shorter growing season to young-of-the-year that could potentially impact the ability for successful overwinter survival and restrict establishment of self-sustaining populations.

Management agencies in the PNW are charged with both conservation of native fishes and supporting a recreational Smallmouth Bass fishery (Carey et al. 2011). Limited information on the distribution and ecology of Smallmouth Bass in PNW streams, however, provides little guidance to support the complex management decisions regarding non-native fish stocking regulations, angling restrictions, and riparian shading restoration efforts aimed to reduce stream warming trends. Overall, our study contributes to an enhanced understanding of how reproduction influences range expansion of nonnative Smallmouth Bass populations in headwater streams, better informing management agencies tasked with minimizing future spread and impacts. Research exploring other mechanisms of range expansion, such as recruitment and dispersal, is suggested to improve future range predictions and to provide managers with new information on specific processes that might be targeted to limit further expansion of Smallmouth Bass.

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1J. Figures

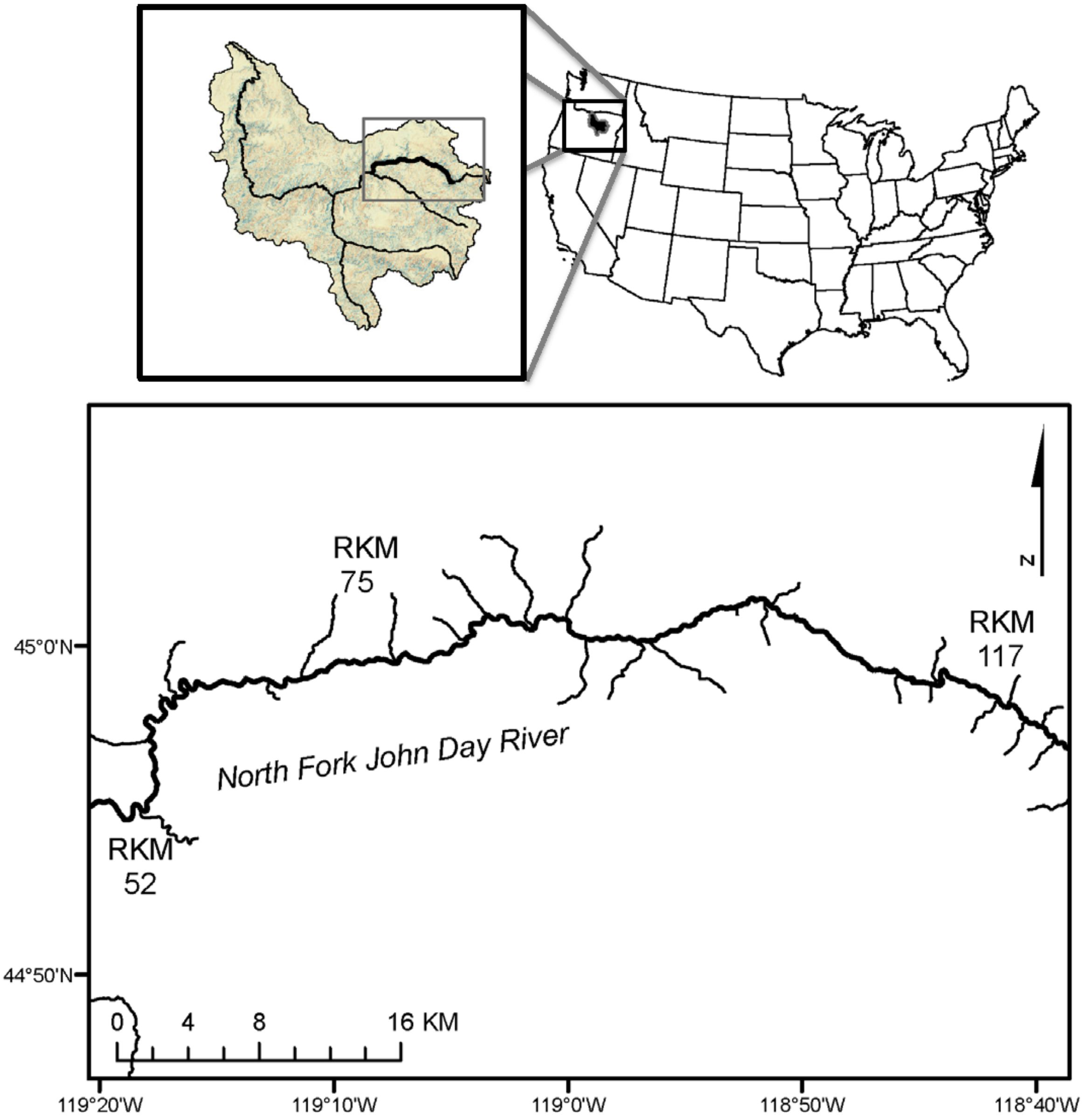

Figure 1.1 Map of the North Fork John Day River, Oregon.

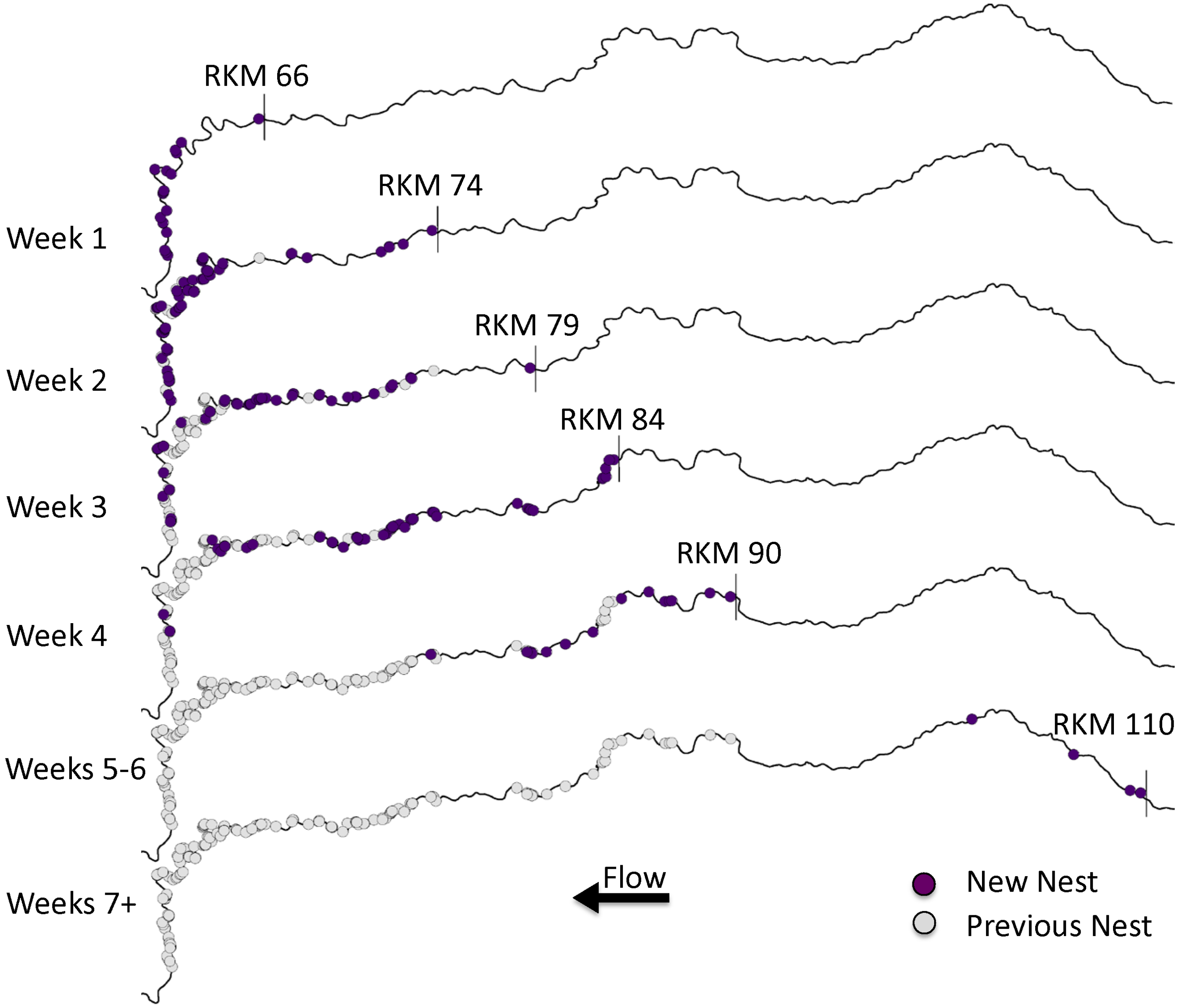

Figure 1.2. Spatiotemporal spawning patterns in the North Fork John Day River for 2014 (10 June – 26 August 2014). Each panel represents the location of active nests during each week of the spawning season. New nests are indicated by dark circles and nests previously observed by light circles. The line demarcates the river kilometer of the most upstream nest during the associated week.

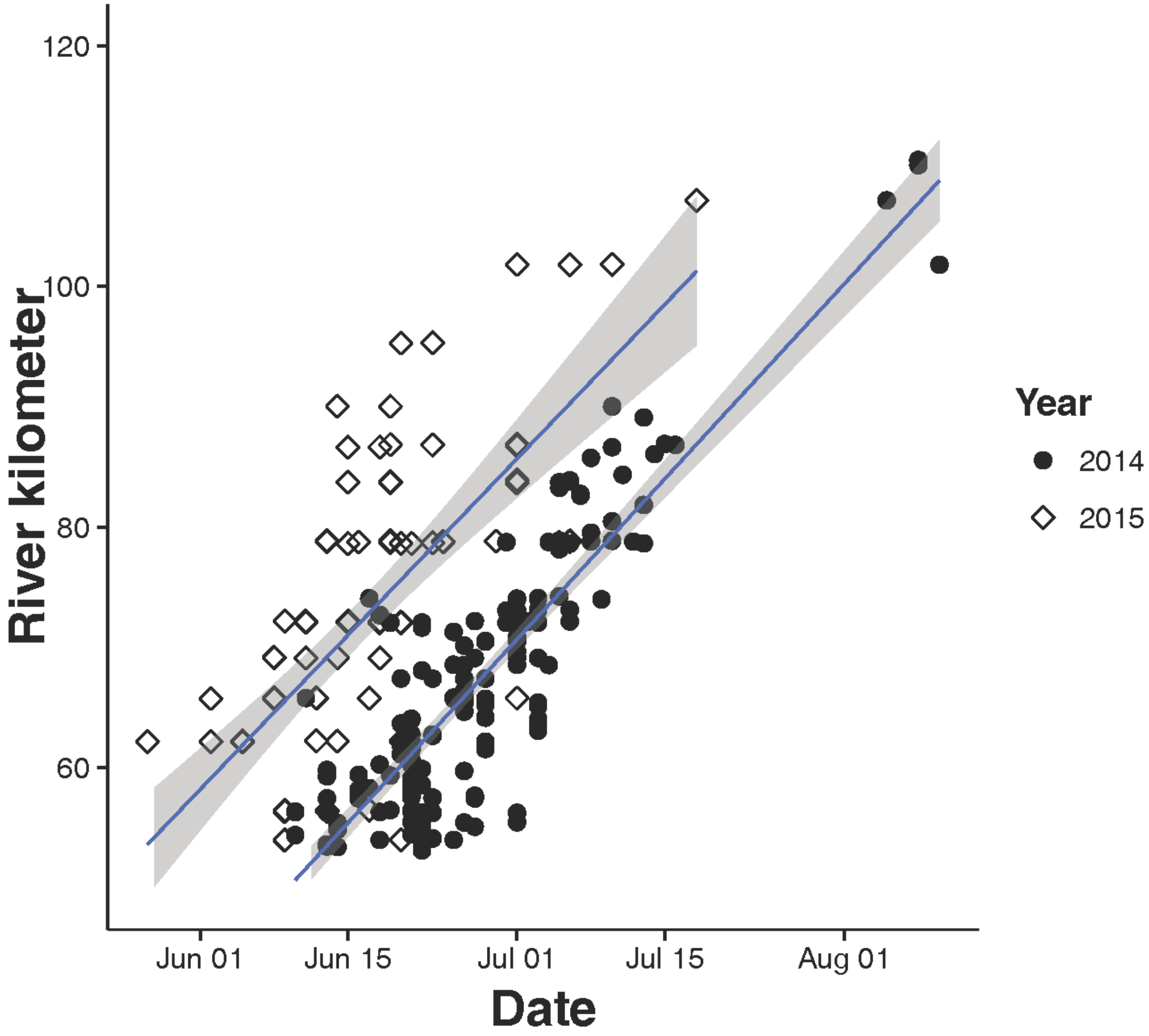

Figure 1.3. The comparison of spatiotemporal spawning patterns in the North Fork John Day River for 2014 (solid dots) and 2015 (open diamonds) with associated linear regression lines. Gray shading represents the 95% confidence interval for each regression line. In both years, spawning date and spatial location were significantly correlated ($R^2 = 0.761$, $P < 0.001$ 2014; $R^2 = 0.498$, $P < 0.001$ 2015). Note that spawning began and ended earlier and that nests occurred further upstream at an earlier date in 2015 relative to 2014.

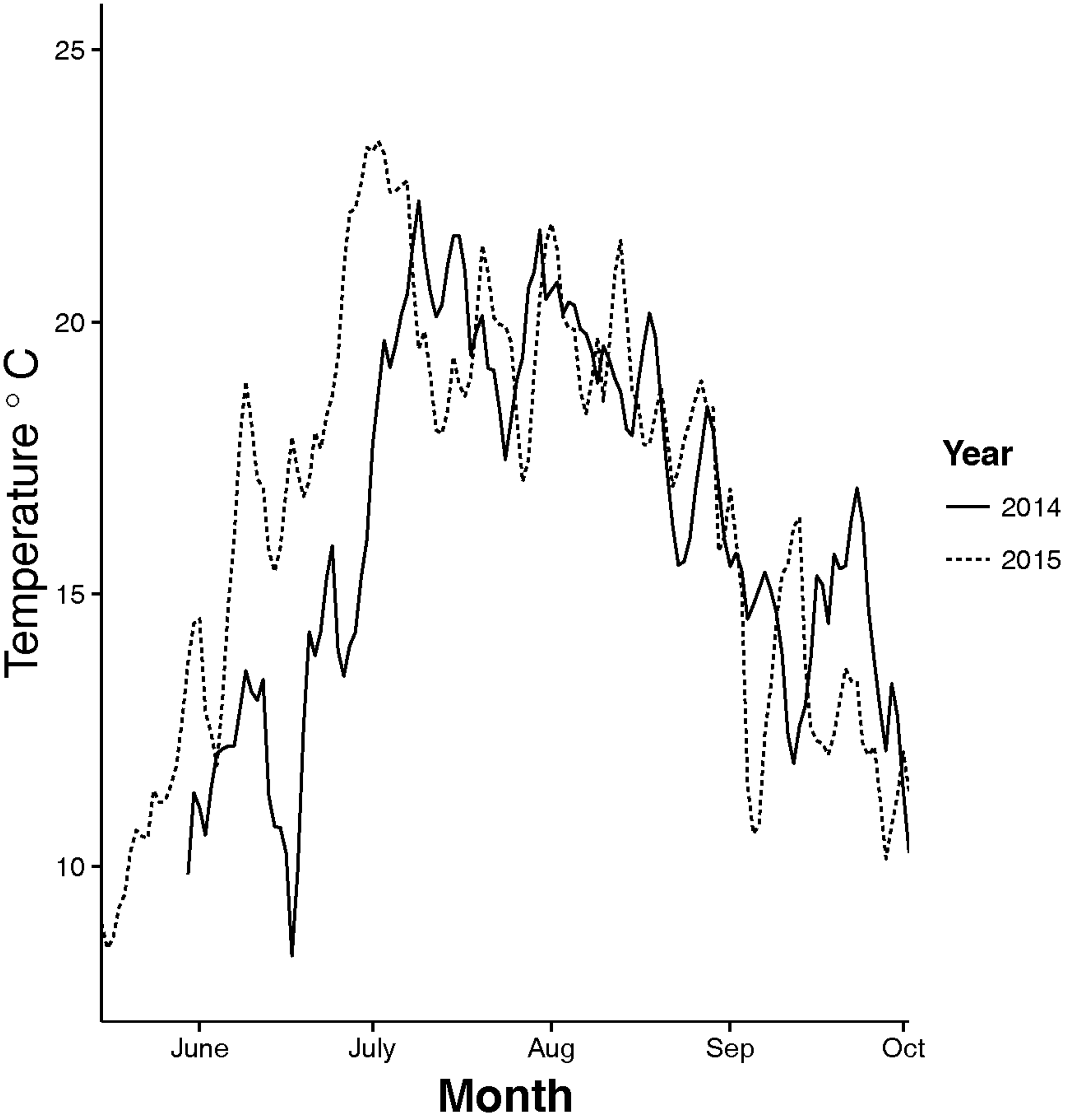

Figure 1.4. Average daily temperature profile for river kilometer 110, highlighting the 2-4°C warmer temperatures in 2015 (dashed line) relative to 2014 (solid line) from May through early July observed throughout the North Fork John Day River.

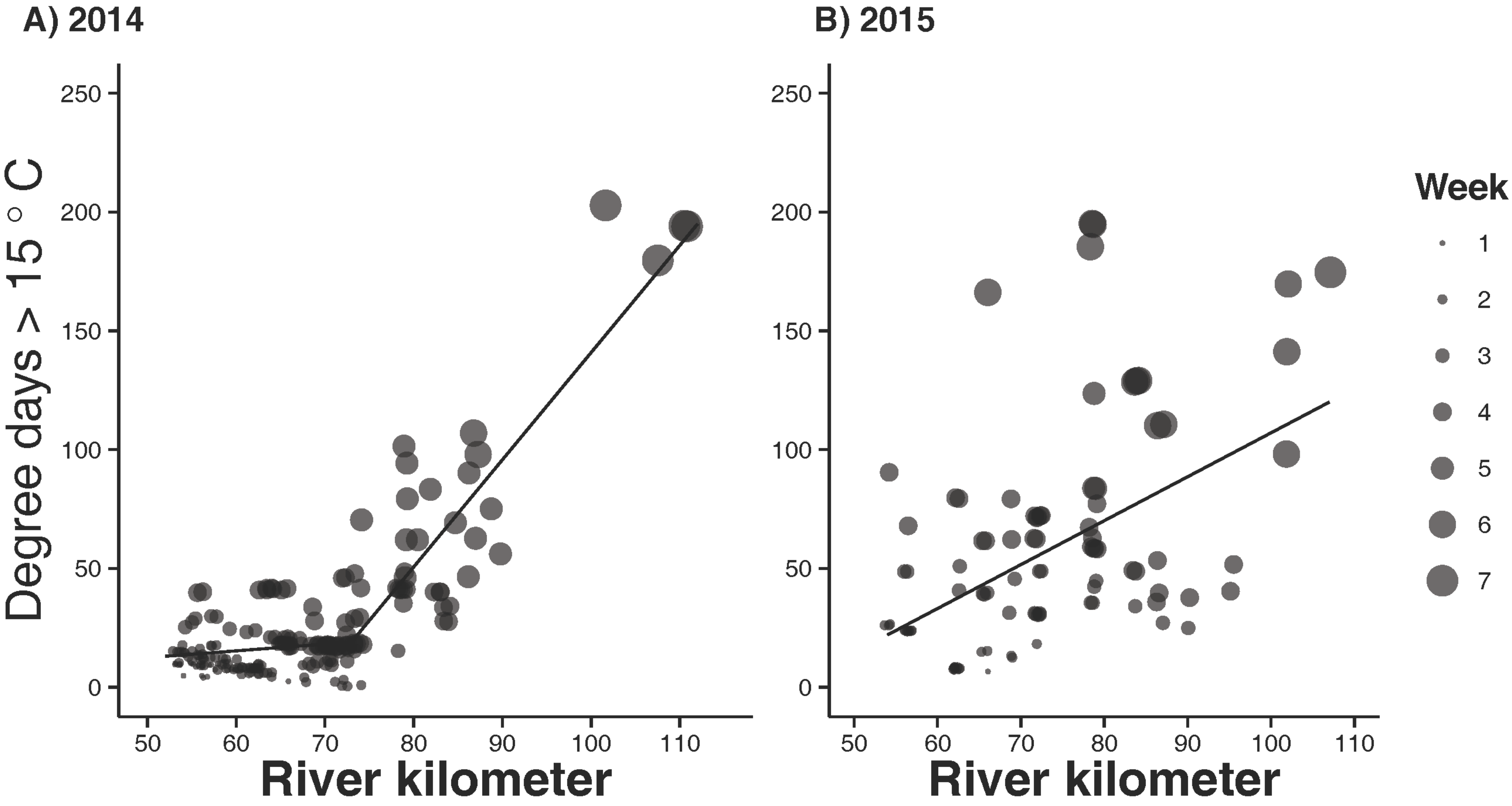

Figure 1.5. The number of degree days > 15°C accumulated prior to the initiation of spawning along the spatial extent of the survey in the North Fork John Day River for **(A)** 2014 and **(B)** 2015. Each point represents a nest (points scaled by the week in which each nest received eggs) over the time period of the survey (May – August). Lines of best fit according to the piecewise regression analysis are reported for 2014 (y = 0.26x – 1.10; y = 4.54x – 312.20), and linear regression analysis is reported for 2015 (y = 1.88x – 79.81).

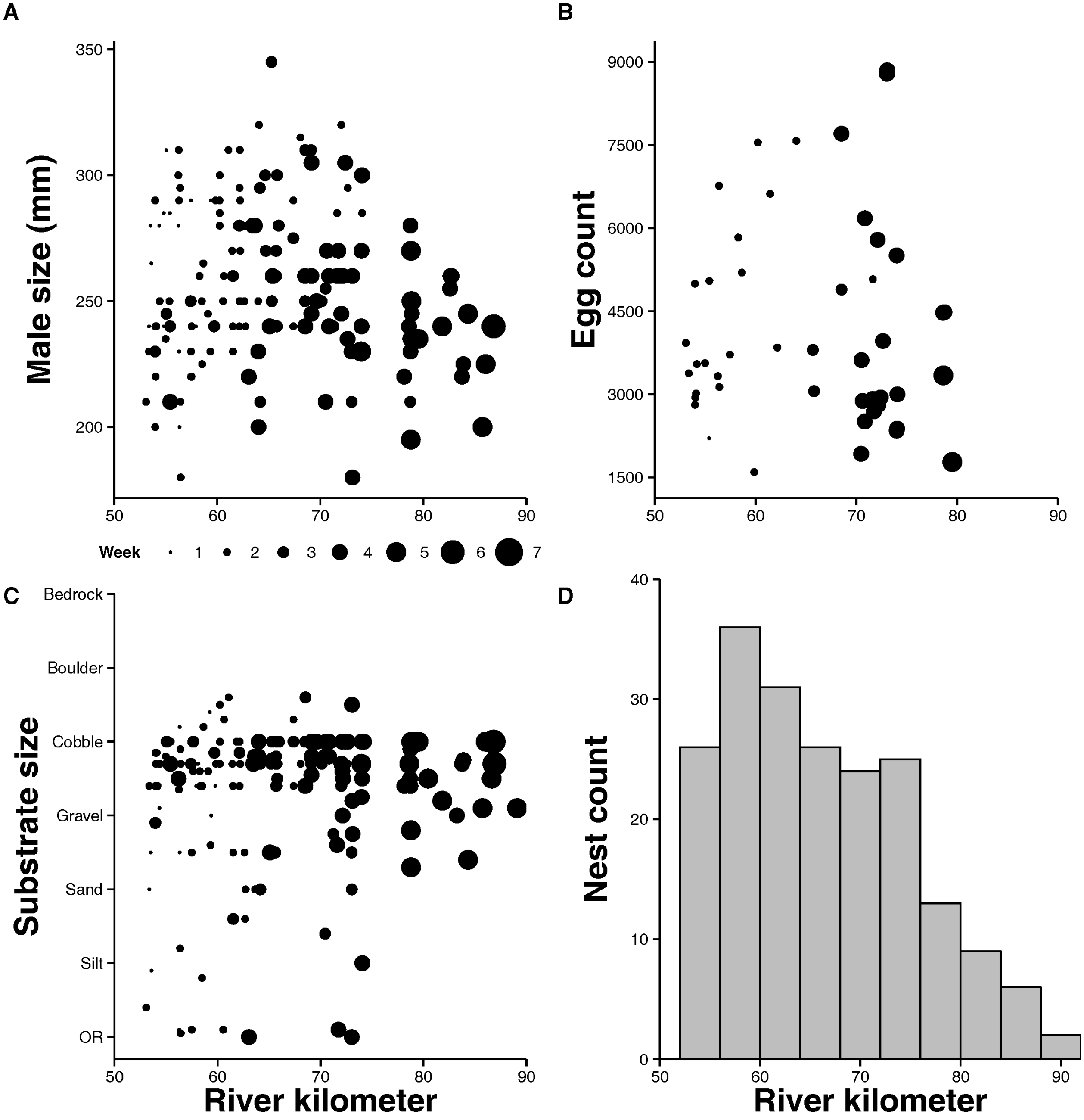

Figure 1.6. Reproductive attributes from nests in 2014. Data points are scaled by week in which the nest received eggs. **(A)** Adult nest-guarding male size (mm) (n = 183) at each river kilometer (RKM). **(B)** Distribution of egg counts for nests (n = 59) at each RKM. **(C)** Average substrate composition of Smallmouth Bass nests at each RKM. Substratum is represented as a composite score determined by multiplying the field-measured substrata percentage (i.e. percentage of dominant and subdominant substratum) by a score for each substrata type. Scores given each substrata are 7 = bedrock, 6 = boulder, 5 = cobble, 4 = gravel, 3 = sand, 2 = silt, 1 = organic matter (OR). **(D)** Number of nests at each RKM.

Chapter 2: Growth and recruitment of nonnative smallmouth bass along the upstream edge of its riverine distribution

Authors: E.S. Rubenson, J.D. Olden

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2A. Abstract

Nonnative species have been widely introduced, and once established, often exhibit secondary spread to new areas. For instance, after its initial introduction in the John Day River, Oregon (USA), smallmouth bass (*Micropterus dolomieu*) has expanded upstream into headwater habitats. Recruitment is a key component of successful range expansion and has been highlighted as a potential bottleneck to continued expansion by smallmouth bass. We explored growth, body lengths, and survival of young-of-the-year (YOY) smallmouth bass in the North Fork John Day River to better understand the recruitment dynamics near its invasion boundary. In 2014-15, we collected YOY across the upstream 63-km of smallmouth bass distribution at the end of the first growth season and after a winter starvation period. We found that growth, body length, and survival showed varied correspondence with patterns in water temperature. Specifically, body lengths matched temperature predictions in upstream sites (after accounting for spawning delays) where smallmouth bass density is low. By contrast, individuals achieved smaller than predicted body lengths in downstream sites where density is relatively high. Model selection revealed that temperature and age ≥ 1 density were the most important predictors of body length. Additionally, individuals predicted to be too small to survive a winter starvation period were present. Our findings reveal nuanced recruitment dynamics at the invasion boundary, where departures from temperature-based predictions point to multiple mechanisms affecting growth and survival. Understanding mechanisms operating at invasion boundaries may help develop management strategies to prevent future spread of smallmouth bass into headwater salmon habitat.

2B. Keywords

Secondary spread, range expansion, invasive species, headwater, young-of-the-year

2C. Introduction

Nonnative species have been widely introduced, including freshwater ecosystems of the northwestern United States (Sanderson et al. 2009). Although rates of intentional introductions by agencies have generally slowed in recent decades, many species are continuing, or have the potential, to expand their range in response to continued environmental change (Vander Zanden and Olden 2008). One of the primary determinants for a species to successfully expand its range is the ability for young-of-the-year (YOY) to survive (i.e., recruit) in new habitat (Angert et al. 2011, Blackburn et al. 2011). Examining patterns of YOY growth and survival near range boundaries may provide useful insight into mechanisms, and thus management strategies that constrain species' range expansion.

In the Columbia River Basin, nonnative Smallmouth Bass (*Micropterus dolomieu*) was introduced in the early 20th century and now supports a multi-million-dollar sport fishing industry (Carey et al. 2011). Smallmouth Bass, a cool water predator native to central and part of the eastern United States and Canada, has also been implicated in the decline of wild populations of native Pacific salmon (Fritts and Pearsons 2004, Carey et al. 2011). Recent decades have seen Smallmouth Bass expand its range into headwater habitats of the Columbia River Basin, calling attention to the need to understand mechanisms that enable successful range expansion (Lawrence et al. 2012).

The John Day River is the largest undammed tributary of the Columbia River and home to a prized Smallmouth Bass fishery. Initially, Smallmouth Bass from the Columbia River were unable to colonize the John Day River due to an impassable (for Smallmouth Bass) waterfall near the John Day River mouth (Tumwater Falls at river kilometer [RKM] 16). In 1971, however, 80 individuals were introduced upstream of that barrier at a location approximately 42 km downstream of the mouth of the North Fork John Day River (Shrader and Gray 1999). Smallmouth Bass have since expanded approximately 150 km upstream into the North Fork John Day River and have encroached on endangered, wild, juvenile spring Chinook Salmon (*Oncorhynchus tshawytscha*) rearing habitat (Lawrence et al. 2012).

The spatial congruence of Smallmouth Bass populations and Chinook Salmon rearing habitat varies across time in the North Fork John Day River (Lawrence et al. 2012, Rubenson and Olden 2017). Recent surveys indicate that the invasion edge of Smallmouth Bass shifts both seasonally and inter-annually, but that there has been an overall trend of upstream expansion since 2009 (Rubenson and Olden 2017). Each spring, some adult Smallmouth Bass move upstream, spawn, and then return downstream in the late summer and early fall. Although there are detailed data on the adult movement and spawning patterns, little is known about the growth and survival dynamics of the resultant YOY. Based upon previous modeling, it is hypothesized that the growth potential in the most upstream habitats (i.e., > RKM 90) is insufficient to provide adequate energy reserves for YOY to survive the winter (i.e., cold-water period) (Lawrence et al.

2015), but this hypothesis has yet to be tested. Our objectives were to 1) characterize the growth and body length distributions of YOY across the upstream-most 63 km of riverine Smallmouth Bass distribution, 2) explore potential determinants of YOY length, 3) examine post winter length distributions, and 4) compare observed lengths (pre and post winter) with lengths predicted by temperature-dependent models of growth and survival (Shuter et al. 1980).

2D. Methods

Study Area

Our study examined the growth and survival of YOY Smallmouth Bass in the upstream 63 km of its distribution in the North Fork John Day River (i.e., upstream of RKM 54) (Figure 2.1). The John Day River is an undammed major tributary of the Columbia River that originates in the Blue Mountains of northeastern Oregon. The hydrology is characterized by a snowmelt flow regime, resulting in peak discharges in the spring that decline to a summer base flow that remain low until early winter (Figure 2.2). In the North Fork John Day River, mean daily water temperature ranges from 0°C (i.e., ice over) in winter to > 25°C in summer (Figure 2.2). Channel gradients range from 2.4 m km^{-1} to 30 m km^{-1}, producing a thermal gradient that cools ca. 0.3°C every 5 km. This results in a 4.5°C difference in daily mean temperature between the most upstream (RKM 117) and downstream (RKM 54) temperature of our study extent (Figure 2.2). Guided by previous snorkel surveys, this also encompassed portions of the river that have had year-round presence of Smallmouth Bass since at least 2009 (i.e., below RKM 72) and continued upstream to 5 km beyond the most upstream presence of Smallmouth Bass observed in 2014 (Rubenson and Olden 2016).

Temperature and Discharge

Water temperature was recorded using a series of pendant temperature loggers (HOBO pendant, Onset Electronics, Onset, Massachusetts) distributed across the upstream 63 km of Smallmouth Bass distribution in the North Fork John Day River. Loggers were placed approximately 5 km apart. Temperatures were recorded at 1-hour intervals from June 2014 to October 2015. Linear models were then fit to estimate hourly water temperature at a 1-RKM scale ($R^2 = 0.958$ to 0.992, all $P < 0.001$). Smallmouth Bass are considered to rarely feed once temperatures drop below 10°C (Shuter et al. 1980). As such, we quantified the winter starvation period by tallying the number of days below a mean daily temperature of 10°C, generally beginning in fall and ending in spring. Daily mean temperature for tributaries of the North Fork John Day River were obtained from the Integrated Status and Effectiveness Monitoring Program (ISEMP) database (https://www.webapps.nwfsc.noaa.gov/apex/stem/f?p=168:2:14227893538131), which measured daily temperature using loggers from June to October 2001-2003. Data was extracted from paired temperature loggers in tributaries and their confluence with the mainstem North Fork John Day River. Finally, discharge ($m^3 s^{-1}$) was collected from the USGS gauge station 14046000, located at RKM 24 of the North Fork John Day River.

Collection and Processing of YOY

Individuals spawned in spring 2014 were collected at pre-selected glides distributed approximately every 3-7 km along the upstream-most 63 km of Smallmouth Bass distribution (Figure 2.1). Glides were selected to be similar in length (between 100-200 m), width (20-40 m), and to have substrate and vegetation characteristics conducive to Smallmouth Bass spawning. Upstream of RKM 90, Smallmouth Bass occurrence was extremely rare, and surveys in 2014 indicated only 4 Smallmouth Bass nests that received eggs (Rubenson and Olden 2016). As such, the sample sites upstream of RKM 90 were aligned with nest locations. The spring 2014 fish cohort were collected twice; once as YOY during the fall of 2014 as mean daily temperatures declined toward 10°C – the threshold below which feeding reportedly stops (Table 2.1; Figure 2.3a) and again as age-1 fish in the spring of 2015 as mean daily temperatures warmed above 14°C – the threshold at which growth begins (Table 2.2; Figure 2.3b). Between 10-14°C, feeding occurs, but not at sufficient levels to achieve growth (Shuter et al. 1980). Two attempts were made to capture age-1 fish during the spring; one in May and one in June. During the May survey, water temperatures were below 14°C, but the water was too deep and discharge too swift to efficiently capture age-1 fish. In addition, a two-week rain event increased the discharge and water depths during the survey, preventing further sampling at that time (Figure 2.2). As such, only 18 individuals were captured in four glides. These 18 individuals, however, provided a useful baseline for body lengths before spring growth could occur. A growth correction was applied to individuals captured in June (see below), and resultant sizes were compared with the 18 individuals captured in May.

Two survey methods were used in every unit to capture the spring 2014 cohort. For shallow (depth < 0.5 m) areas characterized by cobble, silt, or vegetated substrate, we used a 3-m beach seine (3 mm bar mesh) to capture individuals. In depths > 0.5 m or areas with boulder substrate, we set dog-food baited Gee minnow-traps (23 x 44 cm, 2.5 cm opening, 0.6 cm mesh) overnight. Traps were set between 0.5 m and 1 m depths approximately every 10 m along both shorelines (as appropriate). Smallmouth Bass were extracted from the nets or traps, and all other fishes released. Smallmouth Bass were killed using an overdose of MS-222 and total length (mm) (hereafter, body length) was measured. Individuals were then placed in individual bags and frozen on dry ice until transport back to the laboratory.

Aging and Growth of YOY

The otoliths from 95 YOY were extracted for aging (Table 2.1). These YOY were subsampled from the total number of Smallmouth Bass collected during the fall surveys in select sample sites distributed across the survey extent (RKM 57 [n = 24], RKM 69 [n = 25], RKM 78-87 [n = 24], RKM 102-110 [n = 22]) (Table 2.1). Otoliths were extracted by Washington Department of Fish and Wildlife (WDFW) and mounted in thermoplastic resin, ground using successive grits of lapping film (30-5 µm grit size), and polished using aluminum oxide slurry (1 µm grit size). Otoliths were imaged (20X) using a Leica DM100 compound scope paired with a DFC 450 camera and increments enumerated using Image Pro software. Daily increments were independently enumerated by two readers beginning at the hatch check out to the dorsal edge (Hill and Bestgen 2014). The average percent error index (Beamish and Fournier 1981) between readers was 2.2% and the mean difference between readers was 3 days (d) (min = 0 d, max = 9 d). After completing increment analysis, days from hatch date was calculated as the mean of all reads for each fish, rounded to the nearest integer. Growth rate (mm d^{-1}) was then calculated for each fish based on number of days from hatch date (d) and body length at capture. Growth rate was calculated using the equation:

$$\textit{Growth (mm } d^{-1}\textit{)} = \textit{Length at capture (mm)} - 5.6 \textit{ (mm) / Days from hatch date (d)}$$

where 5.6 mm is the average body length at hatching (Hill and Bestgen 2014).

Predicted Length and Survival

Predicted body length (mm) was calculated using the Smallmouth Bass YOY daily growth model from Shuter et al. (1980):

$$G = 0.0,\ T < 14°C;\ T > 35°C;$$

$$G = -1.7 + 0.12T,\ 14°C \leq T < 25.5°C;$$

$$G = 1.4,\ 25.5°C \leq T < 31.5°C;$$

$$G = 1.4 - 0.04T,\ 31.5°C \leq T \leq 35°C$$

where T is daily mean temperature (°C) and G is daily growth (mm). This model assumes maximum feeding rates, and therefore reflects the maximum body length achievable based upon the thermal conditions. It also accounts for limitations to growth at high temperatures (i.e., > 35°C). To determine predicted lengths at the end of the growth season (i.e., fall 2014), modeled daily growth was summed over the growing season, ending at YOY capture date, using daily mean temperatures at each sample site.

Three curves predicting the potential growth of YOY Smallmouth Bass across the study area were developed. The first curve calculated potential growth assuming that spawning began on the first day mean daily water temperatures warmed above 15°C (the temperature at which spawning is typically initiated) (Rubenson and Olden 2016). We use this curve to provide a benchmark for the maximum size that could be attained had spawning occurred as soon as conditions became amenable. Here, we also assumed immediate brood development, adding 9.1 mm to the summed daily growth increments to account for the average length of fry when they rise from the nest (Shuter et al. 1980). The second and third curves calculated potential growth from the first and last date of observed spawning, which were often delayed relative to when water temperatures first exceeded 15°C. To determine this timing, a comprehensive assessment of spawning events was conducted. Spawning was assessed via repeat snorkel surveys by two snorkelers on opposite shorelines (Rubenson and Olden 2016). All nests were geo-referenced, enumerated, and then monitored for success throughout the spawning season (i.e., June to August) (Table 2.1). Spawning initiation was defined as the date a nest received eggs. When there was a discrepancy between observed spawning dates and spawning dates calculated from otolith ages (Table 2.1), the most conservative date was used. Again, we added 9.1 mm to the summed daily growth increments based on early spawn timing but elected to not make this correction for the estimates for late spawn timing. This delivers a more conservative estimate of minimum body length predicted at that location. Predicted body lengths were then compared with observed body lengths.

Predicted survival was calculated using the Shuter et al. (1980) survival index. This index describes the probability that a YOY Smallmouth Bass will survive a winter (i.e., cold-water) starvation period based upon its body length at the end of the growing season. It was calculated as:

$$L_0 = 0.188d + 14.8$$

where L_0 represents the length (mm) below which survival is predicted to be 0% and d is the duration of winter (daily mean temperature < 10°C) in days. Observed body lengths (mm) of age-1 fish collected in the spring of 2015, after a winter starvation period, were compared with the predicted minimum body length required for survival (L_0) to test the accuracy of this model in predicting survival in this system. In some sample sites, the age-1 fish were collected after temperatures were sufficiently warm for post-winter growth to occur. In these cases, a length correction was applied using sizes calculated from the modeled growth equations (Table 2.2). Specifically, the potential growth incurred during spring 2015 was subtracted from size at capture. Resultant lengths were then compared with individuals collected in May 2015 (i.e., before temperatures warmed to above 14°C when growth begins) to validate the use of this correction.

Smallmouth Bass Density

The linear density of Smallmouth Bass YOY was measured using quantitative surveys in the fall of 2014. Each sample site was divided into 20 m transects within 5 m of both shorelines. Surveys were conducted a minimum of 20 m from the previous transect to ensure independent counts. The number of transects surveyed was proportional to the length of the sample site (105 – 250 m), with a maximum of 10 transects surveyed (divided evenly between both shorelines). For each transect, a snorkeler swam in the center and reported counts of YOY Smallmouth Bass to an above water observer. Density was then calculated by dividing the sum of the YOY Smallmouth Bass observed by the total length surveyed in each sample site.

Linear density of Smallmouth Bass ≥ age 1 was estimated using two separate snorkel surveys in the spring and fall of 2014. Two snorkelers sampled individual sample sites on opposite shorelines, surveying in an upstream direction. Snorkelers reported the number of ≥ age-1 Smallmouth Bass (> 100 mm) in each sample site to an above water observer. Counts between the two surveys were averaged, and density was calculated by dividing the average count by the length of the sample site surveyed.

Statistical Analysis

All statistical analyses were conducted in R version 3.3.2 (R Foundation for Statistical Computing, Vienna). We used linear modeling to examine the determinants of body length (mm) at each sample site as a function of age estimated from otoliths (days), mean temperature (calculated for each individual based upon its age and sample site) (°C), and intra-specific density of both age ≥ 1 and YOY. Models were built using the 95 individuals that had age determined via otoliths. All possible candidate models (n = 15) were considered, and model support was evaluated using Akaike's information criterion (AIC) (Burnham and Anderson 2002). Residual and diagnostic plots were examined for evidence of departures from model assumptions. Piecewise linear regression was applied to assess longitudinal trends in mean body lengths (n = 13) and mean growth rates estimated from otoliths (n = 8) using the *segmented* library in R (Muggeo 2008). Piecewise linear regression identifies breakpoints at which an abrupt change in slope occurs, highlighting departures from linearity.

2E. Results

Contrary to our prediction of a linear, temperature-determined decrease of YOY Smallmouth Bass lengths from downstream to upstream, we found a more nuanced pattern at the upstream distribution of Smallmouth Bass (Figure 2.3a). We captured 426 YOY Smallmouth Bass across its upstream distribution in the fall of 2014 (Table 2.1) and 230 age-1 Smallmouth Bass the following spring (2015) (Table 2.2). No individuals were captured at RKM 95 or 117, so these sample sites were not included in our analyses. In contrast to an upstream linear decrease in body lengths as predicted by the temperature-dependent growth models, we found a significant breakpoint at RKM 90 (piecewise regression $R^2 = 0.870$, $P = 0.000321$, n = 13) with a non-significant slope downstream of this breakpoint ($P = 0.693$; $y = 49.6 + 0.05*x$). This result appears to be caused by the majority of individuals in downstream sample sites not achieving the predicted body length based upon spawn timing (Figure 2.3a). Upstream of RKM 90, individuals achieved the predicted body length based upon observed spawn timing, but appeared constrained by a delay in spawn timing relative to when water temperatures warmed above 15°C (Figure 2.3a).

Ages of YOY generally reflected the protracted spawning season observed in 2014. Spawning was initiated in early June in downstream sample sites as water temperatures reached 15°C, with a progressive delay (relative to water temperature) with increased RKM (Figure 2.3a). The latest spawning event was observed August 10th at RKM 110, well after water temperatures reached 15°C (Table 2.1, Figure 2.2). Individual YOY aged from otoliths ranged from 56 to 99 days old (mean = 73 d, SD = 6 d), corresponding to spawning dates of June 22 – August 5. When comparing the ages in each sample site, however, there were some exceptional divergences from the spawn timing observed (Table 2.1). Specifically, spawning surveys indicated that spawning ceased in June or early July, but otolith ages suggest additional spawning that was not detected in snorkeling surveys.

The density of both ≥ age 1 and YOY Smallmouth Bass declined in an upstream direction (Figure 2.4). The maximum observed density of YOY was 0.6 fish m^{-1}, which declined exponentially toward < 0.1 fish m^{-1} in the uppermost sample sites ($y = 1.8 – 0.03*x + 0.0002*x^2$, $R^2 = 0.525$). The maximum observed density of ≥ age 1 fish was 0.3 fish m^{-1}, which declined toward 0.0 fish m^{-1} moving upstream ($y = 0.9 – 0.02*x + 0.00009*x^2$, $R^2 = 0.834$). The top-performing models that predicted individual lengths included mean daily water temperature (°C) over the growth season and the density of ≥ age 1 Smallmouth Bass (Table 2.3). This highlights a potential interaction between the length of a growth season and the temperature experienced, but also suggests a negative relationship between the density of ≥ age 1 Smallmouth Bass and YOY growth in downstream sample sites.

Contrary to our predictions, growth rates did not exhibit a consistent longitudinal pattern (Figure 2.5). Across the sample sites in which YOY were aged, growth rates ranged from 0.4-0.7 mm d^{-1} (mean = 0.6 mm d^{-1}, SD = 0.1 mm d^{-1}). We hypothesized that there would be a decreasing linear trend in growth across the study sites based upon growth scope (Figure 2.3a). Instead, piecewise regression revealed a significant breakpoint at RKM 86 ($R^2 = 0.974$; $P = 0.00188$, n = 8), with a non-significant slope downstream from this breakpoint ($P = 0.833$; y = 0.7 – 0.0003*x). This matches the trend observed in body lengths, with similar lengths in sample sites downstream of RKM 90 and smaller individuals upstream.

We found that the length of the winter starvation period (i.e., the number of days < 10°C) did not predict the minimum size of age-1 fish post winter. Instead, 23% of the age-1 fish collected in the spring (after the cold-water winter period) were smaller than the size below which we predicted 0% survival (Figure 2.3b). In the sample sites upstream of RKM 100, however, no recruitment was observed, which matched the modeled prediction. By contrast, mean and maximum sizes remained relatively unchanged after winter. In all but one sample site (RKM 90), measurements of mean and maximum body lengths of YOY before and age-1 after winter were within 5 mm of each other (Figure 2.3b). At RKM 90, however, we found that age-1 individuals were on average 9 mm larger than YOY captured the previous fall, and we found a difference of 21 mm between the maximum length of YOY and age-1.

2F. Discussion

Our study presents the first assessment of Smallmouth Bass YOY growth and recruitment at its upstream-most distribution. We show that the factors that influence growth rates, body length, and overwinter survival vary considerably across the upstream 63 km of its distribution.

The most upstream sample sites were predictably constrained by season length and temperature, but were also substantially influenced by a delay in spawn timing (Figure 2.3a). By contrast, the most downstream sample sites were not well predicted by season length and temperature, but instead revealed evidence for additional constraints on growth related to bass density. Furthermore, we found that some small individuals were able to survive an overwinter starvation period despite not attaining the predicted minimum survival size (> L_0) according to temperature-dependent growth models (Figure 2.3b).

In the most downstream sample sites, YOY body length at the end of the growth season fell short of model benchmarks (Figure 2.3a). Models predicted that YOY in downstream sample sites would receive growth benefits from warmer temperatures and a longer growth season (Figure 2.3a). Instead, the majority of observed body lengths were either below or near the minimum predicted lengths, creating a departure from the hypothesized linear relationship between body length and RKM (Figure 2.3a). This was also exhibited in the non-significant slope across growth rates in the downstream sample sites (Figure 2.5). Although it is not surprising that some individuals fell below the modeled predictions (due to the assumption of maximum consumption and potential errors in calculating the last spawning date), this result suggests that other mechanisms may be in operation. One explanation could be that smaller YOY from upstream sample sites were displaced into lower sample sites, potentially confounding the data. Dispersal of YOY was not measured and recent research indicates that Smallmouth Bass juveniles may undergo frequent, albeit short-distance, movements in lotic systems (Humston et al. 2017). In addition, passive dispersal during high flow conditions can force YOY downstream (Humston et al. 2010). Although there were no high discharge events between 1 June 2014 (before spawning began) and 27 September 2014 (the last collection date in the fall) (Figure 2.2), even small discharge events could displace fry or small individuals (Simonson and Swenson 1990). The potential that YOY were mobile could also explain some of the discrepancies between observed spawn timing and calculated ages. The influence of YOY movement on our results remains an open line of research. Dispersal from upstream, however, would also not explain the paucity of large individuals in the most downstream sample sites and research suggests that YOY generally remain within a few hundred meters from their natal nest site (Langhurst and Schoenike 1990, Scott et al. 1997). Another explanation for the lack of a longitudinal relationship could be that biological interactions – specifically competition or risk of predation – may be limiting growth in this region of the invasion boundary. We show that intra-specific density declines from downstream to upstream (Figure 2.4), which corresponds with our observations that body lengths are smaller than predicted in downstream sample sites. This hypothesis is supported by our modeling exercise, which indicated a significant negative relationship between adult density and YOY body lengths (Table 2.3). This mechanism is supported by Ridgway et al. (2002) who found that first year growth was negatively correlated with abundance of age 1-4 Smallmouth Bass. In addition, pools with high densities of largemouth bass juveniles have been shown to reduce juvenile growth when compared to low density pools (Ludsin and DeVries 1997), and results from individual based modeling of Smallmouth Bass show that high densities consistently lead to smaller juvenile size (DeAngelis et al. 1991). The potential that adult abundance in downstream sample sites has a negative effect on YOY growth could have important management implications. Specifically, any efforts to remove adults in these sites could result in increased growth of YOY, potentially confounding control efforts. By contrast, adult removals further upstream where abundances are lower may be more effective at controlling the population. In order to better understand the effects of intra-specific density on YOY growth, however, additional food web and bioenergetics analyses are required.

In two sample sites (RKM 72 and RKM 90), a few individuals achieved larger than predicted body lengths (Figure 2.3a). The age-1 individuals captured in the spring at RKM 90 were also substantially larger (mean = 9 mm larger, maximum = 21 mm larger) than YOY captured in the fall at this location (Figure 2.3b). This is in contrast to the rest of the study sites and perplexing based upon the model assumption of maximum consumption, therefore maximum potential growth. One explanation is that larger individuals may have moved up from warmer, downstream regions. The lack of larger YOY captured in nearby downstream sample sites, however, makes this less plausible. Another explanation is these sample sites might be in close proximity to a thermal refuge. For instance, RKM 90 is located at the confluence of a warm water tributary (i.e., Camas Creek) (Figure 2.1). On average, the mouth of Camas Creek is approximately 0.6°C warmer than the North Fork John Day River just upstream of the confluence with Camas Creek. This suggests that warm water tributaries may confer growth benefits to individuals assuming adequate access to these habitats. The growth opportunities provided by warm water refuges during the winter may provide a number of advantages, such as reduced risk of predation (Miller et al. 1988, Post et al. 1998), quicker transition to piscivory (Ludsin and DeVries 1997, Phelps et al. 2008) and lower risk of mortality from starvation (Shuter et al. 1980, Post et al. 1998). In addition, perennial tributaries, such as Camas Creek, may provide overwinter refuges where growth is still possible despite cold temperatures in the main channel. Smallmouth Bass has been shown to use thermal refuges in other streams during cold-water periods, demonstrating a propensity for thermoregulation (Peterson and Rabeni 1996, Carter et al. 2012, Westhoff et al. 2014). Refuges can act to buffer populations from fluctuations in inter-annual conditions, potentially acting as a source population after prohibitively cold winters (Peterson and Rabeni 1996). In addition to warm water tributaries, groundwater upwelling and deep pools may also provide thermal refuge in the North Fork John Day River (Torgersen et al. 1999). It is unknown if a thermal refuge exists near RKM 72.

In the most upstream sample sites (i.e., > RKM 90), body lengths matched our modeled predictions according to temperature-dependent growth, but were also heavily constrained by spawn timing (Figure 2.3a). In 2014, adults did not spawn upstream of RKM 90 until late July; a month after temperatures had exceeded 15°C (Rubenson and Olden 2016). This delayed initiation of spawning truncated the length of the overall growing season and reduced the maximum body length that YOY could achieve entering into the winter. Had spawning occurred as temperatures warmed above 15°C, the YOY upstream of RKM 90 could have potentially grown 5-15 mm larger before water temperatures began to cool in the fall (Figure 2.3a). This highlights the interaction between adult overwintering location and spawning habitat. Specifically, a previous survey found that adults abandon the upper reaches of the North Fork John Day River in the early fall – overwintering downstream (Rubenson and Olden 2017). Adult Smallmouth Bass often exhibit strong site fidelity to overwinter sites that, in cold-water habitats, are characterized by deep pools or nearby lakes. This suggests a high metabolic cost of remaining resident in spawning and summer feeding habitats in the upstream edge of Smallmouth Bass distribution. The mismatch between overwintering habitat and spawning habitat likely contributed to spawning delays, therefore a better understanding of limitations to adult overwintering locations may provide a key piece to the recruitment potential of Smallmouth Bass at its invasion boundary.

The observed minimum body length required to survive an overwinter starvation period did not match modeled predictions according to the survival equations of Shuter et al. (1980) downstream of RKM 90 (Figure 2.3b). At least two reasons may explain this result. First, our analysis relied on daily mean temperatures measured at a single location in the channel, thus not accounting for small-scale thermal refuges for fishes caused by groundwater upwelling, vegetation, interstitial spaces in the substrate, stratified pools, and/or tributaries (Peterson and Rabeni 1996, Torgersen et al. 1999, Torgersen et al. 2006, Westhoff et al. 2014). There are multiple deep pools, rock crevices, and thick vegetation in the North Fork John Day River that may support warm water microhabitats important to overwintering fish. Second, it is possible that the survival equations of Shuter et al. (1980) are not appropriate for rivers, specifically in the Pacific Northwest. These equations are based on temperate populations of lacustrine Smallmouth Bass in Canada, which may have different physiological responses based on adaptive differences (Shuter et al. 1980). For instance, populations of largemouth bass from high, intermediate, and low latitudes were shown to be locally adapted to winter severity, causing low-latitude juvenile largemouth bass to experience increased mortality over high-latitude juveniles under the same winter conditions (Fullerton et al. 2000). Even if local adaptation cannot account for differences in survival, however, the severity of the winter may play some role in the observed differences. Specifically, the winters in the John Day River do not exhibit rapid declines in temperature below 10°C, but instead hover between 7-10°C for 30-60 days (Figure 2.2). This is in sharp contrast to lacustrine habitats that often show rapid temperature shifts post turnover of the lake water column. There is evidence that although Smallmouth Bass activity level declines rapidly below 10°C, it is not until 7°C that they remain completely inactive and do not feed. Shuter et al. (1980) states that, "Experiments showed that the activity of the young of year drops markedly as the temperature falls below 10°C. At 7°C and below, the fish seek shelter, remain inactive for long periods, and do not feed." Although the 10°C threshold is typically used in the models, the starvation experiments conducted by Shuter et al. (1989) used temperatures less than 7°C. We found that using a 7°C threshold better predicted survival in 2015, with the estimated 0% survival line (L_0) falling reasonably close to the smallest observed age-1 fish that survived to the spring (Figure 2.3b). This suggests that the survival equations are appropriate and effective for YOY Smallmouth Bass in the North Fork John Day River, but that 7°C is a more accurate temperature threshold to use when calculating winter length. Examining the mechanism of mortality, however, remains an open line of research in the leading edges of riverine invasions.

In comparing the size of fish before and after the winter, it is important to note that the winter period in the North Fork John Day River in 2014 was characterized by several high discharge events that could have displaced individuals downstream (Figure 2.2). During these periods, however, water temperatures were cold, which affects Smallmouth Bass behavior. In temperatures below 7°C, YOY seek shelter and remain inactive (Shuter et al. 1980) – behavior that was observed during our surveys. The benthic nature of YOY during cold temperatures may make them less susceptible to passive transport during high discharge events in the winter. This, however, remains an open line of research. Even if YOY were displaced downstream, however, it does not explain how fish predicted to have insufficient lipid reserves to survive a winter starvation period (i.e., fish smaller than L_0) were present in downstream sample sites. Had the smaller individuals been displaced from upstream units, they still should have accumulated additional days of winter to survive based on models using a 10°C threshold.

In the most upstream sample sites (i.e., > RKM 90), we were unable to locate any YOY post winter, suggesting little to no survival. The individuals in these locations were the smallest and experienced the longest winter (Figure 2.3b). Several mechanisms can account for high mortality rates. First, these small individuals had vastly insufficient energy reserves available to survive a long winter, regardless of whether the winter threshold was at 10°C or 7°C, falling well below the predicted 0% survival line (Figure 2.3b). In addition, small size also likely increased their susceptibility to predation. Smaller individuals are more prone to predation risk due to being within the gape limitation of more predators and having reduced mobility than larger conspecifics (Post et al. 1998, Hurst 2007). These two factors were coupled with very low reproductive activity upstream of RKM 90, leading to low abundances relative to downstream. Specifically, only four nests were observed over the most upstream 27 km of our survey extent (Rubenson and Olden 2016). Between these four nests, the two closest singular nests were distributed at least 300 m apart, leading to extremely low densities of YOY fishes (Figure 2.4). These low abundances made complete mortality more likely.

One major limitation to our study is the scarcity of data above RKM 80. Although the sample sizes are small, they are representative of the density of Smallmouth Bass, particularly above RKM 80. There were very low abundances of adults upstream of RKM 80 and only a few successful nests (Figure 2.4, Table 2.1). We present these data to highlight potential differences in mechanisms that affect growth and survival at the upstream distribution of invasive Smallmouth Bass. In downstream sample sites, we depict an environment in which there is likely a complex interaction of factors influencing the growth scope of YOY. By contrast, growth in upstream sample sites is more easily predicted from spawn timing and the thermal regime alone. These differences are important to understand to appropriately tailor future management strategies.

Although there is some evidence that the temperature threshold most appropriate to predict 100% overwinter mortality was closer to 7°C than 10°C based on fish collections in May and June (as discussed above), combining a calculation of predicted lengths and the minimum size predicted to survive based on local daily mean temperature can potentially predict the edge of recruitment to within 10-20 km (Rubenson and Olden 2017). Thus, using only temperature data, managers have access to an easy, inexpensive, and quick method to locate the approximate recruitment boundary in an unstudied system where Smallmouth Bass has been introduced. This may hold utility when predicting the invasion extent of a long-established population where data are scarce or if predicting the potential invasion extent of a newly introduced population of Smallmouth Bass. Knowing the potential for invasion can provide incentives for control or eradication efforts before an expanding population achieves the physiological range boundary.

2G. Conclusion

Managers throughout the Pacific Northwest are charged with conservation of native fishes in addition to supporting a healthy and economically viable Smallmouth Bass fishery (Carey et al. 2011). Understanding recruitment dynamics may provide new management tools that can protect critical headwater habitats from range expansion of Smallmouth Bass while still preserving fishing opportunities downstream. Here we demonstrate that growth potential at the most upstream edge of Smallmouth Bass distribution is strongly influenced by temperature, although mediated by biotic conditions such as spawn timing and the density of ≥ age 1 Smallmouth Bass. This highlights the sensitivity of the YOY life-history stage to temperature. Initiatives that reduce the growing season such as riparian zone restoration (which can cool streams) (Lawrence et al. 2014), timing dam releases of cold water to deter or delay spawning

(Olden and Naiman 2010), and other control strategies, including inducing spawning failure via nest destruction (Loppnow et al. 2013) may deter recruitment in new habitat. Understanding the mechanisms that enable the secondary spread of nonnative species can ultimately improve our ability to manage streams that are now home to both native and nonnative species.

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2J. Tables

Table 2.1. Fall 2014 survey data at each sample site, including river kilometer (RKM), sampling date, mean daily temperature (°C) at time of sampling, date when growth ceased (i.e., when daily mean temperature fell below 14°C), number of young-of-the-year (YOY) captured, number of captured YOY that also had otoliths analyzed, observed spawn timing, calculated spawn timing from aged YOY, and the number of observed nests that produced swim-up fry.

RKM	Sampled	T (°C) @ sampling	T < 14°C	# YOY	# Otoliths	Observed spawn	Calculated spawn	# Nests
54	22 Sep	19.4	2 Oct	58		10-25 Jun		4
57	21 Sep	19.4	2 Oct	54	25	13-27 Jun	22 Jun – 18 Jul	5
62	20 Sep	18.4	2 Oct	23		20-28 Jun		8
66	20 Sep	18.8	1 Oct	40		11 Jun-3 Jul		6
69	23 Sep	18.6	1 Oct	41	25	22 Jun-3 Jul	8 – 22 Jul	5
72	24 Sep	18.6	1 Oct	111		19 Jun-6 Jul		6
78	25 Sep	16.4	1 Oct	50	17	30 Jun-13 Jul	8 – 23 Jul	9
84	26 Sep	14.6	1 Oct	4	4	5-11 Jul	10 – 23 Jul	3
87	26 Sep	14.3	30 Sep	4	4	10-16 Jul	14 – 26 Jul	3
90	27 Sep	13.0	30 Sep	5		10 Jul		1
95	27 Sep	13.2	30 Sep	0				0
102	27 Sep	12.8	27 Sep	7	3	10 Aug	1 – 2 Aug	1
107	27 Sep	12.8	26 Sep	9	9	5 Aug	18 – 25 Jul	1
110	27 Sep	12.8	26 Sep	21	13	5-8 Aug	25 – 29 Jul	2
117	27 Sep	12.6	26 Sep	0				0

Table 2.2. Spring 2015 survey data at each sample site, including the river kilometer (RKM), sampling date, mean daily temperature (°C) at time of sampling, date when growth began (i.e., when daily mean temperature increased above 14°C), number of young-of-the-year (YOY) captured, and a length correction for the potential growth may have occurred prior to sampling.

RKM	Sampling	T (°C) @ sampling	T > 14°C	# YOY	Length Correction (mm)
54	22 Jun	21.0	27 May	52	15
57	17 May	11.6	27 May	3	
	22 Jun	20.9	27 May	50	14
62	19 Jun	19.7	28 May	2	11
66	19 Jun	19.9	28 May	28	11
69	16 May	10.8	28 May	3	
	19 Jun	19.8	28 May	36	10
72	16 May	10.8	28 May	9	
	19 Jun	19.8	28 May	23	10
78	18 May	10.8	29 May	3	
	20 Jun	19.6	29 May	11	10
84	20 Jun	18.9	29 May	2	9
87	20 Jun	18.9	30 May	1	8
90	21 Jun	18.8	30 May	10	7
95	21 Jun	18.8	30 May	0	
102	21 Jun	18.6	30 May	0	
107	21 Jun	18.1	31 May	0	
110	21 Jun	18.0	31 May	0	
117	21 Jun	17.6	31 May	0	

Table 2.3. Summary of the top 5 regression models predicting body length (mm) of aged young-of-the-year (YOY) smallmouth bass (n = 95) collected in the fall 2014 as a function of mean daily water temperature (°C), age (days), and density of YOY and adult smallmouth bass at each sample site. The null model AIC is 740.3. All candidate models provided in Supplemental Table 1.

Model	R^2	AIC_i	ΔAIC_i
Temp*, Adult Density*, Age	0.597	656.9	0.0
Temp*, Adult Density*	0.592	657.2	0.3
Temp*, Adult Density*, YOY Density	0.594	657.6	0.7
Temp*, Adult Density*, Age, YOY Density	0.594	658.4	1.5
Temp*, Age	0.563	663.6	6.7

* $P < 0.05$

2K. Figures

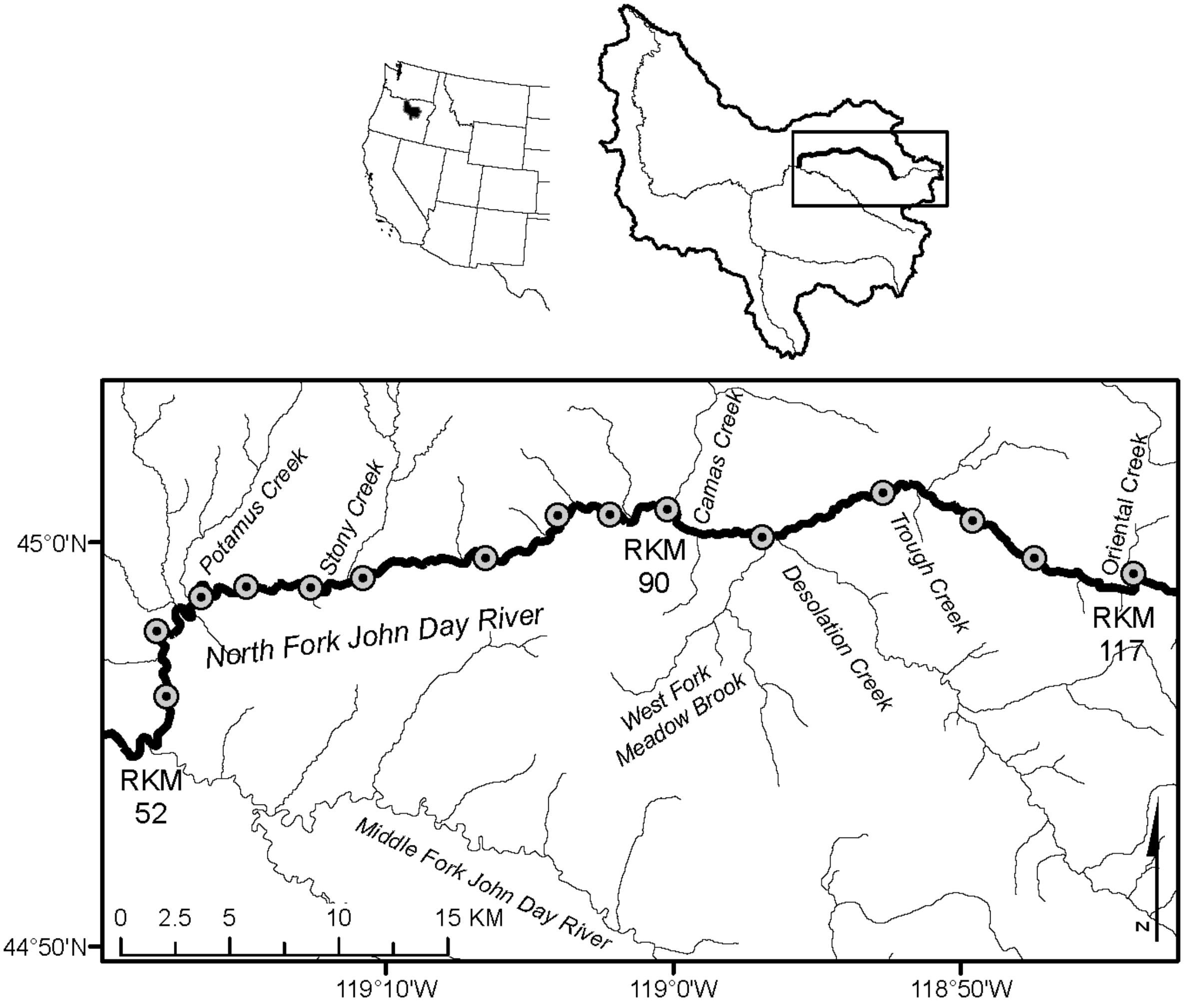

Figure 2.1. The location and watershed of the North Fork John Day River, Oregon. The survey extent is highlighted in the lower box, with river kilometer (RKM) and large tributaries annotated. The gray circles indicate the distribution of sample sites where young-of-the-year and age-1 smallmouth bass were collected in 2014-2015.

Figure 2

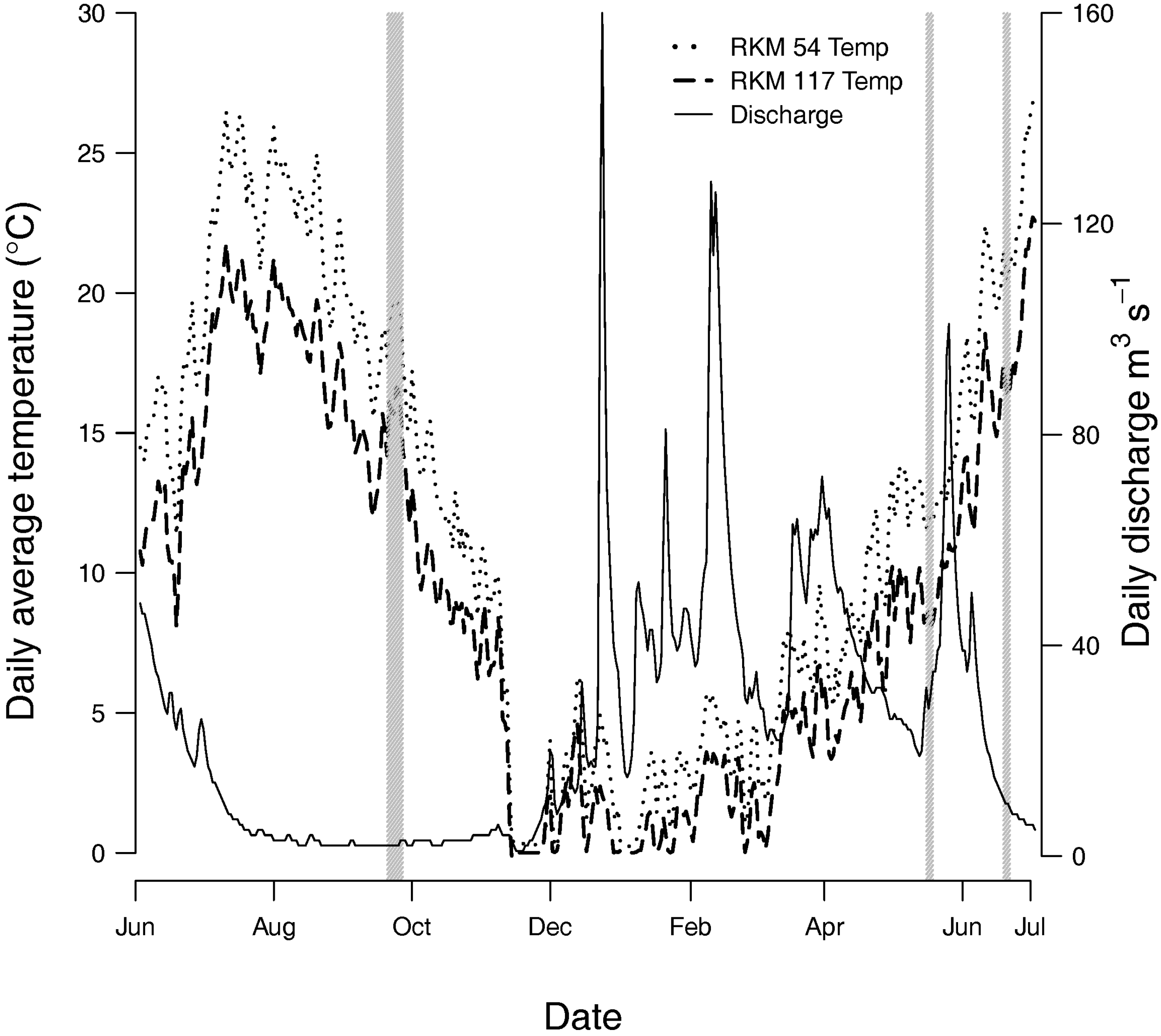

Figure 2.2. Daily mean temperature (°C) and discharge ($m^3 s^{-1}$) over the study period (June 2014 – July 2015). On the left y-axis is daily mean temperature in the river kilometers (RKM) at the lower (RKM 54) and uppermost (RKM 117) sampled sample sites. On the right y-axis is daily discharge from the USGS Gauge Station 14046000, measured at RKM 24 of the North Fork John Day River. The three shaded rectangles represent the dates when young-of-the-year smallmouth bass were sampled.

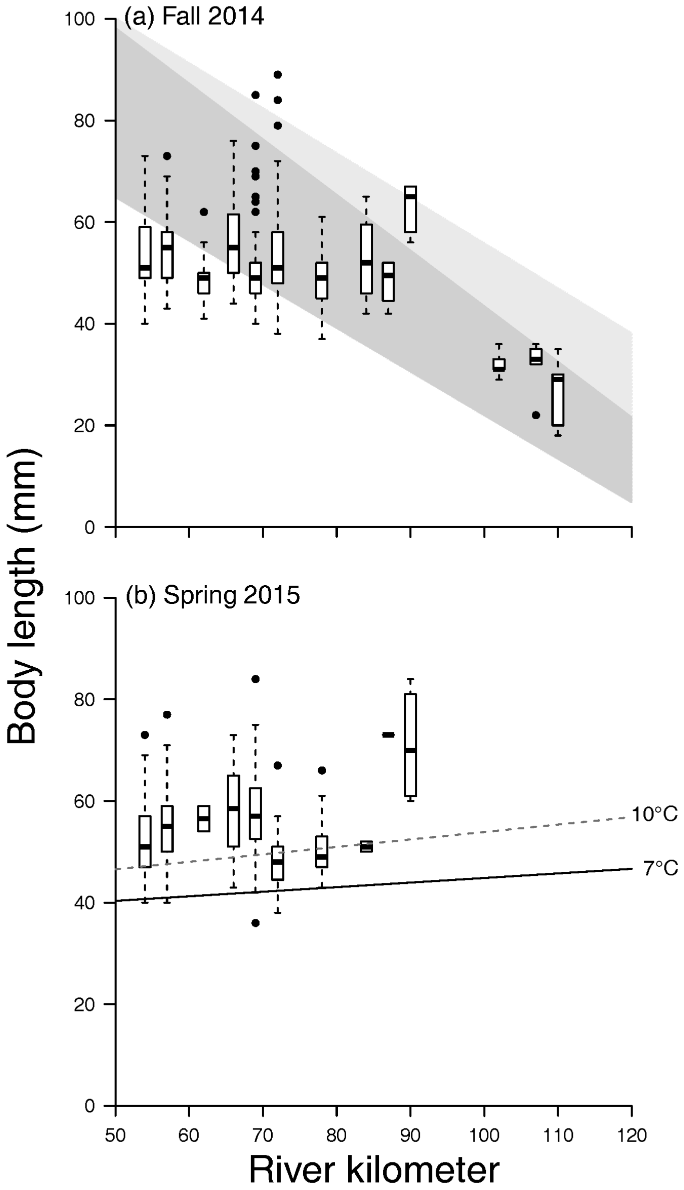

Figure 2.3. Body length (mm) of young-of-the-year (YOY) and age-1 smallmouth bass captured across the upstream distribution in the a) fall 2014 and b) spring 2015. The boxes represent the first and third quartile of all the individuals collected, with the midline representing the median. The whiskers represent the highest and lowest values excluding outliers (i.e., individuals more than 1.5 times the interquartile range). The points are individual outliers. a) The grey polygons represents the predicted body lengths based upon previously published growth models (Shuter et al. 1980). The upper limit represents the maximum body length predicted assuming spawning initiation when water temperatures warmed above 15°C. The dark grey represents predicted body lengths based upon observed spawning at each sample site. The light grey represents the lost growth opportunity due to delays in spawning relative to water temperature. b) The lines represent the modeled minimum length a YOY must achieve to survive an overwinter starvation period (L_0) using a 10°C (dashed) and 7°C (solid) threshold for defining winter.

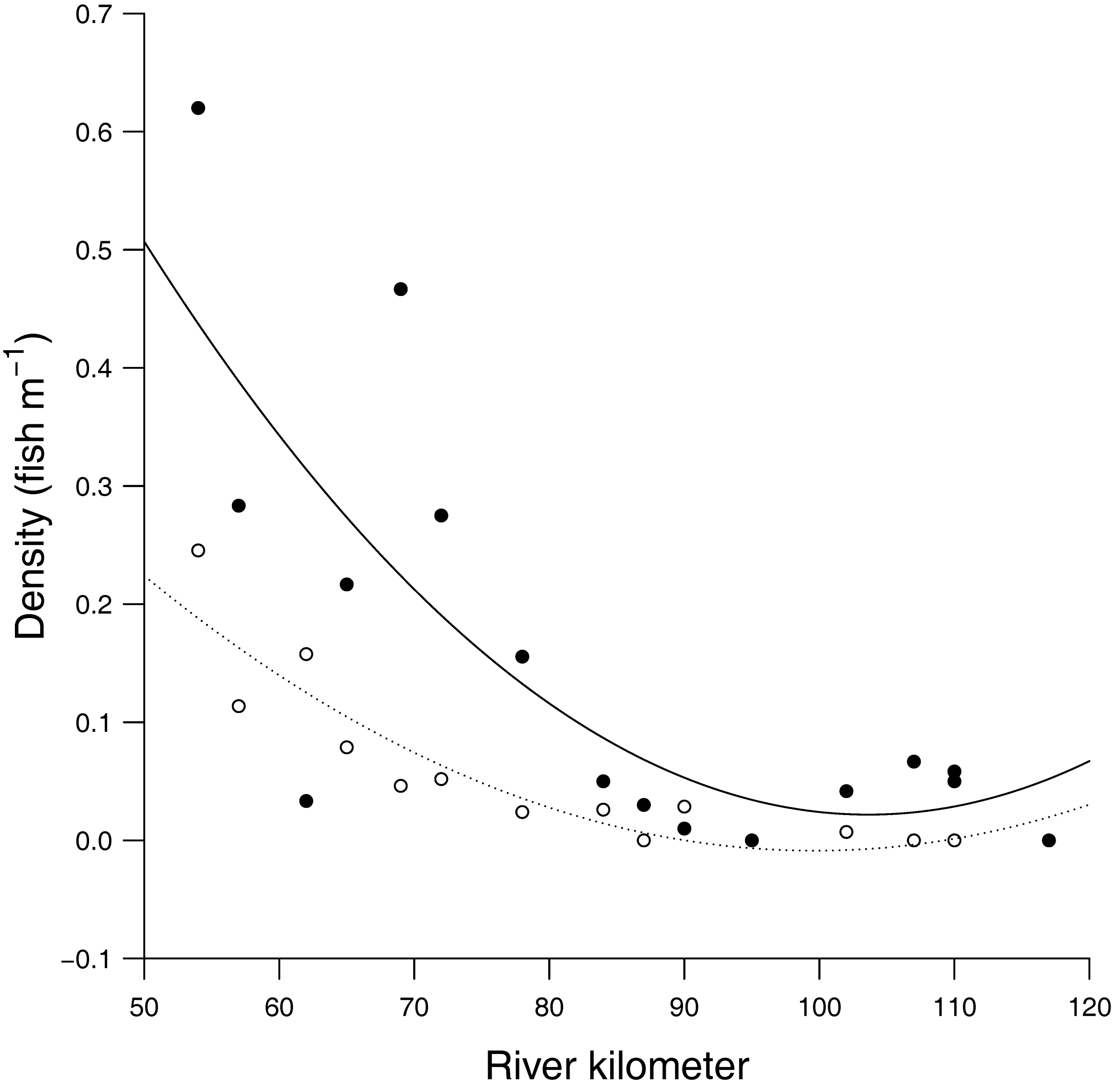

Figure 2.4. Density (fish m^{-1}) of young-of-the-year (YOY) (closed circles) and age ≥ 1 smallmouth bass (open circles) across the invasion boundary. Density was calculated by taking the sum of the smallmouth bass observed and divided by the total length surveyed. The fit lines represent the exponential decrease in density as river kilometer increases (YOY: $y = 1.8 - 0.03*x + 0.0002*x^2$, $R^2 = 0.525$; Age ≥ 1: $y = 0.9 - 0.02*x + 0.00009*x^2$, $R^2 = 0.834$).

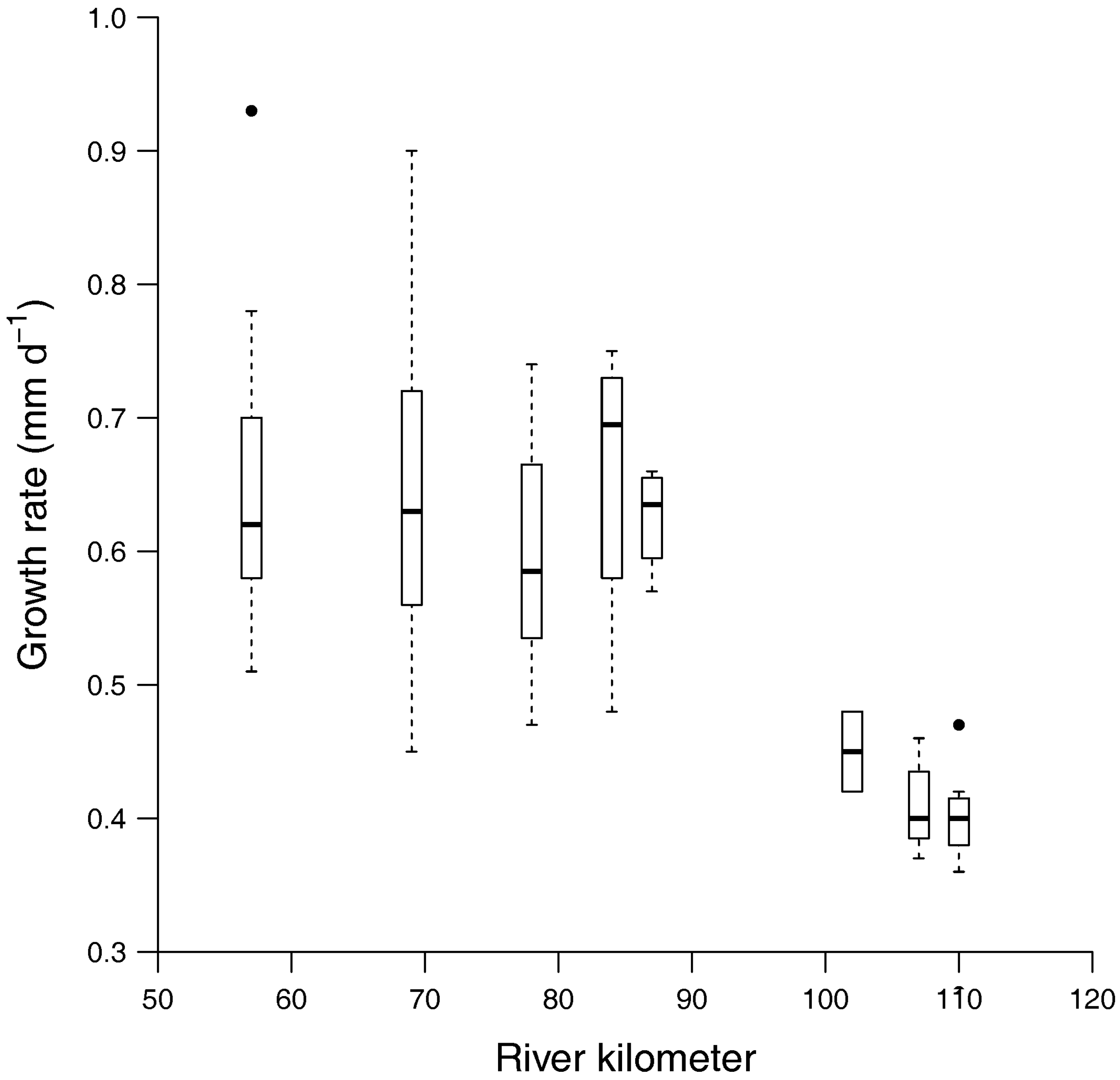

Figure 2.5. Growth rates (mm d^{-1}) for individual young-of-the-year smallmouth bass across subsampled habitats at the upstream distribution of smallmouth bass (n = 95). The boxes represent the first and third quartile of all the individuals collected, with the midline representing the median. The whiskers represent the highest and lowest values excluding outliers (i.e., individuals more than 1.5 times the interquartile range). The points are individual outliers.

2L. Chapter 2 Supplement

SUPPLEMENTAL TABLE 2.1. Equations for the regression models predicting body length (mm) of aged young-of-the-year (YOY) smallmouth bass (n = 95) as a function of mean daily water temperature (°C) (**T**), age (days) (**A**), and density of YOY (**YOY**) and adult smallmouth bass (**AD**) at each sample site. The null model AIC is 740.3.

Model	R^2	AIC_i	ΔAIC_i
-106.1 + 7.2***T** – 115.7***AD** + 0.2***A**	0.597	656.9	0.0
-106.9 + 8.1***T** – 125.7***AD**	0.592	657.2	0.3
-123.5 + 9.1***T** – 142.8***AD** – 8.8***YOY**	0.594	657.6	0.7
-116.3 + 8.0***T** – 128.2***AD** + 0.2***A** – 5.3***YOY**	0.594	658.4	1.5
-60.5 + 4.3***T** + 0.3***A**	0.563	663.6	6.7
-56.0 + 3.9***T** + 0.4***A** + 4.5***YOY**	0.560	665.3	8.4
-56.2 + 5.2***T**	0.550	665.4	8.5
-56.8 + 5.3***T** – 0.5***YOY**	0.545	667.4	10.5
-12.6 + 44.2***AD** + 0.7***A** + 21.1***YOY**	0.497	678.0	21.1
-20.5 + 0.9***A** + 25.3***YOY**	0.487	678.8	21.9
-11.4 + 82.4***AD** + 0.8***A**	0.438	687.6	30.7
-28.7 + 1.1***A**	0.382	695.5	38.6
36.7 + 109.1***AD** + 22.8***YOY**	0.374	697.8	40.9
40.1 + 153.1***AD**	0.306	706.5	49.6
38.2 + 37.5***YOY**	0.262	712.5	55.6

Chapter 3: Dynamism in the upstream invasion edge of a freshwater fish exposes range boundary constraints

Authors: E.S Rubenson, J.D. Olden

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3A. Abstract

Studying the dynamics of species' borders can provide insight into the mechanisms limiting or promoting range expansion in response to environmental change. In the John Day River, Oregon (USA), rising stream temperatures are facilitating the upstream expansion of invasive smallmouth bass *Micropterus dolomieu*. Here, where smallmouth bass occupy the upstream limit of its thermal tolerance, we explore population structure and seasonal movement patterns to elucidate the environmental conditions and individual traits that define front edge (where individuals reside year-round) and leading edge (where individuals colonize, but may not establish) limits to its upstream distribution. Reporting on a multi-year, spatially extensive riverscape survey, our results show dramatic ebbs and flows of seasonal occupancies due to individual movement with an overall trend of upstream expansion. We revealed distinct front and leading edge invasion extents, each constrained by different ecological conditions. The front edge is largely constrained by the ability for juveniles to survive an overwinter starvation period whereas the leading edge is associated with adult growth potential and seasonal discharge patterns. We also found key morphological traits associated with more mobile individuals. By

providing mechanistic insight into the factors that promote or limit range expansion of an invasive riverine species, our study enhances the ability to predict future range shifts and provides critical information to managers tasked with restricting further expansion.

3B. Keywords

Smallmouth bass, secondary spread, range dynamics, expansion, morphology

3C. Introduction

Environmental change, including shifts in climate and habitat conditions, has required many species to move to avoid extinction (Parmesan 2006, Ackerly et al. 2010). In recent history, dramatic shifts to species' ranges have been documented throughout the world (Chen et al. 2011), creating challenges for conserving native species (Lawson et al. 2012) and for slowing the spread and impact of nonnative invasions (Diez et al. 2012). Predicting the rate and magnitude of range shifts, however, has proven difficult – in part because of unexpected responses of individuals to novel environmental conditions at the edge of their range (Dytham 2009, Chuang and Peterson 2016).

Investigating the dynamics of individuals that inhabit the edge of an ongoing expansion presents a unique opportunity to understand the factors that promote or inhibit species' ranges. At range boundaries, species reside at the limit of their physiological tolerances and tend to experience large fluxes in habitat suitability caused by changing seasonal and inter-annual environmental conditions (Holt et al. 2005, Sexton et al. 2009). These range boundary conditions impose unique selection pressures on individuals, often resulting in different dispersal, life-history or ecological strategies relative to conspecifics in the core distribution (Dytham 2009, Burton et al. 2010, Volis et al. 2016). Examining the patterns, behaviors and traits of individuals in the expanding edge, therefore, can provide critical insight into the mechanisms that determine the rate and magnitude of future range shifts (Lindstrom et al. 2013).

Range boundaries are characterized by repeated colonization attempts, successes and failures through time (Sexton et al. 2009, Kubisch et al. 2010). By examining the ebb and flow of species' edges in both space and time, it is possible to differentiate areas where populations persist and grow (i.e., core population and front edges, respectively) from areas that are being actively colonized but are not yet established (i.e., leading edge) (Lenoir and Svenning 2013). This distinction provides insights into the conditions that enhance or restrict colonization success, and ultimately better inform conservation and management practices at critical boundary regions (Rehm et al. 2015).

Freshwater fishes face unique movement and range expansion challenges. Streams and rivers constrain fishes into the wetted boundaries of their dendritic network (Grant et al. 2007), and individuals can only respond to environmental change within these confines (Olden et al. 2010). As a result, fish have demonstrated longitudinal shifts towards more upstream habitats in response to climate warming (Heino et al. 2009, Comte and Grenouillet 2013). However, understanding range expansion dynamics in riverine systems is constrained by how little is known about the movement patterns of non-anadromous freshwater fishes (Radinger and Wolter 2014). Natural history data, for example, have limited ability to reveal the mechanisms that underpin range changes, as they cannot directly assess the environmental conditions that initiate movements or account for failed colonization attempts. Here, we leverage new tracking technology and the study of invasive species to address some of these knowledge gaps.

Invasive species can act as large-scale experiments that enable real-time investigation of the ecological processes influencing range expansion (Sax et al. 2007, Moran and Alexander 2014). The colonization dynamics of secondary spread from an initial introduction location and natural colonization are biologically similar (Hoffman and Courchamp 2016), and consequently represent a powerful, yet still under-utilized, opportunity to advance our understanding of range expansion in changing environments. Recognizing that established nonnative species are often, by definition, good dispersers, they still face ecological and biological constraints that limit their range and contribute to a greater understanding of range boundary dynamics (Moran and Alexander 2014, Hoffman and Courchamp 2016).

In the present study we examine an established, yet actively expanding, invasive population of smallmouth bass *Micropterus dolomieu* at the upstream extent of its range boundary in a major Columbia River tributary. Smallmouth bass is a popular sport fish native to central and eastern North America that has been widely introduced throughout the world (Loppnow et al. 2013). As a voracious predator, there is concern about the rate and magnitude of climate-driven range expansion into headwaters of Pacific Northwest rivers that provide critical rearing habitat for several endangered and threatened salmonids (Sanderson et al. 2009, Lawrence et al. 2014).

Smallmouth bass in riverine ecosystems are generally described as a sedentary species (Todd and Rabeni 1989) that establish home ranges and exhibit strong inter-annual homing tendencies (Ridgway and Shuter 1996). Populations subjected to large fluctuations of habitat suitability – such as range boundary populations – however, have exhibited dramatic differences in dispersal behaviors, undertaking long journeys to attempt colonization in novel habitats (Lyons and Kanehl 2002, Tabor et al. 2012). In the Pacific Northwest, evidence suggests that such large seasonal movements occur each year at the upstream extent of smallmouth bass' riverine range (Lawrence et al. 2012).

The goal of our study is to explore the environmental conditions (i.e., extrinsic factors) and individual traits (i.e., intrinsic properties) that initiate and inhibit movement responses. We examine two range boundary populations of smallmouth bass in the North Fork and Middle Fork John Day River, Oregon (USA), employing spatially extensive surveys to monitor population and individual movement patterns over four years. Our objectives were 1) to quantify spatiotemporal changes to abundance and extent of smallmouth bass at inter-annual and seasonal time scales, 2) to characterize individual movement patterns of adults and assess individual traits associated with movement, 3) to determine the consistency of the front edge (where individuals reside year-round) and leading edge (where individuals colonize, but may not establish) over time, and 4) to evaluate the potential environmental drivers of invasion edge dynamics. We specifically define the front edge as the most upstream portion of the river in which smallmouth bass are present each spring after a winter (cold-water) period (i.e., spring invasion edge), whereas the leading edge is defined by the most upstream location of smallmouth bass during any time of the year (i.e., summer invasion edge). Through these objectives, we investigate the effect of seasonal movement on range boundary dynamics and evaluate the mechanisms that promote and limit smallmouth bass range in Pacific Northwest rivers.

3D. Methods

Study area description

Our study examines range boundary dynamics at the upstream extent of smallmouth bass distributions in the North Fork (NFJDR) and Middle Fork (MFJDR) tributaries of the John Day

River (JDR) (Fig. 3.1). The JDR basin originates in the Blue Mountains of northeastern Oregon and drains an area of 21,000 km^2. It is the largest undammed tributary of the Columbia River, and is the third longest free-flowing river in the contiguous United States. The JDR is also home to a population of endangered wild-only, spring Chinook salmon *Oncorhynchus tshawytscha* and threatened steelhead *Oncorhynchus mykiss*. In 1971, smallmouth bass was introduced into the JDR, approximately 42 km downstream of the NFJDR confluence (Shrader and Gray 1999). Since that time, smallmouth bass has expanded its range over 150 km upstream and is now resident in both the NFJDR and MFJDR (Lawrence et al. 2012).

Flowing through colluvial and alluvial canyons and valleys, the NFJDR is characterized by channel gradients ranging from 2.4 m km^{-1} to 30 m km^{-1}, and the less steep MFJDR ranging from 0.6 m km^{-1} to 15 m km^{-1}. As a result, the NFJDR also has a steeper thermal gradient (cools ca. 0.3°C every 5 km gained in an upstream direction) than the MFJDR (cools ca. 0.2°C every 5 km). Discharge in both systems peak in Apr and May in response to snowmelt, decline through Jun and Jul and generally exhibit summer base flow until late Sep (Online Resource 3.1). Water temperatures range from 0°C in winter (partial ice coverage) to mean summer temperatures > 25°C that generally peak in Jul, with a 4.5°C (NFJDR) or 3°C (MFJDR) difference between the most upstream and most downstream temperatures of our survey extent (Lawrence et al. 2012, Rubenson and Olden 2016).

Temperature and discharge monitoring

Water temperature was measured from Jun-2009 to Sep-2010 (Lawrence et al. 2012) and again May-2014 to Jun-2016 using an array of 15 Hobo temperature loggers (Onset Electronics, Onset, MA, USA) in the NFJDR and 13 loggers in the MFJDR. Each logger was placed approximately

5 km apart and distributed over 66 and 58 river kilometers (RKM) in the NFJDR and MFJDR, respectively. Additionally, water temperatures for 2008-9 and 2013-14 were obtained from an array of temperature loggers managed by the U.S. Forest Service and MFJDR Intensively Monitored Watershed program. Water temperatures for individual RKMs (the study units) were assigned using a linear extrapolation of recorded temperatures as a function of river kilometer; r^2 values for all models ranged from 0.958 to 0.992 (all $p < 0.001$). Growing degree-days for young-of-the-year were calculated by summing mean daily temperatures exceeding a 10°C threshold (the temperature at which smallmouth bass initialize feeding: Shuter et al. 1980) for each day beyond when daily mean temperature first exceeded 15°C (the temperature at which smallmouth bass initiate spawning: Shuter et al. 1980, Lawrence et al. 2015). Days of winter were calculated by summing the number of days less than 10°C beginning when first achieved in late fall the year prior to the survey until temperatures again first attained 10°C in the spring. Discharge was measured from a U.S. Geological Survey (USGS) long-term flow gauge (station 14046000) located on the NFJDR at RKM 24 and a gauge (station 14044000) located at RKM 24 on the MFJDR. The number of days of low discharge during the spring and summer was calculated by summing the number of days below mean daily discharge thresholds of 28 m^3 s^{-1} for NFJDR and 9 m^3 s^{-1} for MFJDR between 1-Apr and 1-Aug. These thresholds were determined by the mean daily discharge observed at the time of first smallmouth bass spawning activity for each study year. These values generally represent the receding limb of the hydrograph and demarcate a shift from high magnitude snowmelt discharge to lower flow conditions amenable to smallmouth bass spawning and movement (Online Resource 3.2).

Growth and survival models

Predicted size of young-of-the-year smallmouth bass was calculated using mean daily temperatures and the daily growth equations in Shuter et al. (1980):

$$G = 0.0,\ T < 14°\text{C};\ T > 35°\text{C};$$

$$G = -1.7 + 0.12T,\ 14°\text{C} \leq T < 25.5°\text{C};$$

$$G = 1.4,\ 25.5°\text{C} \leq T < 31.5°\text{C}$$

where T is temperature (°C) and G is daily growth (mm). The average starting length of fry was assumed to be 9.1 mm, to which the summed daily growth increments were added at the end of each season. Additionally, we used the Shuter et al. (1980) survival index that describes the minimum size young-of-the year smallmouth bass need to attain to survive a winter (cold-water) starvation period:

$$L_0 = 0.188d + 14.8$$

$$L_1 = 0.328d + 33.9$$

where L_0 represents the length (mm) below which survival is predicted to be 0% and L_1 represents the length above which survival is predicted to be 100%; and d is the duration of winter in days. Previous smallmouth bass bioenergetics modeling has demonstrated the validity of the daily growth and survival equations of Shuter et al. (1980) in the JDR (Lawrence et al. 2015).

Longitudinal surveys

In 2009, 2010 (Lawrence et al. 2012) and 2014, seasonal spatially continuous snorkel surveys were conducted in the NFJDR; one in the spring as temperatures warmed above 15°C – when smallmouth bass typically initiate spawning (Shuter et al. 1980, Lawrence et al. 2015) – and the second in the late summer as temperatures began to decline (late Jul to early Aug). The MFJDR was similarly surveyed in 2009 and 2014. Surveys consisted of two snorkelers on opposite shorelines simultaneously assessing each reach in an upstream direction. Snorkelers reported the number and size class (< 100 mm, 100-200 mm, 200-300 mm, > 300 mm total length) of all observed smallmouth bass to an above water data recorder. In 2015, glide-like pool habitats in both the NFJDR and MFJDR were selected at approximately 5 km intervals. In these subsampled reaches, snorkel surveys were repeated 4 times (04 to 05-Jun, 19 to 22-Jun, 08 to 12-Jul and again 16 to 18-Sep) to monitor seasonal changes in abundance of smallmouth bass populations. Additionally, the four most upstream reaches in each river were snorkeled twice more (22 to 23-Jul and 12 to 14-Aug) to gain resolution at the upstream extent during peak summer temperatures.

Movement study

During 15 to 19-May and 6 to 9-Jun-2015, a total of 100 adult smallmouth bass (195-353 mm total length) were captured between RKM 57 and 69 of the NFJDR using hook-and-line sampling. Fish were anesthetized using a 30 mg L^{-1} dosage of AQUI-$S^{®}$ 20E until surgical plane was achieved (measured by loss of equilibrium, lack of response to external stimulus and slowing of operculum movement), which took ca. 5 minutes. Fish were then connected to a flowing anesthesia solution to irrigate the gills, and a ca. 15 mm shallow incision was made on the ventral surface just anterior to the pelvic girdle offset of the linea alba. A shielded catheter needle was inserted through the incision aft into the abdominal cavity and guided through the lateral side body of the fish. The transmitter antenna was then guided through the catheter shield and transmitter placed in the incision. Incisions were closed using three simple interrupted sutures (PDS 3-0 absorbable monofilament) placed 3-5 mm apart.

Fish were then placed in a recovery bath, photographed, weighed and measured. Additionally, 5-7 scales were removed from behind the pectoral fin for aging. Scales were placed directly onto wax paper and stored until laboratory processing. In the laboratory, impressions of the scales were made on acetate slides and viewed under a dissecting microscope at 48x magnification. Scale annuli were then counted to determine age (DeVries and Frie 1996). Once fish were fully processed post-surgery (5-7 minutes), fish were monitored in the bath until full recovery (ability to maintain body equilibrium and resumption of normal coordinated swimming) (15-20 minutes). Once fully recovered, fish were released in slow moving water with ample cover.

Smallmouth bass were implanted with an ATS F1440 body implant transmitter with trailing whip antenna (Advanced Telemetry Systems, Inc. Isanti, MN, USA). Each transmitter weighed 2.1 g (dry weight) and had a battery life of 68-158 days. Tagged fish were tracked using an ATS 4500C telemetry receiver (Advanced Telemetry Systems Inc., Isanti, MN, USA) and a three- or six-element yagi antenna from a truck, canoe or on foot. Tracking occurred every 3-16 days from Jun to Oct and once in Nov (n = 18 total periods). Using signal gain, fish were pinpointed to within 100 m, and location was recorded using a handheld Global Positioning System (GPS).

To calculate movement, GPS locations of individual fish were translated into RKM using a Geographic Information System (ArcGIS, version 10.2). Distance moved was then calculated as change in RKM between each tracking period (upstream indicated by positive values and downstream as negative values). At the end of the season, the absolute values of all movements were summed to determine cumulative movement distance over a season for each individual.

Fish morphology

Geometric morphometrics were used to characterize body shape of tagged individuals. When anesthetized, each fish was placed on a photo board, straightened with fins extended and photographed. Landmark coordinates were sampled from the digital photographs using tpsDig version 2.18 (Rohlf 2006) and input into R using the *geomorph* (Adams and Otarola-Castillo 2013) package. Only fish that were consistently tracked throughout the season (n = 87) were used for the analysis. An additional 21 specimens were removed due to lack of proper alignment in the photograph (i.e., tail not straight, body bent, etc.). Of the remaining 66 specimens, 11 fixed landmarks were selected to represent overall body morphology. A single person digitized all landmarks to reduce the potential for systematic biases.

Using *geomorph*, specimens were superimposed using a Generalized Procrustes analysis (Rohlf and Slice 1990). From the superimposed specimens, 22 principal components (PC) were extracted, representing the overall change in shape between all specimens. The top 5 PC from the morphometric analysis (representing 68% of the total shape variation) were then each analyzed for relationships with cumulative movement using linear regression analysis.

Aspect ratio of the caudal fin is often used as a proxy for swimming performance and has been suggested to be a significant predictor of dispersal distance in fishes (Radinger and Wolter 2014). Aspect ratio of the caudal fin is defined by the equation:

$$AR = \frac{h^2}{s}$$

where h is the height of the caudal fin and s is the surface area of the caudal fin. To determine aspect ratio of the caudal fin, photos for the 87 tracked fish were re-examined to determine which had caudal fins that were properly spread for analysis (n = 47). In order to ensure the subsample of 47 fish was a random and representative sample, Mann-Whitney *U* tests of all body measurements and movement metrics were conducted between the 47 subsampled individuals and the remaining 40 individuals. No differences were found between the groups (all $p > 0.2$).

In addition to photographs, direct measurements were taken of individual fish. While anesthetized, total length (mm), weight (g), body depth (mm) (dorsal to ventral measurement at pectoral fin), caudal peduncle depth (mm) (dorsal to ventral measurement at narrow point in caudal peduncle), head height (mm) (dorsal to ventral at head insertion to jaw opening) and girth (mm) (side to side at pectoral fin) were measured (Online Resource 3.3). For statistical analyses, body measurements were standardized by total length to account for the influence of allometry.

Statistical analysis

Statistical analyses were conducted in R version 3.2.2 (R Foundation for Statistical Computing, Vienna). Pearson's correlation coefficients were computed to assess the relationships between environmental variables (i.e., temperature, discharge) and upstream distributional limits. We used linear modeling to determine the relationship between individual movement and morphology. We considered 59 candidate models, including every combination of 6 body shape predictor variables (i.e., weight, body depth, caudal peduncle depth, head height, girth and aspect ratio of the caudal fin). Akaike's information criterion (AIC) was then used to evaluate the support for each model. As movement data were not normally distributed, we used Mann Whitney *U* tests to compare groups of fish that moved at different time periods.

3E. Results

Trends in population abundance and upstream distributional limit (2009, 2010, 2014, 2015)

Across all years and both rivers, a seasonal shift in upstream distribution was revealed. Each year, the upstream extent of distribution increased between spring surveys (conducted as temperatures increased above 15°C) and late summer snorkel surveys (conducted as temperatures began to decline from peak summer temperatures). In the NFJDR, the spring invasion edge (i.e., front edge) was closely associated with minimum young-of-the-year growth requirements (Table 3.1). By contrast, the spring invasion edge in the MFJDR remained highly consistent despite inter-annual variability in conditions. In late summer, the distribution of smallmouth bass in both rivers increased 5-33 km upstream, depending on the year. This summer invasion edge (i.e., leading edge) in the NFJDR was correlated with the number of days of low discharge during the spring and early summer (1-Apr to 1-Aug) ($r = 0.941$, $P = 0.058$, n = 4) and was consistently located at a longitudinal position exhibiting mean daily Jul temperatures of 19.4-21°C (Table 3.1). These trends hold in the MFJDR, but statistical analyses were not possible due to limited sample size (n = 3 years). Finally, a pattern of steady increase to all distributional limits (i.e., spring and summer) were observed, indicating that smallmouth bass successfully expanded both its inter-annual and seasonal range in both the NFJDR and MFJDR during the study period (Fig. 3.1). The two exceptions were 1) late summer 2010, which was characterized by low temperatures and late recession of peak discharge relative to other years (Table 3.1 and Online Resource 3.2) and 2) early spring 2014, which was preceded by a season of cold upstream temperatures relative to 2010.

In 2015, repeat snorkel surveys revealed that both abundance and upstream extent increased throughout the season, peaking in late Jul (Fig. 3.2a,b). Initial shifts upstream of the spring invasion edge occurred as mean daily stream temperatures increased above 15°C and spawning was initiated (simultaneous in 2015). Each subsequent survey revealed higher numbers of smallmouth bass in each subsampled reach with more reaches occupied. In mid-Aug, mean daily temperatures began a steady downward trend from the summer seasonal highs (Online Resource 3.1). After this time, smallmouth bass abandoned upstream reaches of both rivers and abundances began to decline in downstream reaches. By the middle of Sep, no smallmouth bass were observed above RKM 84 in the NFJDR and RKM 39 in the MFJDR.

A marked longitudinal trend in the distribution of size classes was also revealed during the snorkel surveys. Downstream of the spring invasion edge, all size classes of smallmouth were represented in the abundance counts, with individuals < 100 mm constituting 25-43% of the population. By contrast, individuals < 100 mm were notably absent from the summer invasion edges. Instead, 100-300 mm fish made up the majority (83% - 100%) of total observed individuals each year, with the remainder being in the > 300 mm size class.

Individual movement patterns (2015)

Smallmouth bass were found to make considerable seasonal movements in the NFJDR. In total, 87 individuals were tracked from early Jun through late Nov-2015, represented by 1,450 valid tracks, averaging 17 locations out of 18 tracking periods per individual. The tracked fish ranged from 195-311 mm (SD = 26 mm) total length and 105-375 g (SD = 62.5 g) wet mass. Total (cumulative) distance moved varied substantially between individuals, ranging from 0 km (i.e., fish that remained within the same 100 m pool throughout the entire study) to 109 km. Distributions of individual movements were such that there was no clear distinction between a mobile and stationary component of the population. Specifically, 25% (n = 22) moved less than 1 km, 16% (n =14) moved 1-5 km, 24% (n = 21) moved 5-20 km, 15% (n = 13) moved 20-40 km and 20% (n = 17) moved 40-109 km.

Age structure in the headwaters of the NFJDR and MFJDR was relatively young compared to the mainstem JDR (unpublished data, Oregon Department of Fish and Wildlife) and native smallmouth bass populations (Beamesderfer and North 1995). Of the 100 fish caught using angling and implanted with a tracking device, 66% were age 3 (n = 66), 25% age 4 (n = 25), 1% age 5 (n = 1), and less than 5% age 6-8 (n = 5) (3 individuals could not be conclusively aged due to scale regrowth). An additional 41 individuals were captured that were too small to tag (< 200 mm), and presumed to be predominantly age 2 based on consistent size-age relationships in the system. Similarly, fish > 300 mm (representing older age classes) were rarely observed during snorkel surveys. The fish that were tracked through the season (n = 87) had a distribution of ages similar to all aged fish. There were no significant differences in cumulative movement between age 3 and age 4+ individuals (Mann-Whitney $U = 556$, $n_1 = 61$, $n_2 = 24$, $P = 0.086$); the only two age classes with sufficient sample size to analyze.

Individuals that moved > 1 km generally exhibited complex movement patterns. Specifically, two-thirds (68%, n = 44) of individuals moved in an upstream direction in the spring and downstream direction in the late summer (Fig. 3.3). The remaining one-third (32%) either moved only upstream (n = 11), primarily in the spring, or only downstream (n = 10) at different times during the study. Tagged individuals were observed as far upstream as RKM 107 and as far downstream as RKM 22. Only 9% (n = 6), however, exhibited site fidelity to the initial tagging location (i.e., returned to within 500 m of where they were tagged). Instead, 46% (n = 30) of individuals were last observed downstream and 45% (n = 29) upstream from the initial tagging location. All fish that occupied habitats upstream of RKM 81 (n = 15), however, eventually returned to a location downstream of RKM 81 later in the year.

Upstream movement throughout the system was highly coordinated, occurring predominantly in two main phases (Fig. 3.3a,b). Upstream movement was first observed in early Jun and generally ceased the second week in Jul when peak summer water temperatures were reached. The vast majority of individuals that moved greater than 1 km upstream (n = 43) initiated movement either in the period spanning 2 to 18-Jun (n = 23) (Fig. 3.3a) or a second period spanning 24-Jun to 7-Jul (n = 17) (Fig. 3.3b) (only 3 fish initiated upstream movement between 18 to 24-Jun). The first period encapsulates mean daily temperatures above 15°C (and the initiation of spawning), whereas the second period correlates with the cessation of spawning for the year and an increased rate of stream warming (Online Resource 3.1). There was a slight, but not significant, size difference between fish that initiated movement 2 to 18-Jun (mean TL = 240 mm, n = 28) and those that waited until 24-Jun to 7-Jul (mean TL = 249, n = 17) (Mann-Whitney $U = 143$, $n_1 = 28$, $n_2 = 17$, $P = 0.090$). When comparing fish that made large upstream movements (i.e., > 5 km) (n = 39), however, the trend becomes significant. Specifically fish that initiated large upstream movements in the first time period (2 to 18-Jun) (n = 21) were significantly smaller (mean = 237 mm) than those in the second period (24-Jun to 7-Jul) (n = 11) (mean = 258 mm) (Mann-Whitney $U = 37$, $n_1 = 21$, $n_2 = 11$, $P = 0.0020$). Additionally, there was an anomalous period 6 to 22-Oct in which 6 individuals initiated large upstream movements (Fig. 3.3f). Of these, 3 individuals returned to their tagging location after having initiated downstream movements earlier in the season. The other 3 individuals moved into deep (i.e., >4 m) pool habitat upstream of where they had been previously located in the season.

Downstream movements were less coordinated than upstream movement. These movements occurred over a more protracted period beginning in late Jul and ending early Oct (Fig. 3.3c-f). During the first 4 weeks, 3-5 individuals per week moved downstream (n = 15), with some individuals moving multiple times. During this period, no large temperature deviations were observed (daily mean temperatures oscillated around 22-25°C in downstream segments and 17-20°C in upstream segments) (Online Resource 3.1). In the middle of Aug, daily mean temperatures began trending downward. At this time, increased numbers of individuals (7-15) moved downstream each week for the remainder of the season (n = 48) (Fig. 3.3d,e). By early Oct, most downstream movements ceased (except 1 fish that moved downstream in Nov), at which point mean daily temperatures were below 10°C upstream and 15°C downstream (Fig. 3.3f). When comparing individuals that moved each week, those that moved downstream earliest were largest with a steady decline in average size over each subsequent week. There was a significant difference in size and a slight difference in age between individuals that initiated downstream movement in late Jul (n = 9) (mean size = 265 mm, mean age = 3.8 years) and those that first initiated downstream movement in Oct (n = 5) (mean size = 228 mm, mean age = 3.0 years; Mann-Whitney $U = 42.5$ (size), $n_1 = 9$, $n_2 = 5$, $P = 0.0092$; Mann-Whitney $U = 35$ (age), $n_1 = 9$, $n_2 = 5$, $P = 0.058$).

Several body size metrics were significantly correlated to cumulative movement, but the top performing model included only body depth (standardized by total length) and aspect ratio ($R^2 = 0.287$ (multiple regression), $P = 0.00058$). Body depth and aspect ratio were included in all top candidate models (Table 3.2), whereby deeper bodied fish were positively correlated (Fig. 3.4a) and fish with larger aspect ratios of the caudal fin were negatively correlated (Fig. 3.4b) to cumulative movement. PC 2 from the Generalized Procrustes Analysis was also significantly correlated with cumulative movement. PC 2 primarily described shape variation in body depth (Fig. 3.5), with deeper body depths again correlated with increased movement distance ($r^2 = 0.103$ (single regression), $P = 0.0085$).

3F. Discussion

Through detailed longitudinal monitoring of range boundary dynamics of smallmouth bass, we reveal unique population responses to changing ecological conditions, intrinsic traits (i.e., body shape) that predispose some individuals to be more exploratory of the riverscape, and an overall trend of range expansion over time. At an inter-annual scale, we expose distinct front (i.e., spring invasion edge) and leading (i.e., summer invasion edge) edges (*sensu* Lenoir and Svenning 2013); each limited by different ecological conditions that restrict upstream establishment of smallmouth bass. The front and leading edges of the NFJDR and MFJDR demonstrate a clear trend of upstream progression during the 7-year study period (2009-2015) (Fig. 3.1), responding to changes in inter-annual environmental conditions. The NFJDR population of smallmouth bass, with its steeper thermal gradient, however was found to be considerably more sensitive to seasonal environmental conditions leading to increased distributional volatility relative to the MFJDR population.

A key attribute of front edges is population growth (Lenoir and Svenning 2013). Although recruitment was not directly measured, a unique characteristic of this region in the NFJDR and MFJDR was the consistent presence of all size classes up through the front edge, but not beyond. Additionally, the front edge location was associated with the combination of antecedent growing degree-days and the length of the preceding winter. Combined, these metrics describe the requirements for young-of-the-year to grow to a sufficient size to survive an overwinter starvation period (Shuter et al. 1980); a pattern consistent with bioenergetics modeling suggesting that age-0 survival constrains the upstream distribution limits of smallmouth bass (Lawrence et al. 2015). Indeed, predicted size based on available growing degree-days generally fell just above the minimum size required to survive the upcoming winter for all study years except 2009 (Table 3.1). Improved conditions in 2015 – characterized by a late cooling period at the end of 2014 (i.e., extended growing season) and temperatures rising much earlier in the spring of 2015 (i.e., shorter winter) – resulted in a 6 RKM increase to the front edge in the NFJDR and 1 RKM in the MFJDR. Here, increased duration of the growing season and decreased duration of the winter starvation period appears to have removed perhaps the most important environmental constraint to establishment, especially in the NFJDR, which has a steeper thermal gradient than the MFJDR. By contrast, colder temperatures and a longer winter in 2014 (relative to 2010) resulted in a decrease to the front edge in the NFJDR (no data available for MFJDR in 2010).

There are obvious exceptions to these patterns and differences in association strength between rivers. In the MFJDR, the consistency of the edge location across different years (and hence, different environmental conditions) suggests a strong secondary mechanism that restricts front edge expansion. This could include limited habitat suitable for reproduction, strong Allee effects associated with mate limitation, competitive interactions with other fish species, and low prey availability. Smallmouth bass were also located farther upstream than expected (based on stream thermal conditions) in 2009. One explanation is that the complex depth profile and thermal heterogeneity of the NFJDR and MFJDR provided warm-water thermal refugia during the winter (Torgersen et al. 1999). Although a secondary mechanism seems to be exhibiting influence on the location of the front edge in the MFJDR, we demonstrate that climatological events alone do play a primary role in defining the invasion edge, portending future expansion under certain climate change scenarios (Lawrence et al. 2014).

The location of leading edges between years was much more dynamic compared to the front edges. Depending on current year environmental conditions, the leading edge extended from as little as 5 km to as much as 33 km beyond the front edge during summer months (Jun through Aug) (Fig. 3.1). The location of the leading edge was correlated with the number of days of low spring discharge, where earlier spring run-off timing was associated with greater increases to the front edge (Online Resource 3.2). This, along with the individual movement patterns observed, lends support to the idea that the leading edge is recolonized each year by smallmouth bass during the receding limb of the spring hydrograph. The absence of individuals < 100 mm at the leading edge suggests that recolonization is limited to larger-bodied individuals demonstrating greater dispersal abilities. This mechanism is further supported by the observation that earlier timing of peak spring runoff leads to earlier spawning in both the NFJDR and MFJDR (Rubenson and Olden 2016), thus providing a longer time period for spawning-initiated movement.

The location of the leading edge was also highly associated with water temperature. For both rivers across all years, the respective edge locations occurred at different longitudinal positions but were consistently found where the mean Jul temperatures were 19.4-21°C (Table 3.1). This temperature window corresponds to the optimal growth temperature for adult bass of 20-22°C (Lawrence et al. 2015), suggesting that adult growth potential plays an important role in determining the location of the leading edge. Although a direct comparison between growth rates of individuals that over-summered in the front versus the leading edge is not available, bioenergetics modeling predicts age 3 bass to maintain growth scope and age 4 and above to increase growth scope in cooler habitats during summer months (Lawrence et al. 2015). In support, we saw the greatest increases to leading edge extent in 2015, which had warmer upstream Jul temperatures compared to previous years. It is important to note, however, that although temperatures upstream of the leading edge are colder (decreasing by ca. 0.5°C every 5 km), temperature alone is not a constraint to farther expansion. Further research into additional constraints such as habitat availability, resource limitations and biotic interactions is required to more fully understand the constraints to leading edge populations of smallmouth bass in temperate streams.

Our study provides strong evidence that large seasonal movements by individuals are a dominant component of range boundary demographics in the headwaters of the JDR. The increased rate and magnitude of dispersal relative to other core population movement dynamics (Todd and Rabeni 1989) is a distinctive trait of population edges (Burton et al. 2010, Chuang and Peterson 2016), and is typically influenced by environmental variability, competition, and potential for kin inbreeding (Dytham 2009). Previous research in the NFJDR and MFJDR demonstrated that abundances of smallmouth bass and nest densities are high in the front edge relative to the leading edge, and that there was no reduction of nesting success along the longitudinal gradient (Rubenson and Olden 2016). These conditions paired with the alignment of upstream movement with spawning initiation and the tendency for smaller sexually mature individuals (therefore less competitive) to initiate large upstream movements early, suggest that competition for spawning habitat is a critical driver influencing distribution changes at the range boundary. This is not unexpected as nesting smallmouth bass males aggressively defend their nests, potentially excluding less dominant mature males from spawning nearby (Ridgway et al. 1991). If these outcompeted individuals disperse upstream, they can receive reproductive benefits of available spawning habitat and less competition. Reproductive benefits, such as these, can outweigh the costs and risks associated with dispersal, and are considered a key component of successful range expansion (Dytham 2009, Chuang and Peterson 2016).

Upstream movement, however, was not solely confined to spawn timing, but instead continued until temperatures began to decline off summer maximums. In 2015, a rapid rise in temperature occurred as broods were dispersing off nests, which was paired with a second mass initiation of upstream movement (Fig. 3.3b, Online Resource 3.1). These movements, in addition to the continuous increase in upstream abundances observed during single-season repeat snorkel surveys (Fig. 3.2a,b), suggest some level of behavioral thermoregulation, which has been demonstrated in other smallmouth bass populations (Westhoff et al. 2016). Above 22°C, growth scope for smallmouth bass begins to decline due to high costs of metabolic activity (Whitledge et al. 2002). In the front edge, mean daily temperatures regularly reach 25-27°C (maximum temperatures > 31°C), whereas mean daily temperatures in the leading edge are 20-22°C (maximum temperatures < 26°C). This temperature gradient may be sufficient to select for upstream movement and further supports the hypothesis that adult growth scope drives the location of the leading edge (Lawrence et al. 2015). The ability to thermoregulate and track areas of suitable habitat is advantageous for species to respond to climate change (Henry et al. 2013). Under continued warming scenarios, selection would benefit those individuals better able to disperse into the leading edge and escape the high metabolic costs of warmer temperatures downstream.

After a period of expansion in the spring and summer, the range boundaries in the NFJDR and MFJDR contracted in the fall. Elastic boundaries, such as demonstrated here, tend to occur when mortality and growth rate at the leading edge no longer benefit the individuals that dispersed (Kubisch et al. 2010). Evidenced by the abandonment of upstream habitats as temperature drops, it appears that declining temperatures trigger movement downstream. The habitat in the leading edges of the NFJDR and MFJDR experience partial to full ice cover in the winter months and have only a few deep pools (Lawrence et al. 2012), which may limit overwinter survival in the leading edge. The specific trigger that initiates downstream movement, however, is still unclear. It is interesting that larger, older individuals were prone to move earlier. One potential explanation is that previous years' experience influenced current behavior; or perhaps, more simply, that larger individuals are in better condition earlier to make another large seasonal movement compared to smaller individuals.

In addition to environmental influences and body size, morphology may provide important clues into the intrinsic factors that promote movement at range boundaries (Hudina et al. 2012). Individuals in the upper NFJDR and MFJDR smallmouth bass populations are relatively homogenous in size (< 350 mm) and age (< age 6), suggesting that our study area did not include the core population. Even so, we found significant differentiation between movement and individual body shape. Movement was significantly positively correlated to individuals that were deeper bodied with smaller aspect ratios of the caudal fin. Other fish movement studies have also shown body depth to be a significant predictor of movement in centrachids (Hanson et al. 2007) and aspect ratio a significant predictor of movement in fishes (Radinger and Wolter 2014). However, the direction of the association with body depth and aspect ratio in our study is opposite to that shown in previous studies. In terms of aspect ratio of the caudal fin, most studies compare movement and aspect ratio between species – highlighting differences in function between extremely different forms (Webb 1984, Radinger and Wolter 2014). When comparing intraspecific differences in the aspect ratio of the caudal fin, more nuanced results are to be expected – especially in a species such as smallmouth bass that is labeled as a locomotor generalist (Webb 1984). Our results may provide a key clue into determining the dominant drivers behind dispersal along an invasion gradient. On the invasion front, advantageous morphology for movement is only one factor selecting for dispersal – along with competition for resources and spawning habitat (Henry et al. 2013). In smallmouth bass, nest guarding requires burst acceleration and quick turning ability (Cooke et al. 2002). These specific swimming traits have been correlated with individuals with narrow bodies and large caudal regions (Hanson et al. 2007). As such, the individuals with these morphological traits may be those better able to compete for spawning habitat downstream, thus selecting for the subdominant forms (i.e., individuals with deeper bodies and smaller aspect ratio) to disperse upstream. The combination of subdominant forms moving farther and the timing of movement aligning with spawning again supports that range expansion is driven, in part, by competitive pressure to spawn.

Another hypothesis that warrants further exploration is that differences in body shape of mobile individuals is the result of longitudinal genetic differentiation. Several studies have shown a single population of smallmouth bass to contain two groups of individuals that exhibit distinct life-history patterns year-to-year, suggesting the potential for genetic differentiation within a single population. For instance, Barthel et al. (2008) reported that a population of smallmouth bass that overwintered in a common lake habitat contained individuals that spawned in an adjoining river and those that spawned in the lake. Similarly, Tabor et al. (2012) found distinct evidence of reproductive isolation between individuals that were residents of Lake Washington (Washington, USA) from those that migrated into connected tributaries. Our findings of significant morphological differences in a relatively homogenous group of individuals suggest that selection for movement traits may be occurring at relatively confined spatial scales located at the range boundary. We encourage additional research to fully understand the costs and benefits of differing body morphologies in range boundary populations.

Although the habitat at the leading edges of the NFJDR and MFJDR does not, yet, maintain self-sustaining populations, the elasticity at the range boundary may have both ecological and evolutionary benefits. Ecologically, dynamics at the range boundary provide opportunities for individuals to establish in new upstream habitat. Specifically, we found that close to half (45%) of the individuals that moved remained upstream of their initial tagging location. Interestingly, all of these individuals settled inside the front edge boundary, and there were no tagged individuals that attempted to remain upstream of this boundary. This implies strong selection for successful overwinter survival of juvenile bass. From an evolutionary perspective, seasonal movement dynamics may pre-dispose the invasive population for future upstream secondary spread. The NFJDR and MFJDR are predicted to experience stream warming under various climate change scenarios (Lawrence et al. 2014), which may provide opportunities for continued range expansion. High dispersal characteristics enable individuals to exploit new habitat and benefit from fewer competitive pressures relative to core populations (Dytham 2009, Burton et al. 2010).

3G. Conclusion

Understanding the movement patterns of species is essential for the accurate prediction of future range shifts and forecasting the speed of invasion (Kokko and Lopez-Sepulcre 2006, Radinger and Wolter 2014). As streams continue to warm into the future, smallmouth bass are well poised to take advantage of improving conditions in these headwater streams. This has important implications for managers that are tasked with minimizing the impact of this top predator on juvenile salmonids that use these headwater habitats to rear. Understanding the mechanisms of expansion, however, may help mitigate this challenge. Here we show that seasonal adult movement occurs at a predictable time. This presents a unique opportunity to find means to delay or inhibit these movements, be that through restricting movement itself or perhaps increasing fishing pressure to reduce downstream densities. By contrast, expansion might be restricted through efforts to cool streams through riparian restoration that increases channel shading (Lawrence et al. 2014) or by altering the timing or temperature of water released from dams when feasible (Olden and Naiman, 2010). Cooler water deters movement, spawning, decreases the growing season, and increases the length of winter cold-water conditions. Ultimately, understanding the intrinsic and extrinsic factors that influence individual movement patterns within the range boundary at multiple timescales are critical components to understanding the mechanisms behind range expansion. With these data, we can both improve predictions of future range expansion and better prevent secondary spread of an invasive species.

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3J. Tables

Table 3.1. Environmental variables associated with the location of the front and leading edges in the North Fork John Day River (NFJDR) and Middle Fork John Day River (MFJDR)

River	Front edge							Leading edge		
	Year	RKM	Jul Mean T	GDD	Days of Winter	Size	L_0/L_1	RKM	Jul Mean T	Days of Low Discharge
NFJDR	2009	72	21.9	638	210	43	54/103	100	20.4	39
	2010	84	19.8	912	245	67	61/114	89	19.4	29
	2014	77	22.2	921	239	66	60/112	110	20.0	48
	2015	90	21.5	901	208	59	54/102	117	19.6	72
MFJDR	2009	45	22.1	650	213	46	55/104	64	20.9	44
	2014	47	22.1	1081	239	79	60/112	64	21.0	51
	2015	48	21.8	997	186	70	50/95	72	20.7	97

RKM represents the river kilometer where the front or leading edge of smallmouth bass occurred during that year. GDD represents the number of growing degree-days > 10°C accumulated the year prior to the survey. Days of Winter represents the number of days < 10°C accrued the year prior to the survey. Size represents the predicted size (mm) young-of-the-year should achieve given the GDD available the year prior. L_0 represents the minimum size (mm) a young-of-the-year bass would need to be in order to survive the winter. L_1 represents the size (mm) above which probability of overwinter survival is 100%. July Mean T is the mean monthly temperature (°C) for Jul at the corresponding RKM. Days of Low Discharge depicts the number of days (between 1-Apr and 1-Aug) in which discharge was less than 28 $m^3 s^{-1}$ (NFJDR) and 9 $m^3 s^{-1}$ (MFJDR). The discharge threshold levels were chosen based upon the mean discharge observed when spawning was first initiated in each system. This generally represents the receding limb of the hydrograph and demarcates a shift from high magnitude snowmelt discharge to lower flow conditions amenable to smallmouth bass spawning and movement

Table 3.2. Summary of the top five ranked regression models predicting cumulative individual movement as a function of body morphology.

Model	R^2	P	**AIC**	**ΔAIC**
BD + AR	0.287	0.0006	429.4	0
G + BD + AR	0.297	0.0015	430.7	1.3
CPD + BD + AR	0.292	0.0018	431.0	1.6
HH + BD + AR	0.287	0.0021	431.4	2
W + G + BD + AR	0.314	0.0028	431.6	2.2

Total movement distance (km) (response variable) was calculated as the cumulative sum of the absolute values of change in river kilometer between each tracking period (upstream indicated by positive values and downstream as negative values). Morphological metrics (predictor variables) included in the analysis were body depth (BD), aspect ratio of the caudal fin (AR), girth (G), caudal peduncle depth (CPD), head height (HH) and weight (W). All measurements, with the exception of aspect ratio of the caudal fin, were standardized by total length to minimize the effects of allometry

3K. Figures

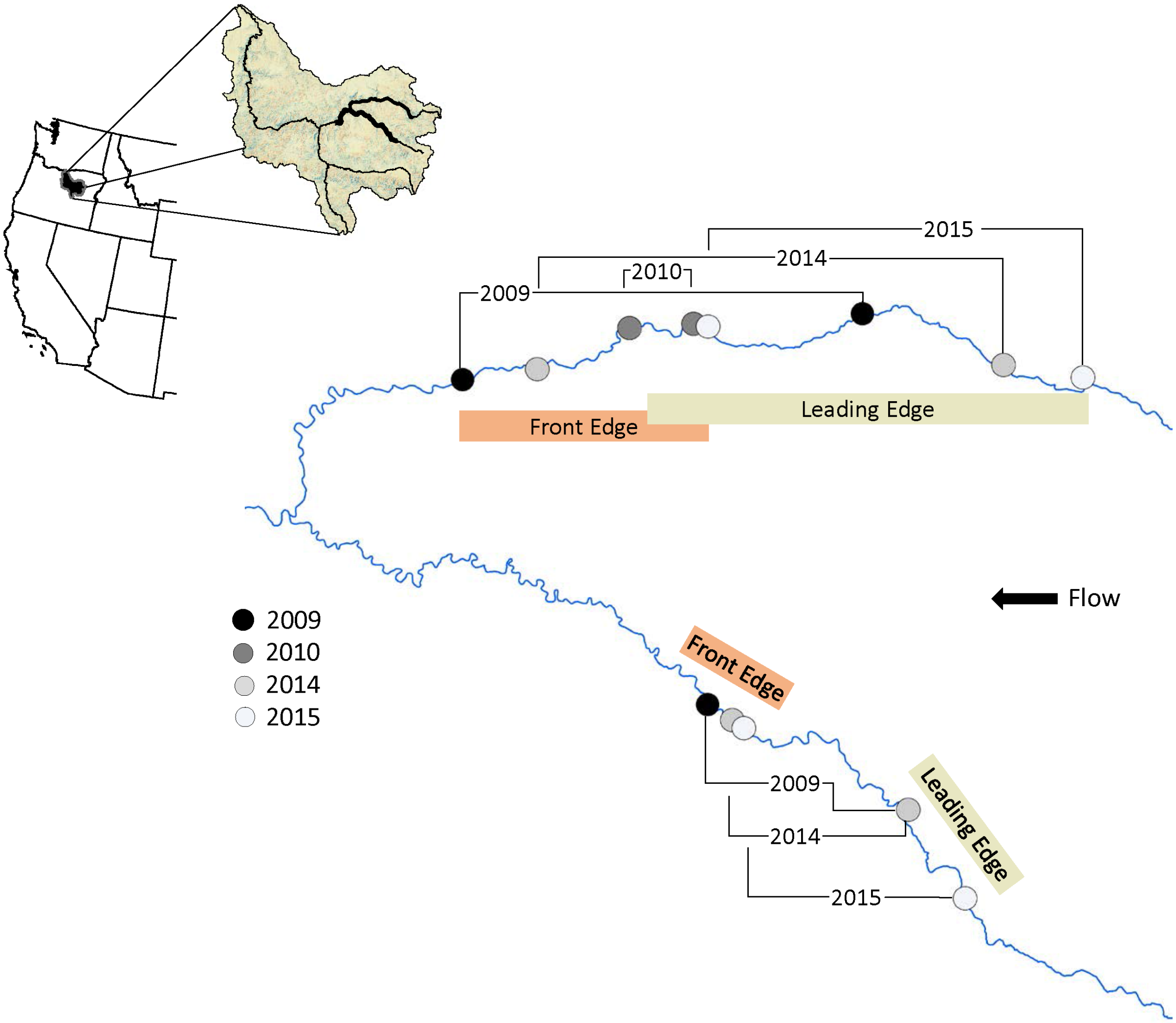


Figure 3.1. The John Day River Basin in northeastern Oregon of the United States. The surveyed portions of the North Fork John Day River (upper tributary) and Middle Fork John Day River (lower tributary) are highlighted in bold and magnified. Each circle represents the location of the front and leading edges for 2009 (black), 2010 (dark grey), 2014 (light grey), and 2015 (white) in both rivers

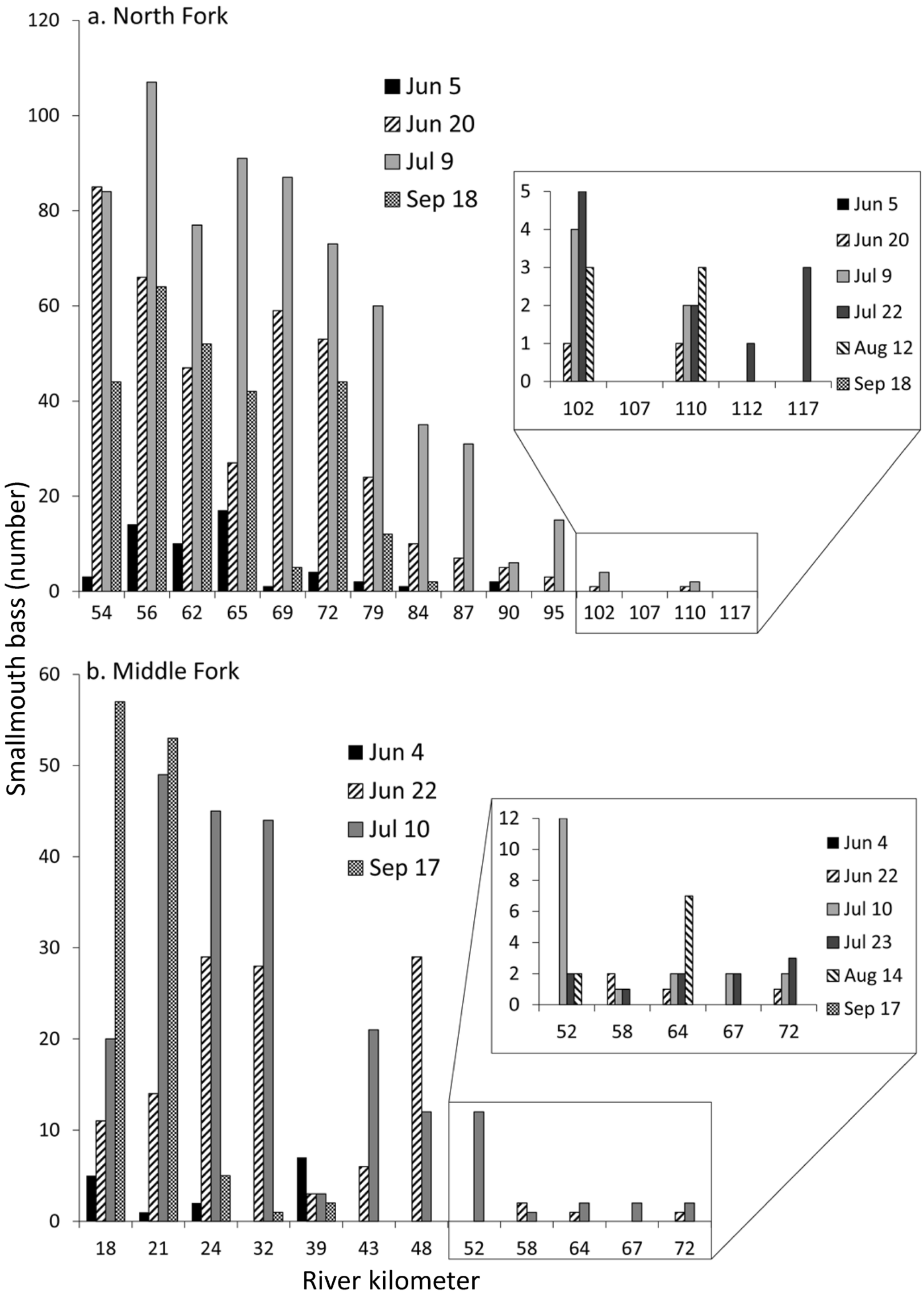


Figure 3.2. The change in smallmouth bass counts during repeated snorkel surveys in subsampled reaches in the (a) North Fork John Day River and (b) Middle Fork John Day River. Bars are indicated according to the date in which the survey was conducted. The most upstream river kilometers were also surveyed 22 to 23-Jul-2015 and 12 to 14-Aug-2015 as depicted in the boxes. Additional surveys were conducted in these regions to gain resolution at the leading edge

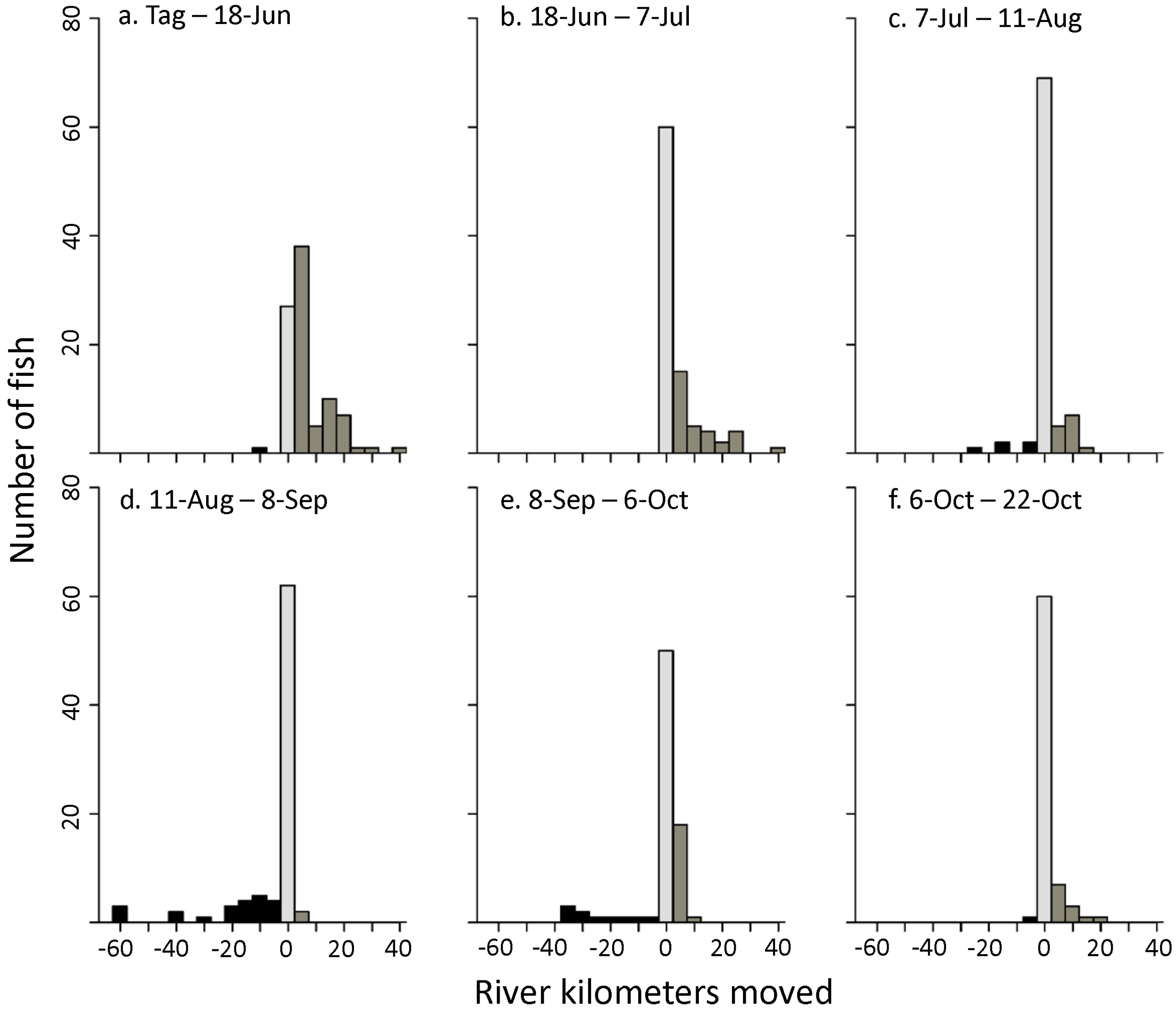


Figure 3.3. Individual movement patterns in 2015 for (a) Tag – 18-Jun (b) 18-Jun – 7-Jul (c) 7-Jul – 11-Aug (d) 11-Aug – 8-Sep (e) 8-Sep – 6-Oct and (f) 6-Oct – 22-Oct. The x-axis depicts the number of river kilometers moved in the time period displayed. Positive values (dark grey) indicate upstream movement whereas negative values (black) indicate downstream movement. Individuals that did not move are shaded in light grey. They y-axis depicts the number of fish observed

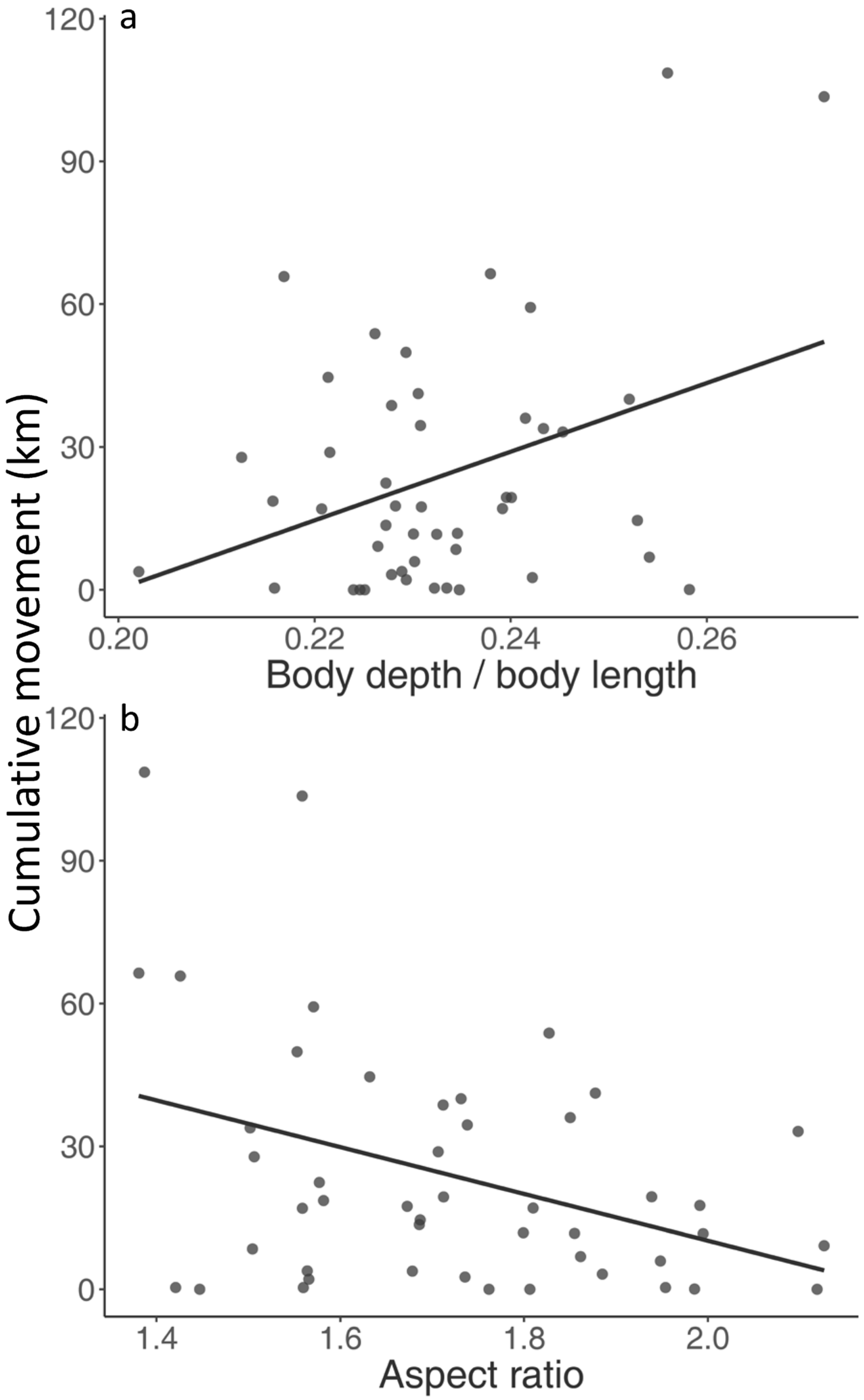


Figure 3.4. (a) The relationship between body depth standardized by total length and cumulative movement ($r^2 = 0.133$, $P = 0.012$, n = 47). (b) The relationship between aspect ratio of the caudal peduncle and cumulative movement ($r^2 = 0.146$, $P = 0.0080$, n = 47). Cumulative movement was calculated as the cumulative sum of the absolute values of change in river kilometer between each tracking period. The top model predicting movement included both body depth and aspect ratio (Table 2)

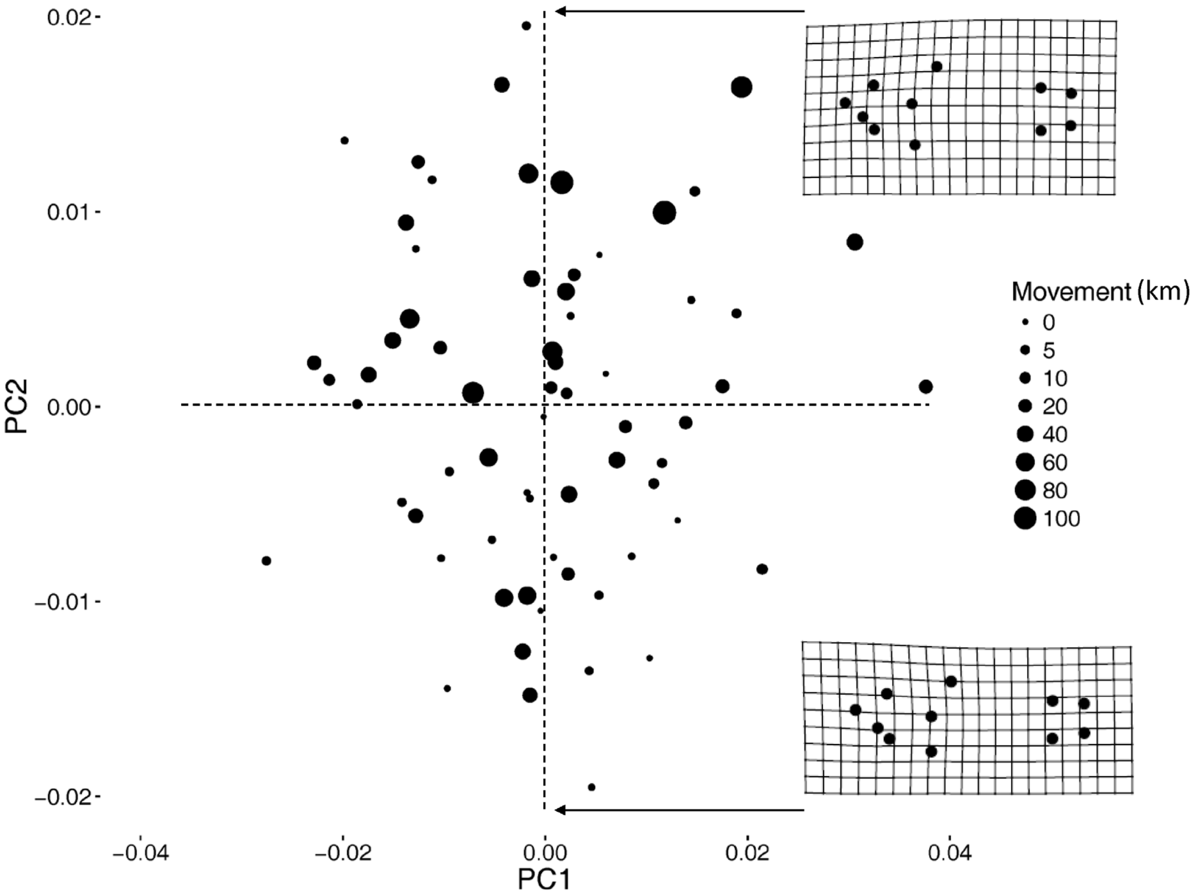
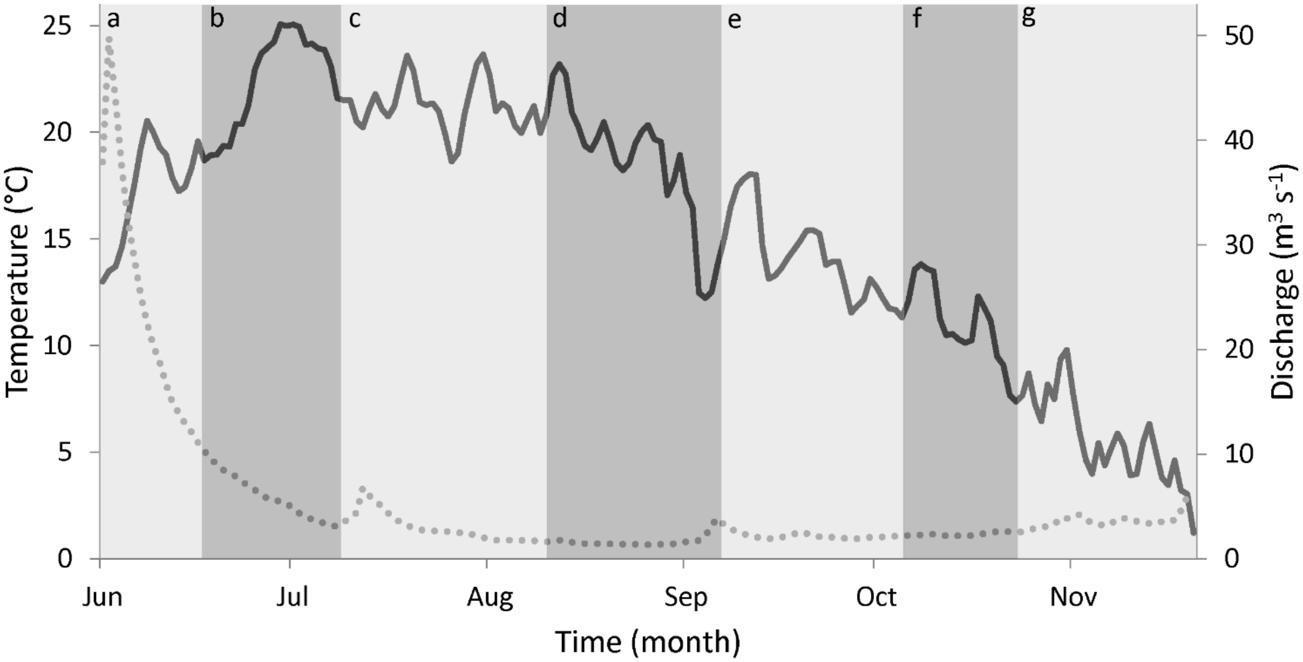


Figure 3.5. Ordination plot from a Principal Component Analysis describing shape variation among Procrustes-aligned specimens. The circles are scaled by cumulative movement distances (km) moved by individuals. Principal Component (PC) 1 is associated with curvature in the caudal peduncle (not correlated to cumulative movement (P = 0.71)) and PC 2 is associated with changes in body depth. PC 2 is significantly correlated to cumulative movement (r^2 = 0.103, P = 0.0085, n = 66).The warp grids are visual representations of the hypothetical shapes created from the extreme differences between the minimum (lower) and maximum (upper) PC2 values

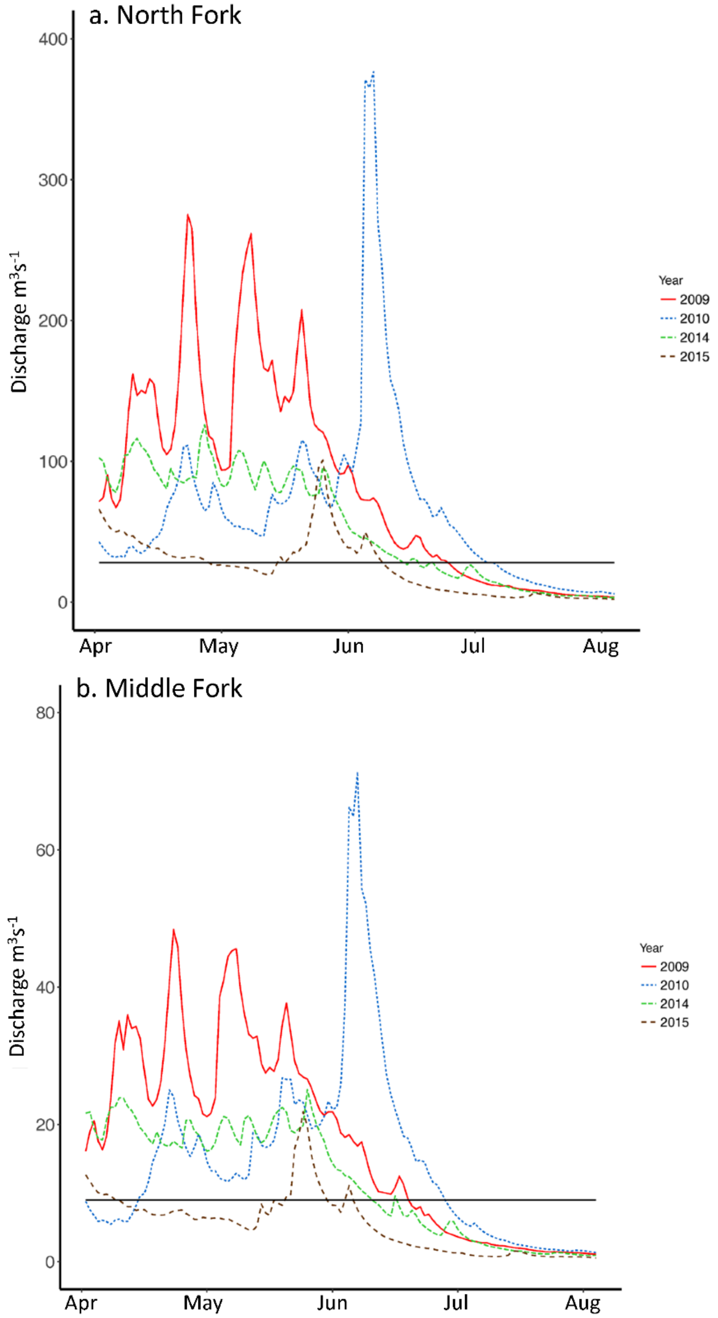
3I. Chapter 3 Supplemental Material

Online Resource 3.1



Online Resource 3.1. Mean daily temperature (°C) (black solid line) at river kilometer 83 and discharge ($m^3 s^{-1}$) (grey dotted line) (USGS station 14046000) from Jun to Dec-2015 in the North Fork John Day River. Shaded areas correspond to time periods over which movement was aggregated in Fig. 2(a) Tag – 18-Jun (b) 18-Jun – 7-Jul (c) 7-Jul – 11-Aug (d) 11-Aug – 8-Sep (e) 8-Sep – 6-Oct and (f) 6-Oct – 22-Oct, as well as the conditions during the final tracking period (g) 22-Oct – 23-Nov.

Online Resource 3.2



Online Resource 3.2. Discharge (m^3 s^{-1}) from 1-Apr to 1-Aug for the a) North Fork John Day River (NFJDR) and b) Middle Fork John Day River (MFJDR) in 2009 (red), 2010 (blue), 2014 (green), and 2015 (brown). The horizontal lines are the thresholds of 28 m^3 s^{-1} (NFJDR) and 9 m^3 s^{-1} (MFJDR). The discharge threshold levels were chosen based upon the mean discharge observed when spawning was first initiated in each system. This generally represents the receding limb of the hydrograph and demarcates a shift from high magnitude snowmelt discharge to lower flow conditions amenable to smallmouth bass spawning and movement.

Online Resource 3.3

Body shape metrics and cumulative movement distance for the 87 individuals tracked from early Jun through late Nov-2015. Cumulative movement was the sum of all distance moved over the season, calculated as the sum of the change in river kilometer between each tracking period. Body depth is the dorsal to ventral distance at the pectoral fin. Head height is the dorsal to ventral distance at head insertion to jaw opening. Girth is the side-to-side distance as measured at the pectoral fin. Caudal peduncle depth is the dorsal to ventral measurement at the narrow point in the caudal peduncle. Aspect ratio is the height of the caudal fin (squared) divided by the surface area of the caudal fin (AR = h^2/s).

Fish ID	Cumulative Movement (km)	Sex	Age (yr)	Length (mm)	Weight (g)	Body Depth (mm)	Head Height (mm)	Girth (mm)	Caudal Peduncle Depth (mm)	Aspect Ratio
F001	26.23	NA	4	260	230	63.0	39.3	31.0	27.2	NA
F004	16.99	NA	3	251	170	55.4	36.8	30.0	25.9	1.56
F007	66.38	NA	3	253	212	60.2	40.1	32.1	28.5	1.38
F008	0.35	Female	4	270	210	62.7	39.9	33.3	27.1	1.56
F009	19.28	Female	4	274	250	66.1	41.7	36.0	28.7	NA
F010	86.79	Male	4	277	286	66.6	43.8	37.6	31.5	NA
F012	3.81	NA	4	240	138	48.5	35.8	30.8	25.2	1.68
F013	11.86	Female	NA	243	180	57.0	39.2	31.9	27.1	1.80
F014	0.00	NA	3	217	100	48.6	34.1	27.7	24.4	2.12
F015	2.81	NA	6	311	370	73.0	51.9	44.0	34.3	NA
F017	54.87	NA	3	227	140	51.5	34.9	28.9	25.2	NA
F018	22.42	NA	3	242	185	55.0	36.7	28.7	26.0	1.58
F019	65.78	Female	4	249	162	54.0	39.7	28.2	25.5	1.43
F020	27.82	NA	3	215	110	45.7	32.5	26.7	23.1	1.51
F022	5.16	NA	3	236	188	59.9	38.0	30.6	27.0	NA
F023	108.60	Male	4	259	232	66.3	42.3	34.4	29.0	1.39
F024	6.86	Male	3	242	232	61.5	40.1	35.1	29.1	1.86
F025	41.78	NA	3	260	258	65.6	42.6	33.5	32.5	NA
F026	33.88	NA	4	263	260	64.0	45.9	36.4	31.5	1.50
F027	0.00	NA	3	199	100	44.7	33.1	25.7	20.7	1.81
F028	1.13	NA	3	206	108	48.5	32.3	26.0	23.8	NA
F031	0.05	NA	3	210	105	44.9	33.5	26.6	21.8	NA
F032	21.78	NA	4	270	220	62.0	43.1	34.5	27.4	NA
F033	19.41	NA	3	235	170	56.3	41.3	31.0	27.1	1.94
F034	0.00	Male	3	247	195	60.6	39.5	30.2	28.8	NA
F035	3.85	NA	3	218	121	49.9	34.8	27.7	24.1	1.56
F037	18.62	Male	3	216	137	46.6	34.6	25.5	22.4	1.58
F038	11.73	NA	4	246	233	56.6	36.8	29.8	27.6	1.85
F039	34.51	Male	3	250	200	57.7	40.7	32.0	27.7	1.74

Fish ID	Cumulative Movement (km)	Sex	Age (yr)	Length (mm)	Weight (g)	Body Depth (mm)	Head Height (mm)	Girth (mm)	Caudal Peduncle Depth (mm)	Aspect Ratio
F040	0.36	NA	3	220	135	47.5	31.9	26.7	22.3	1.95
F044	38.71	NA	3	219	175	49.9	34.7	38.4	24.5	1.71
F045	3.71	NA	3	260	164	51.0	35.1	29.0	24.2	NA
F047	8.68	NA	3	215	159	49.0	34.2	29.4	23.1	NA
F048	103.60	NA	3	239	193	65.0	38.5	35.0	26.3	1.56
F049	32.81	NA	NA	280	296	67.0	46.2	34.0	33.7	NA
F051	0.88	NA	3	209	155	47.0	33.9	24.0	23.2	NA
F052	36.04	NA	3	265	257	64.0	42.9	31.0	30.8	1.85
F054	3.64	NA	3	211	145	45.7	33.0	27.2	22.2	NA
F056	20.13	NA	4	285	282	68.4	50.3	39.0	34.0	NA
F057	0.05	NA	3	237	180	57.0	38.9	31.9	28.1	NA
F058	11.67	NA	3	228	157	53.0	38.4	31.6	26.7	1.99
F059	0.05	NA	3	213	160	55.0	36.7	28.3	25.5	1.99
F060	14.93	Male	3	205	144	48.0	34.2	27.1	23.3	NA
F061	42.09	NA	3	245	193	58.8	40.2	33.9	27.3	NA
F068	17.41	NA	6	301	325	69.5	49.0	37.0	32.2	1.67
F069	28.87	NA	3	246	170	54.5	42.1	28.0	26.8	1.71
F070	35.84	NA	3	207	110	48.5	33.8	25.5	22.6	NA
F071	14.56	Male	3	257	245	65.0	45.2	34.0	31.0	1.69
F072	2.74	Male	3	239	170	52.5	39.5	30.5	26.5	NA
F073	0.30	NA	3	245	166	57.0	40.8	31.5	25.7	NA
F074	4.44	NA	3	229	152	54.4	36.8	30.0	25.4	NA
F076	1.94	Male	3	236	169	54.5	38.4	29.0	26.5	NA
F077	11.29	Female	4	258	221	60.8	42.8	34.0	27.9	NA
F078	13.56	NA	3	231	155	52.5	36.5	27.0	24.6	1.69
F082	9.15	NA	4	242	185	54.8	40.4	33.0	27.7	2.13
F083	3.18	NA	4	237	170	54.0	38.4	32.7	26.4	1.89
F084	5.93	Female	4	255	220	58.7	43.3	35.6	28.8	1.95
F087	89.70	Male	3	229	155	54.8	37.4	29.0	26.5	NA
F092	0.70	Male	3	211	110	47.2	35.2	28.3	23.1	NA
F093	1.19	NA	3	201	105	46.0	33.7	29.1	22.4	NA
F099	49.86	NA	4	266	255	61.0	41.9	39.1	29.3	1.55
F100	0.00	Female	4	249	200	56.6	41.7	33.4	29.4	NA
F101	17.60	NA	3	223	140	50.9	36.4	28.3	26.6	1.99
F102	3.82	Male	5	301	320	67.5	50.7	36.1	34.3	NA
F104	0.00	Male	3	233	150	54.7	39.1	29.7	26.3	1.76
F105	0.00	Male	3	195	100	44.8	33.7	27.2	23.3	NA
F106	2.10	Female	6	310	375	71.1	52.6	41.1	36.7	1.57
F112	0.00	NA	3	223	140	50.2	37.4	29.1	25.6	1.45

Fish ID	Cumulative Movement (km)	Sex	Age (yr)	Total Length (mm)	Weight (g)	Body Depth (mm)	Head Height (mm)	Girth (mm)	Caudal Peduncle Depth (mm)	Aspect Ratio
F114	0.00	Male	3	207	115	48.5	34.2	26.4	23.4	NA
F116	44.62	Female	4	248	175	54.9	40.1	30.4	28.6	1.63
F117	2.57	NA	3	225	165	54.5	37.6	32.7	27.1	1.74
F118	0.88	Male	3	216	136	49.2	32.7	28.1	21.5	NA
F119	4.93	NA	3	202	105	47.0	30.7	25.3	19.8	NA
F120	0.37	Male	3	230	155	53.7	34.7	29.9	22.4	1.42
F121	40.02	Male	3	238	187	60.0	35.8	29.2	25.3	1.73
F123	53.78	Male	4	260	229	58.8	42.7	22.5	27.8	1.83
F124	52.73	Male	3	218	124	49.1	33.7	22.2	22.8	NA
F125	70.64	NA	3	212	143	53.5	35.1	33.1	24.4	NA
F126	1.19	Male	3	203	114	45.1	31.2	28.1	22.6	NA
F132	41.20	Female	3	229	162	52.8	37.3	29.9	25.6	1.88
F133	17.05	Male	3	217	145	51.9	36.2	31.7	25.2	1.81
F134	59.30	NA	3	257	250	62.2	46.0	35.2	30.5	1.57
F135	8.48	NA	3	215	142	50.4	34.3	28.6	24.3	1.50
F137	1.51	NA	3	245	207	58.3	38.8	36.7	27.0	NA
F140	1.23	Male	3	224	145	50.9	36.9	28.1	24.4	NA
F143	33.16	NA	4	256	350	62.8	44.5	37.8	30.3	2.10
F144	19.38	Male	3	232	180	55.7	36.5	31.9	27.3	1.71

Chapter 4. An invader in salmonid rearing habitat: current and future distributions of smallmouth bass (*Micropterus dolomieu*) in the Columbia River Basin

Authors: E.S. Rubenson, J.D. Olden

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4A. Abstract

Nonnative species and climate change are leading threats to freshwater ecosystems. In the Columbia River Basin (CRB), nonnative species are a critical component in salmon recovery efforts, but little is known about species' current or future distributions. Combining a species distribution model with environmental DNA (eDNA), we locate range boundary regions of nonnative smallmouth bass (Micropterus dolomieu) and evaluate its overlap with native salmonid species. A combination of thermal, hydrological, and geomorphic variables predict that smallmouth bass is distributed across approximately 18 000 river kilometers and overlaps with 3-62% of spawning and rearing habitat of salmonids (species dependent). Under a moderate climate change scenario, smallmouth bass is predicted to expand its range by two-thirds (totaling ca. 30 000 river kilometers) by 2080. Our model generally located boundary regions to within 15 kilometers, and eDNA was successful in verifying range boundary locations across diverse tributaries. Using a unique combination of species distribution models and eDNA, we determine the extent of an invasion at multiple scales and highlight an important threat to salmonid conservation in the CRB.

4B. Keywords

Invasive species, salmon conservation, range boundary, eDNA, species distribution model

4C. Introduction

Freshwater ecosystems remain extremely vulnerable to the combined threats of multiple stressors (Craig et al. 2017). Climate-induced stream warming is causing widespread changes to species assemblages, promoting the secondary spread of nonnative, and often invasive, species (Comte et al. 2013). Consequently, understanding current and projected future distributions of nonnative species is fundamental to strategic conservation planning for freshwater ecosystems (Bush et al. 2014). Modeling efforts, however, are challenged by the simultaneous need to be both generalizable to capture a species' broad distribution while also being adequately specific to inform local scale management and conservation practices (Dormann et al. 2012). Although myriad methods to improve model performance continue to be developed, data availability remains limited, prompting the continued use of correlative species distribution models that utilize only species occurrence and spatial environmental data. Advancements in the performance of correlative models, however, are possible by integrating diverse datasets that span different spatial scales and sources (Ibáñez et al. 2014). Although this does not necessarily capture processes that set range constraints, spatially diverse datasets may improve model accuracy at critical range boundaries where management relevance is the greatest.

The Columbia River Basin (CRB) once supported large populations of ecologically, culturally, and socioeconomically important native salmonids (salmon, trout, and char), but habitat alteration, nonnative species proliferation, and ongoing climate change have contributed to dramatic declines in many parts of their range (Rieman et al. 2015, Hand et al. 2018). Recovery efforts are complicated by the broad expanse and diverse ecology of the CRB, which spans portions of seven states (Washington, Oregon, Idaho, Montana, Nevada, Utah, and Wyoming), two countries (United States and Canada), and countless management agencies that operate at different spatial scales. Although $300 million is spent annually on salmonid recovery in the CRB, a disproportionate focus on habitat restoration has been highlighted as insufficient to produce meaningful benefits (Naiman et al. 2012, Rieman et al. 2015, Hand et al. 2018). By contrast, landscape scale assessments and factors that impact food webs, such as presence of nonnative species, are cited as critically overlooked components in salmon recovery efforts (Naiman et al. 2012, Rieman et al. 2015).

Smallmouth bass (*Micropterus dolomieu*) is a prevalent nonnative recreational fish in the CRB, management of which is highlighted as a critical conservation priority for salmon recovery plans (Sanderson et al. 2009, Carey et al. 2011). A high trophic-level predator, smallmouth bass is capable of consuming large proportions of salmon runs (up to 35%) when co-occurring with small salmonids in water sufficiently warm for activity (Fritts and Pearsons 2004, Sanderson et al. 2009, Carey et al. 2011). Smallmouth bass was first introduced into the Willamette and Yakima Rivers in the 1920s (Lampman 1946). Secondary introductions, such as the introduction into the John Day River in 1971 (Shrader and Gray 1999), have occurred throughout the region since that time, to include extensive stocking efforts by multiple state agencies (Carey et al. 2011). Today, substantial local abundances (up to 2 300 fish per river kilometer) and voracious consumption rates (up to 0.623 salmonids per smallmouth bass per day) have been found in parts of the CRB (Erhardt et al. 2018), making smallmouth bass one of the most frequent predators of juvenile salmonids in the CRB (Carey et al. 2011). These estimates come from mainstem rivers or reservoirs where the predominant threat for salmonids occurs during outmigration events. Recent evidence suggests that smallmouth bass is also capable of colonizing some portions of sensitive salmonid spawning and rearing habitat in Columbia River tributaries in spite of having warmer thermal preferences (Lawrence et al. 2014, Rubenson and Olden 2017). This portends a year-round predation and competition threat during a sensitive life-history stage for salmonids. Despite this, very little information is available on current or predicted future distributions of smallmouth bass and its potential overlap with native salmonid species' spawning and rearing habitat in the CRB.

Upstream range boundaries of smallmouth bass (which constitute the regions of most probable overlap with spawning and rearing salmonids) offer unique management opportunities. For instance, the high threat to salmon confined to relatively isolated locations may assist in gaining support for localized efforts to control (Loppnow et al. 2013) or prevent further upstream invasions (Rahel 2013). In addition, upstream range boundaries represent regions where physiological stress, habitat suitability, and/or dispersal barriers present potentially exploitable constraints on expanding populations (Sexton et al. 2009). These factors make range boundaries critical battlegrounds for invasive species management. Determining the location of range boundaries, however, often requires extensive sampling or accurate species distribution models developed from large datasets, both of which have been prohibitively costly (Urban et al. 2016). Environmental DNA (eDNA), however, may present a new opportunity to supplement correlative modeling efforts at critical boundary regions by providing fine-scale distributional resolution at relatively low costs. Moreover, eDNA samples may be combined with data from traditional surveys, potentially enhancing model performance both range wide and at range boundaries.

In this study we explore the efficacy of developing a correlative species distribution model from existing smallmouth bass records to guide eDNA survey design at range boundaries. These additional distribution data both locate management-relevant range boundary regions in individual streams and provide additional data to inform a basin-wide distribution model for smallmouth bass. Our objectives were to 1) develop and validate a species distribution model for smallmouth bass in the CRB, 2) use eDNA to refine the location of and improve model performance at predicted upstream range boundaries, 3) predict future distributions of smallmouth bass in the CRB under future climate change scenarios, and 4) quantify the degree of spatial overlap of smallmouth bass with critical habitat and designated refugia for CRB salmonid species.

4D. Methods

Smallmouth bass occurrence

We collated smallmouth bass distribution data from a diversified set of databases and individual biologists across Washington, Oregon, Montana, Idaho, and Canada. We refer to this as the "initial" dataset, whereas the dataset that also includes the additional eDNA-derived records is referred to as the "final" dataset. Although distribution data was provided for Canada, the Canadian portion of the CRB was not included due to the lack of compatible environmental predictor variables (i.e., current and future mean August water temperature, Variable Infiltration Capacity (VIC) hydrologic models for spring flow, and waterfall distributions). Distribution in Canada, however, is currently limited and concentrated along the border with the United States.

Data were provided by Washington Department of Fish and Wildlife (WDFW), Idaho Department of Fish and Game (IDFG), Idaho Department of Environmental Quality (IDEQ), Oregon Department of Fish and Wildlife (ODFW), Yakima Nation Fisheries, Confederated Salish and Kootenai Tribes, and the Nez Perce Tribe. Responses to data requests that contained location, confirmed presence or absence of smallmouth bass, and sampling dates between 1981 and 2016 were included in our database. In addition, we included data from the EPA and Regional Environmental Monitoring and Assessment Program (EMAP and REMAP), the EPA National Rivers and Streams Assessment (NRSA), the USGS National Water Quality Assessment Program (NAWQA), the USGS Nonindigenous Aquatic Species Database (https://nas.er.usgs.gov), Montana Fish, Wildlife, and Parks MFish database (http://fwp.mt.gov/fish), and the published literature (Table 4.S1). Database entries were limited to those that were sampled between 1981 and 2016 and labeled as confirmed, established, accurate, and confined to flowing water environments. Different survey techniques were used by the various agencies and sources (i.e., electrofishing, snorkeling, weir trapping, and seining), thus we recognize issues related to differing sampling efficiency and detection probabilities.

Distribution data were assigned to stream segments according to hydrographic flow lines obtained from the National Hydrography Dataset Plus, Version 2 (NHDPlusV2; McKay et al. 2012) (http://www.horizon-systems.com/NHDPlus). Stream segments classified as intermittent hydrology and those not containing all climate and habitat data metrics were removed, resulting in 118 708 total segments, equating to approximately 204 700 river kilometers. After excluding distribution records from the same location (river segment), we identified 240 presence records (Fig. 4.1). Visual investigation revealed no evidence of spatial sampling bias in the presence data, however there was a disproportionate number of absence points in certain regions, including the Willamette River basin. Consequently, to ensure equal representation of all available habitat types across the CRB, absence points were spatially rarefied, resulting in the initial database containing a total of 177 absence records (Fig. 4.1).

We used the predictions from our model based on the initial database to inform the collection location of eDNA samples along the predicted range boundary in 14 major rivers of the CRB (Fig. 4.1). These rivers encompass a subsample of the diverse environmental contexts and disparate invasion histories of smallmouth bass in the CRB (Carey et al. 2011). All samples were collected during the late summer (last 2 weeks of July 2016) to ensure congruence with the stream temperature predictor variable used in the model (described below). We defined the predicted range boundary regions as sections of rivers where the probability of presence exhibited a visible decline from > 0.9 towards probabilities < 0.5. In each tributary, 25-75 km stretches of river that bracketed the predicted range boundary of smallmouth bass were targeted for sampling. For the Salmon River, sampling locations were informed by conversations with local fishery biologists, guiding us to our sampling location. At each sampling location, 5-L of water was filtered through a 1.5 µm pore-sized fiberglass filter using the equipment and protocol of the Rocky Mountain Research Station (Carim et al. 2016b). When possible, we prioritized sampling locations just downstream of pools where flow increased and the channel-width narrowed. We chose these locations because smallmouth bass are known to prefer slower-water habitat, and sampling at knick-points increased the probability that water from both banks of large rivers would be sampled. Smallmouth bass presence was assessed by extracting and amplifying eDNA from the filters using an assay specifically developed to detect smallmouth bass (Franklin et al. 2018). Marker and assay development as well as extraction and amplification were completed by the Rocky Mountain Research Station (U.S. Forest Service) following the methods in Carim et al. (2016a). Finally, to test the accuracy of eDNA detection in these contexts, we conducted snorkel surveys (when flow and visibility allowed) immediately following eDNA collection. Here, two snorkelers swam 100 m upstream from where water samples were collected on opposite banks of the river. Both snorkelers then floated down the thalweg, noting presence or absence of smallmouth bass.

Environmental determinants of smallmouth bass occurrence

Referencing the extensive knowledge of smallmouth bass ecology (e.g., Peterson and Kwak 1999, Sharma and Jackson 2008, Lawrence et al. 2015), we selected seven predictor variables that represent thermal, hydrologic and geomorphic attributes known to influence fish distributions (Table 4.1, 4.S2). Climate and habitat data were georeferenced at the stream segment scale. We provide summary statistics for the entire network (Table 4.1) in addition to statistics at presence and absence sites (Table 4.S2).

Thermal variables were selected to incorporate conditions that are known to physiologically support smallmouth bass. Specifically, evidence suggests that mean summer daily temperatures near 20-22°C constrain the upstream range boundary of smallmouth bass populations in Columbia River tributaries (Rubenson and Olden 2017) and that winter severity limits the establishment of colonizing populations (Lawrence et al. 2015, Rubenson and Olden 2017). As such, we included mean August water temperature (WaterT) and the mean monthly air temperature between October 1 and March 31 (WinterAirT). Historical summer water temperature data were developed by the NorWeST stream temperature project based upon data from 1993-2011, and air temperatures were developed by the University of Washington Climate Impacts Group based upon data from 1916-2006 (Table 4.1). Because water temperature data was developed for NHDPlusV1, we first spatially joined these data to the NHDPlusV2 layer before conducting our analyses. In addition, reservoir temperatures were not modeled in the baseline water temperature dataset, so river segments in mainstem reservoirs were linearly interpolated between the nearest upstream and downstream riverine segment adjacent to the reservoir.

Hydrologic variables were selected based upon smallmouth bass flow affinities. Generally, smallmouth bass thrive in high-order streams and is preferentially found in perennial pools or glides (Dauwalter et al. 2007). We included mean annual flow (MAFlow) to represent stream size and the slope of each river segment (Slope) to capture localized velocities. Slope was extracted from the NHDPlusV2 dataset (Table 4.1). In addition, smallmouth bass show varied responses to spring hydrologic conditions, specifically related to movement patterns, spawn timing, and spawning success (Rubenson and Olden 2017). We thus included the magnitude of flow during the spring (April-May) standardized by mean annual flow (SpringFlow) to enable comparisons between small headwater streams and large mainstem rivers. All flow metrics were extracted from the Western U.S. Stream Flow Metrics dataset, and were based upon data from 1916-2006 (Table 4.1).

Geomorphic variables were included to represent zones of high-velocity flow or barriers such as waterfalls, cascades, or dams that can deter or prevent smallmouth bass upstream dispersal. Geomorphic variables included slope (described above), the density of dams in the watershed (DamDensity) as published by StreamCat (Hill et al. 2016), and a categorical variable accounting for whether or not the stream segment was located above a waterfall or cascade (as defined in the Fish Passage Barriers dataset from StreamNet.org) (Waterfall) (Table 4.1). Recognizing that smallmouth bass stocking has occurred above some major barriers in the CRB in the past, we considered barriers in the waterfall dataset functionally absent in the model (i.e., permeable) in sections of river with a known stocking history. By contrast, although some mainstem dams have fish passage structures, the ability of non-salmonids to utilize these opportunities for upstream movement remains uncertain, so these barriers were retained.

Baseline thermal and hydrological metrics (i.e., WaterT, WinterAirT, MAFlow, SpringFlow) were paired with their associated predictions for 2080 according to an ensemble mean of 10 or more global climate models (GCM) for A1B emission scenarios for 2080 (IPCC, 2007). The A1B emission scenario is considered a moderate estimate for future carbon emission scenarios, and is similar to the 2010 Representative Concentration Pathway 6.0. Although updated emission scenarios are available from the IPCC 2013, only the A1B scenario was consistently modeled for all our metrics for the same time period (i.e., 2080). For mean August water temperature (WaterT2080), the NorWeST stream temperature scenario 32 was used for the 2080 predictions, which incorporates both modeled mean August air temperatures and stream discharge changes for 2080 (specifics in Wenger et al. 2010 and Hamlet et al. 2013). These data also account for differential sensitivity among streams to climate warming (Luce et al. 2014). Predicted air temperatures for winter (WinterAirT2080) were derived by the University of Washington Climate Impacts Group using the ensemble mean from 19 GCMs associated with the A1B emission scenario (specifics in Littell et al. 2011). Predictions for both flow metrics (MAFlow2080 and SpringFlow2080) were developed using the Variable Infiltration Capacity (VIC) macroscale hydrologic model developed by the University of Washington Climate Impacts Group for the stream segments in the western U.S. (specifics in Wenger et al. 2010).

Species distribution model

We used multivariate adaptive regression spline (MARS) models to estimate smallmouth bass probability of occurrence at the reach scale. We used the initial dataset to predict current distribution for identifying eDNA survey locations and the final (i.e., initial and eDNA records) dataset to predict current and future distribution and overlap with salmon habitat. We selected MARS models because they allow for easy interpretation of complex relationships between the response and predictor variables and have been widely shown to be one of the top performing techniques for species distribution modeling (Muñoz and Felicísimo 2004). MARS models describe non-linear species-environment relationships using a series of piecewise linear segments (Leathwick et al. 2005). MARS models, however, are built assuming normal data, and thus needed to be adapted to accommodate the binomial error structure of our presence-absence data. As such, we fit generalized linear models (GLM) to the extracted basis functions from the MARS model following the methods of Leathwick et al. (2005) and Elith and Leathwick (2007). We used source code from Elith and Leathwick (2007) to run the models in R (R Foundation for Statistical Computing, Vienna). This code fits the initial models using the 'mda' package (Hastie and Tibshirani 1996), extracts the set of linear segments that best describe the nonlinear relationships between the response and explanatory variables (i.e., basis functions), and relates species occurrences to these functions by fitting a GLM with a binomial error distribution to the data. The functions extracted during the MARS/GLM modeling process were applied to each stream segment's unique set of predictor variables to determine the probability of species occurrence at that location for both current and future (i.e., 2080) conditions. In addition, response curves (i.e., the basis functions used during the MARS modeling process) were examined to evaluate the primary influences of each predictor variable on species occurrence probability in the model. Multicollinearity between variables was examined using Variance

Inflation Factor (VIF); high multicollinearity is evident when individual VIFs are ≥ 10 or the mean VIF across variables is ≥ 5. No indications of multicollinearity were discovered between any of the predictor variables (all individual VIF < 2.6; mean VIF = 1.8), thus all variables were included in the model. Although VIF was low, we did reveal moderate levels of correlation (r = 0.5-0.6) between WaterT, WinterAirT, and Slope, as well as between WinterAirT and SpringFlow (Table 4.S2).

Model validation was conducted using both threshold-independent and threshold-dependent model statistics. For the threshold-independent test, 10-fold cross-validation (i.e., 90% of the data were used to train the model, and the remaining 10% were withheld for testing) was conducted and model accuracy assessed by calculating the area under the receiver operating characteristics (ROC) curve (AUC) (Olden and Jackson 2002). AUC measures the ability of a model to discriminate between presence and absence sites. A score of 0.5 implies no better than a random guess whereas a score of 1.0 indicates perfect accuracy; intermediate scores are ranked poor (< 0.7), good (0.7-0.9), and excellent (> 0.9) (Olden and Jackson 2002). By contrast, threshold-dependent model statistics were calculated to facilitate comparisons with the distributions of salmonid species. We used the maximum sensitivity plus specificity to determine the threshold value by which species were considered present (Liu et al. 2013). Confusion matrices were then constructed, from which sensitivity (correctly predicted presence), specificity (correctly predicted absence), true skill statistic (TSS) (average of net prediction success), Cohen's Kappa (extent to which the agreement between observed and expected are better than chance alone), and model accuracy (overall probability that either presence or absence were correctly predicted) were calculated. We then calculated the proportion of total river kilometers predicted to be occupied by smallmouth bass and the predicted percent change by 2080 for each of the 9 HUC-4 sub-basins of the CRB.

We used model predictions using the initial dataset to guide our eDNA collection efforts and to determine the effect that combining datasets had on model performance. Predictive performance of the SDMs built using the initial versus final distribution datasets were compared to assess whether model performance was improved with the addition of targeted samples at range boundaries. Model performance was separately evaluated at the range boundaries, comparing initial and final model performance at the eDNA data locations only. To assess the accuracy of our initial model for guiding sampling locations at range boundaries, we calculated the approximate watercourse distance (in river kilometers) between the most upstream presence point and the location where probability of occurrence dropped below the pre-determined threshold value. Finally, we compared eDNA detection with our detection during paired snorkel surveys.

Smallmouth bass overlap with critical salmonid habitat and cold-water refugia

We assessed the amount of spatial overlap between the predicted current distribution of smallmouth bass and the location of critical (i.e., spawning and rearing) salmonid habitat and cold-water refugia. Critical habitats of salmonids were delineated using publically available species distributions for the 5 anadromous species of Pacific salmon that spawn and rear in the CRB and the 4 resident trout species (StreamNet GIS Data 2003). These salmonid distributions were developed by StreamNet.org using data collected by agencies in Oregon, Washington, Idaho, and Montana and represent the best professional judgment of local fish biologists in the Pacific Northwest region. These distributions contained species locations classified by habitat-type (i.e., year-round residence, migration, rearing, and/or spawning), and Pacific salmon distributions organized by “runs” of individual species (i.e., spring, summer, or fall) when appropriate. To determine critical habitat, we excluded segments marked as migration-only, thus including only rearing, spawning, and year-round habitat. We then used ArcGIS 10.2 to quantify the percentage of total critical habitat (% total river kilometers) of each salmonid species predicted to be sympatric with current smallmouth bass occurrence (i.e., probability greater than the determined occurrence probability threshold). The salmonid species distributions were not projected into the future, so we could not calculate the predicted change in overlap with smallmouth bass under future climate conditions.

In addition to the above salmonid distributions, we compared smallmouth bass distributions to cold-water refugia as delineated by the U.S. Forest Service (USFS) Climate Shield project (Isaak et al. 2017). Isaak et al. (2017) identified specific cold water habitat associated with juvenile bull trout (*Salvelinus confluentus*) and cutthroat trout (*Oncorhynchus clarkii*) – both species of conservation concern that have cold thermal niches relative to other salmonids in the CRB (Isaak et al. 2017). These cold-water refugia represent only a subset of the entire ranges of these species, but are considered priorities for conservation and cold enough to prevent encroachment by nonnative species. Because the probability of smallmouth bass occurrence in these regions was always less than the pre-determined occurrence threshold, we calculated the summary statistics of predicted probabilities in each cold-water refuge under current and future climate conditions instead of number of river kilometers likely inhabited by smallmouth bass.

4E. Results

We show that nonnative smallmouth bass is widely distributed throughout the CRB, with established populations spanning most of the Columbia River mainstem and its major tributaries (Fig. 4.1). Smallmouth bass is predominantly (79%) located in high-order streams (i.e., stream orders 5-9 which represent all mainstem habitats and major tributaries of the CRB), as well as some low-order streams (i.e., stream orders 1-4); this suggests that smallmouth bass occupy a diverse spectrum of habitats (Table 4.S3). Although smallmouth bass occurrence was predicted across all stream orders, we do note that the low-order streams that had presence points were all located adjacent to high-order streams (Fig. 4.2a). The eDNA survey included 87 stream locations, resulting in 30 presence records and 57 absence records. At 74 of the sample locations, paired snorkel surveys were also conducted. Smallmouth bass were observed in 18 surveys, and not observed in 56 surveys. We had positive eDNA detection in all 18 sites where smallmouth bass were also observed as well as 8 positive eDNA detections where no smallmouth bass were observed. There were no smallmouth bass visually detected (n=48 surveys) at any of the eDNA absence locations. All eDNA data were located in high-order, major CRB tributaries. These 87 records were subsequently combined with the initial database, and the model was parameterized again.

In concordance with the final distribution dataset, our model predicted a present-day distribution of smallmouth bass that spans a diversity of climates, geomorphology, and hydrography across the CRB (AUC = 0.90, Table 4.2, Fig. 4.2a). Although distributed across a large geographic space, smallmouth bass occurrence is predominantly limited to larger, high-order streams or adjacent low-order streams (Fig. 4.2a). Using the maximum sensitivity plus specificity as a threshold for probability of presence (0.5), we found that approximately 17 660 river kilometers of the CRB were predicted as occupied by smallmouth bass under current environmental conditions (Kappa = 0.62, TSS = 0.62; Table 4.2). The Middle Snake, Middle Columbia, Upper Columbia, and Yakima sub-basins had the highest proportion of total river kilometers predicted to be occupied by smallmouth bass (16%, 13%, 12% and 12% respectively), whereas the Lower Snake, Kootenai-Pend Oreille-Spokane and Lower Columbia had the lowest proportion of occupied riverine habitat (5%, 5%, and 7% respectively; Fig. 4.S1). The model built using the final dataset demonstrated similar performance to a SDM constructed using just the initial database of past survey efforts (Table 4.2).

The primary environmental drivers contributing to model performance included a combination of thermal, hydrological, and geomorphic variables. Mean August water temperature, spring flow, and slope were the most influential with respect to the amount of total deviance explained (Δ Deviance = 80.3, 29.0, 27.4; respectively), followed by mean annual flow and the presence of barriers (Δ Deviance = 11.3, 3.7; respectively) (Fig. 4.3). Dam density and winter air temperature did not contribute to model accuracy (Δ Deviance = 0.0). Habitat suitability was generally negatively associated with slope and spring flow and positively associated with water temperatures and large rivers, suggesting that smallmouth bass benefitted from shallow gradients, large rivers, moderate magnitude spring flows, and warm water temperatures (Fig. 4.3). In addition, the presence of hydrologic barriers was associated with reductions in the probability of smallmouth bass occurrence.

Although our model responses generally matched what is known about smallmouth bass habitat suitability, a number of interesting results emerged. First, increasingly steep gradients did not show a linear decrease in predicted smallmouth bass occurrence (Fig. 4.3). Instead, shallow gradients had a positive effect on smallmouth bass occurrence probability, whereas steeper gradients had no effect. Second, rivers with the most stable flow regimes (i.e., low values of spring flow) were associated with lower probabilities of smallmouth bass occurrence. These more stable flow regimes were predominantly isolated to the coastal regions of the CRB. There was a threshold response to spring flow, however, such that moderate levels of spring flow had a positive impact on smallmouth bass occurrence probability, but as spring flows continued to increase, the relationship changed to a steep, negative association. Third, there was a nonlinear relationship between water temperature and smallmouth bass occurrence probability. A positive association with smallmouth bass occurrence was only evident when mean August water temperatures exceeded 17°C, and this association slightly weakened when temperatures exceeded 20°C.

Predicted changes to future (i.e., 2080) flow and temperature resulted in dramatic increases to smallmouth bass distribution throughout most of the CRB (Fig. 4.2b). Specifically, smallmouth bass are predicted to gain over 12 000 kilometers of river by 2080, representing a 69% increase from predicted current occupied habitat (Table 4.2). In addition, some small headwater tributaries that are currently predicted to be inhospitable to resident smallmouth bass appear vulnerable to invasion under warmer water conditions (Fig. 4.2b). This was most apparent in the Middle Snake, Middle Columbia, and Yakima sub-basins of the CRB, where smallmouth bass are predicted to spread through an additional 10% of the available riverine habitat in each sub-basin (Fig. 4.S1). All other sub-basins are predicted to see gains of 4-6%, with the exception of the Lower Columbia, where there is no predicted change to the distribution of smallmouth bass (Fig. 4.S1).

We found our model based on the initial collation of existing data useful in guiding our localized eDNA sampling efforts. In 10 of 14 sampled rivers, we located a transition from eDNA presence to absence points near the location where the model predicted a range boundary (Fig.

4.5, 4.S2). According to the initial SDM, the watercourse distance between the most upstream eDNA presence point and the model predicted upstream extent (considered the first location where the model probability of occurrence fell below 0.5) averaged 15 kilometers (range = 0 - 40 km) (Fig. 4.5). In the Yakima, Payette, Kootenai, and Salmon Rivers, no smallmouth bass were detected using eDNA. When comparing model performance at these range boundary regions, we note a marked improvement of the model built using the final database that incorporated eDNA data (Table 4.2, Fig. 4.S2). The final model demonstrated overall greater performance (as indicated by Kappa, TSS and Accuracy indices) and was twice as successful in correctly predicting species absence (specificity = 0.61 vs. 0.30) at the range boundaries while demonstrating comparable sensitivity (Table 4.2).

We found various amounts of overlap between the predicted current-day smallmouth bass distributions and critical habitat for all CRB salmonids except Chum salmon (Table 4.3). Percentages of critical habitat overlap across these species ranged from 3-62% (mean = 20%) (Table 4.3). Fall Chinook and Sockeye salmon, in particular, had relatively high percentages of overlapping habitat (i.e., 62% and 39% respectively) (Table 4.3). By contrast, there was little to no predicted overlap between designated cold-water refugia for either bull trout or cutthroat trout. Instead, we see very low (albeit non-zero) probabilities of smallmouth bass presence for both current day (mean = 0.06 for both species) and future climate (mean = 0.06 for both species) predictions (Fig. 4.4).

4F. Discussion

By combining phenomenological distribution modeling with enhanced species detectability at range edges using eDNA, we provide new insight into the present and forecasted future invasion of smallmouth bass in the Columbia River Basin. Models performed well at predicting smallmouth bass distribution at the basin scale (Table 4.2), including a strong ability to determine the approximate upstream range boundary locations at the channel segment (Fig. 4.2a, Fig. 4.5). Species detection using eDNA sampling proved useful to improve model predictions from correlative models at the range boundaries without sacrificing model performance at the broader spatial extent, and successfully refined the location of the leading-edge invasion to within management-relevant regions of multiple rivers (Fig. 4.5, 4.S2).

Empirical data and model predictions depict a broad invasion of smallmouth bass across diverse climates, hydrological conditions, and geomorphologic contexts that define the rivers of the CRB. Combinations of shallow gradients, large rivers that display moderate-magnitude spring flows, and warm water were associated with increased probability of smallmouth bass occurrence. According to these habitat correlates, nearly 18 000 river kilometers are suitable to, or currently, support smallmouth bass populations under current environmental conditions (Fig. 4.2a, Table 4.2). Although the distribution of smallmouth bass is spread broadly across geographic space, we show that smallmouth bass is still relatively confined to warmer, low-gradient rivers in the CRB (Fig. 4.2a). This, however, is not unique to cool-water smallmouth bass. Isaak et al. (2017) demonstrate that across 11 fish species, including several cold-water salmonids, only small proportions of total stream habitat (measured only in Idaho) are accessible or suitable to fish. If a majority of the CRB network is not accessible or suitable to fish, the basin-wide occupancy of smallmouth bass may be much more significant than our results depict.

Although our model depicts a propensity for smallmouth bass to inhabit large, higher-order streams, it also portrays suitable habitat throughout a number of smaller headwater tributaries (Fig. 4.2). As a result, we found that there was spatial overlap ranging from 3-62%

with critical spawning and rearing habitat for native salmonids across the CRB (Table 4.3). This observation denotes a potential challenge for salmon conservation where the invasion of smallmouth bass is rarely considered in large-scale conservation and restoration efforts (Carey et al. 2011, Naiman et al. 2012). For anadromous species, in particular, our estimates of habitat overlap provide a conservative estimate of potential impact given that this only depicts the potential threat of year-round co-occurrence, excluding interactions that may occur during outmigration. Furthermore, forecasted changes to flow and temperature regimes predicted a two-third increase in suitable habitat, equating to over 10 000 more river kilometers of potentially suitable habitat to smallmouth bass in 2080 (Fig. 4.2b, Table 4.2). Despite this striking pattern, there remains only scant research evaluating the predator and competitive impacts of smallmouth bass on juvenile salmonids in critical spawning and rearing habitats. Instead, most literature is focused either on predation on outmigrating salmonids (e.g., Fritts and Pearsons 2004) or effects of habitat degradation and loss, dams and impoundments, harvest, and hatcheries (e.g., Naiman et al. 2012). This exposes a potentially important overlooked impact to threatened and endangered salmonids.

We show that the critical spawning and rearing habitat of most salmonids, especially fall Chinook and Sockeye salmon, overlap with established year-round smallmouth bass populations (Table 4.3) and that much of the CRB will see an increase in suitable smallmouth bass conditions in the future (Fig. 4.2b, Fig. 4.S1). The Middle Snake, Middle Columbia, and Yakima sub-basins, in particular, show high percentages of occupancy by smallmouth bass under current climate conditions, which encompass much of the critical habitat for fall Chinook and Sockeye salmon. These same sub-basins show the greatest projected increase in habitat suitability under future climate conditions (Fig. 4.S1). Smallmouth bass is predicted to increase by 4-6% in the

Willamette, Lower and Upper Snake, Kootenai-Pend O'reille-Spokane, and Upper Columbia. Many of these sub-basins include forested mountain streams that currently support native trout species, including both cutthroat trout and bull trout. A positive implication of our study is that refugia for cutthroat and bull trout (Isaak et al. 2017) appear safe from major encroachment by smallmouth bass both in present day and forecasted futures (Fig. 4.4). Protecting these refugia and focusing efforts on the early detection and prevention of secondary spread, where possible, may prove a cost-effective strategy to minimize future impacts of smallmouth bass on salmonid species in these sub-basins. This will require continued investigation of the spatiotemporal patterns of fish movement at the leading-edge of the invasion (Rubenson and Olden 2017).

Little change to the projected distribution of smallmouth bass was observed in the Lower Columbia basin. This sub-basin appears relatively buffered to climate-induced temperature changes observed in other sub-basins, with only a small proportion of the sub-basin warming above the model-identified breakpoint of 17°C. Interestingly, further analysis reveals that many of the segments predicted to warm do not see large increases to the probability of smallmouth bass occurrence. Here, non-temperature related drivers, such as hydrologic conditions or slope, are likely important for determining the future of smallmouth bass in this basin. For instance, many of the stream segments predicted to warm also have steep slopes. This suggests that steep slopes may prevent smallmouth bass from moving upstream in response to warming temperatures despite the model response curve suggesting only a slight influence of steep slopes on probable occurrence (Fig. 4.3). Gibson-Reinemer et al. (2017) demonstrated that high channel slopes likely limit the ability of fish to track climate change. More research is required to determine what slope constitutes a barrier for movement to smallmouth bass. Knowing where smallmouth bass are unlikely to expand even if climate change increases habitat suitability may help target management funds to regions where secondary spread is more likely.

Correlative associations manifested in the distributional model may facilitate targeted management strategies to prioritize locations for early detection monitoring and prevention of secondary spread of smallmouth bass (Vander Zanden and Olden 2008). Here, we highlight potential mechanisms that may be exploited for management purposes at range boundaries. Warmer water temperatures increased the probability of smallmouth bass occurrence in the CRB (Fig. 4.3). The positive influence of warmer water temperatures on smallmouth bass corresponds to current understanding of this species' physiology and aligns with results from previous modeling efforts (e.g., Peterson and Kwak 1999, Sharma and Jackson 2008). Although this clear and strong relationship presents a challenge under likely climate futures, it also highlights a management opportunity that can both benefit native fishes while deterring smallmouth bass (and other cool or warm-water nonnatives). The model-identified breakpoints of 17°C and 20°C may represent water-quality targets and decision points for management action to potentially prevent or dissuade the upstream secondary spread of smallmouth bass.

Restoring riparian vegetation remains a powerful approach to offset climate-induced losses in suitable salmonid spawning and rearing habitat while concurrently reducing the upstream expansion of smallmouth bass. By using downscaled regional climate-change forecasts of air temperature and streamflow with a fine-scale stream temperature model, Lawrence et al. (2014) showed that complete riparian restoration was effective at reducing temperatures in a major tributary of the CRB by up to 2.5°C (7-day average-daily mean water temperatures). Smallmouth bass were projected to occupy the entire Middle Fork John Day River (Oregon, USA) in late summer by 2080, but simulations of 50% riparian restoration restricted smallmouth bass from invading most of the upper 30+ km of river where spring Chinook salmon rearing-habitat persisted. Other management efforts, such as the creation of deeper pools, reconnecting sites to cold water sources, and even the construction of temporary shade structures and selective seasonal releases of colder water from dams (Kurylyk et al. 2015) near or at range boundaries are also potentially viable solutions to reduce water temperature, but require additional investigation.

Although efforts to reduce local stream warming reveals encouraging opportunities, the broad-scale increase of habitat suitability throughout the CRB necessitates a diversity of management approaches. Our model suggests that smallmouth bass occurrence is driven by both temperature and flow, and research shows that manipulating releases downstream from dams may prove useful in the deterrence of secondary spread (Carey et al. 2011). Interestingly, we found a positive relationship between smallmouth bass occurrence probability and moderate spring flows. This association may be related to a mismatch in timing of high discharge events and the initiation of smallmouth bass upstream movement and spawning. For instance, smallmouth bass typically commence spawning as water temperatures warm above 15°C (Rubenson and Olden 2017) and large spring discharge events in much of the CRB are a result of snowmelt and are thus associated with cold water temperatures. Careful consideration of the timing of dam releases is required to effectively deter spawning or upstream movement. In addition, tactics such as nest destruction and targeted adult removals (Loppnow et al. 2013) may also prove feasible, especially at range boundaries where population abundances are generally lower. Finally, intentional fragmentation using natural or artificial barriers to upstream movement may provide opportunities to protect currently uninvaded habitat for native salmonids (Rahel 2013). For example, gabion dams have been constructed to prevent recolonization of nonnative black basses after their removal from South African streams (Weyl et al. 2014). In our model, steep gradients in headwater streams appeared to have little influence on smallmouth bass occurrence; however, the probability of occurrence was negatively associated with the location of barrier features such as waterfalls or cascades. Designating areas upstream of these features as refugia and instituting regulations, monitoring, and education programs to restrict the human-aided transport of species above these barriers may provide cost-effective safeguards to ensure these areas remain free from nonnative predators. In short, the notion that barriers may be useful to prevent secondary spread of invasive fishes requires careful consideration of current efforts to reconnect currently fragmented upstream habitat (Swan and Brown 2017). Before restoration projects to defragment streams occur (e.g., fish passage at road culverts), it would be wise to determine the risk of a predator invasion (Rahel 2013). If invasion risk is high, the benefits of increasing habitat connectivity for target native species may be overridden by new predation and competition threats.

Environmental DNA enabled rapid local-scale assessments of multiple rivers across a large geographical area, confirming the location of management-relevant range boundary regions in individual streams. We found it encouraging that basin-wide models were sufficiently accurate to identify upstream invasion extents to within 15 km of the field-based boundary (Fig. 4.5). Furthermore, including the eDNA presence and absence data in the final model improved model performance at critical range boundary regions (Table 4.2, Fig. 4.5, Fig. 4.S2). We show that including supplemental eDNA from targeted regions can improve the fine-scale distributional resolution of correlative models at relatively low costs without sacrificing broad-scale model performance.

There were some exceptions to identifying approximate range boundaries using eDNA technology. In the Salmon, Kootenai, and Payette Rivers, sampling occurred upstream of where the initial model predicted the range boundary (Fig. 4.S2), therefore the series of absences was expected. In the Yakima River, presence data from our initial distribution database overlaps with the lower 3 eDNA samples that showed absences, suggesting the range boundary was sampled, but that smallmouth bass was not detected by eDNA. Similarly, eDNA results suggest that the range boundary was located in the Clark Fork, but the initial model depicts that smallmouth bass have a high probability of occurrence hundreds of kilometers farther upstream (Fig. 4.S2); calling into question either eDNA detection accuracy or model performance. Little research currently exists on eDNA detection in large rivers such as the Yakima and Clark Fork, and conditions such as low visibility, swift currents, and deep and wide channels make testing the accuracy of eDNA sampling difficult. Both eDNA detection and factors that influence invasion expansion rates in large rivers are ripe areas for additional research. We do note, however, that the final model improved upon the initial model in all of the aforementioned examples (Fig. 4.5).

One key limitation to our study is that eDNA research in waterbodies caution that spatial inferences made on eDNA could vary dramatically between systems based upon local transport processes (Jane et al. 2014, Perez et al. 2017). We specifically targeted rivers with diverse habitat conditions, to include different sizes, sediment load, and velocities. These factors alter DNA concentrations, the detection probability of eDNA, and the distance from an eDNA source at which detection occurs (Pilliod et al. 2013, Goldberg et al. 2016). Although we concede that there is certain to be variability in the precise river kilometer relative to a positive detection from eDNA that defines the range boundary across these systems, we argue that the precision and insight gained relative to the effort exerted far exceeds other capabilities currently available to managers. In addition, we visually located smallmouth bass at 18 of the 30 eDNA detections and had an absence point upstream of the most upstream presence point in all rivers, further narrowing the potential location of the range boundary (Fig. 4.5). Thus, although there are uncertainties in the range at which a positive eDNA detection might occur, our study illustrates the ability to rapidly narrow our knowledge of an invasion extent to within kilometers in multiple rivers across the diverse and vast CRB in a matter of days using only a correlative species distribution model and eDNA.

4G. Conclusion

Conservation and recovery of endangered salmonid species in the CRB are inextricably intertwined with the management of nonnative species. Successful management, however, is contingent on accurate information about species' distributions – especially at range boundary regions. Here, we show how combining new eDNA technology with broad-scale phenomenological modeling was effective in elucidating the current and potential future distribution of invasive smallmouth bass in the CRB and identified range boundary regions to a management-relevant scale. Furthermore, the flexibility, accuracy, and rapidity of these methods are not unique to smallmouth bass, but can also be applied to nearly two dozen other nonnative predators currently established in the CRB, thus providing essential information to managers tasked with ensuring the long-term future sustainability of salmonids.

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4J. Tables

Table 4.1. List of predictor variables used in the smallmouth bass distribution model, as well as summary statistics.

Variable name	Description	Time Period	Source	Unit	Mean (Min, Max)
WaterT	Mean August water temperature	1993-2011	NorWeST stream temperature [1]	°C	13.2 (0, 29.8)
WaterT2080	Future mean August water temperature	2070-2099	NorWeST stream temperature[1]	°C	15.4 (0, 30.4)
WinterAirT	Mean monthly air temperature October 1-March 31	1916-2006	Climate Impacts Group[2]	°C	0.6 (-8.1, 8.3)
WinterAirT2080	Future mean monthly air temperature October 1-March 31	2070-2099	Climate Impacts Group[2]	°C	3.2 (-4.6, 10.3)
SpringFlow	Mean daily flow between April 1 and May 31	1915-2006	Western U.S. Stream Flow Metrics[3]	$cms \cdot cms^{-1}$	1.0 (0.2, 3.1)
SpringFlow2080	Future mean daily flow between April 1 and May 31	2070-2099	Western U.S. Stream Flow Metrics[3]	$cms \cdot cms^{-1}$	0.7 (0.1, 3.4)
MAFlow	Mean of the yearly cumulative discharge	1915-2006	Western U.S. Stream Flow Metrics[3]	cms	35.3 (1.6×10^{-6}, 7 627)
MAFlow2080	Future mean of the yearly cumulative discharge	2070-2099	Western U.S. Stream Flow Metrics[3]	cms	39.0 (1.7×10^{-6}, 8 282)
Slope	Maximum-minimum elevation / length of the NHD Flowline	NA	NHDPlusV2[4]	$km \cdot km^{-1}$	0.06 (1.0×10^{-5}, 0.7)
DamDensity	Density of georeferenced dams within the upstream watershed	NA	StreamCat[5]	$dams \cdot km^{-2}$	0.003 (0, 23)
Waterfall	Categorical. River segments upstream of a waterfall or cascade in the Fish Passage Barriers dataset by StreamNet assigned value of 1.	NA	StreamNet[6]	NA	NA

1. www.fs.fed.us/rm/boise/AWAE/projects/NorWeST.html
2. http://regclim.coas.oregonstate.edu/index.html
3. https://www.fs.fed.us/rm/boise/AWAE/projects/modeled_stream_flow_metrics.shtml
4. http://www.horizon-systems.com/NHDPlus/NHDPlusV2_home.php
5. https://www.epa.gov/national-aquatic-resource-surveys/streamcat
6. http://www.streamnet.org/online-data/GISData.html

Table 4.2. Comparison of basin-wide and range boundary test statistics using 10-fold cross-validation (AUC) and confusion matrices for the model with ("Final") and without ("Initial") supplemental eDNA data. The range boundary statistics measure model performance at the eDNA sample locations only.

	Basin-wide		Range Boundary	
Test Statistic	Final	Initial	Final	Initial
AUC	0.90	0.93	0.91	0.78
Sensitivity	0.83	0.88	0.93	1.00
Specificity	0.80	0.81	0.61	0.30
TSS	0.62	0.69	0.55	0.30
Kappa	0.62	0.69	0.47	0.23
Accuracy	0.81	0.85	0.72	0.54
Current distribution (km)	17 660	22 209	NA	NA
Future distribution (km)	29 818	33 068	NA	NA

Note: Threshold value (0.5) was determined by maximizing the sum of sensitivity and specificity. Estimated distribution is the total river kilometers occupied by smallmouth bass based upon predicted probabilities of occurrence exceeding the threshold value for both current (1981-2016) and projected future (2080).

Table 4.3. Percentage of the spawning, rearing, and/or year-round habitat of each Columbia River Basin salmonid species predicted to overlap with smallmouth bass and total river kilometers (RKM) of predicted overlap.

Species	Run	% of Habitat	RKM
Chinook salmon	Fall	62	2 125
	Spring	22	3 359
	Summer	9	140
Chum salmon	NA	0	0
Coho salmon	NA	19	1 174
Sockeye salmon	NA	39	116
Steelhead	Summer	11	2 606
Steelhead	Winter	15	1 272
Bull Trout	NA	6	1 160
Redband Trout	NA	14	2 810
Westslope Cutthroat Trout	NA	3	1 361
Yellowstone Cutthroat Trout	NA	4	172

4K. Figures

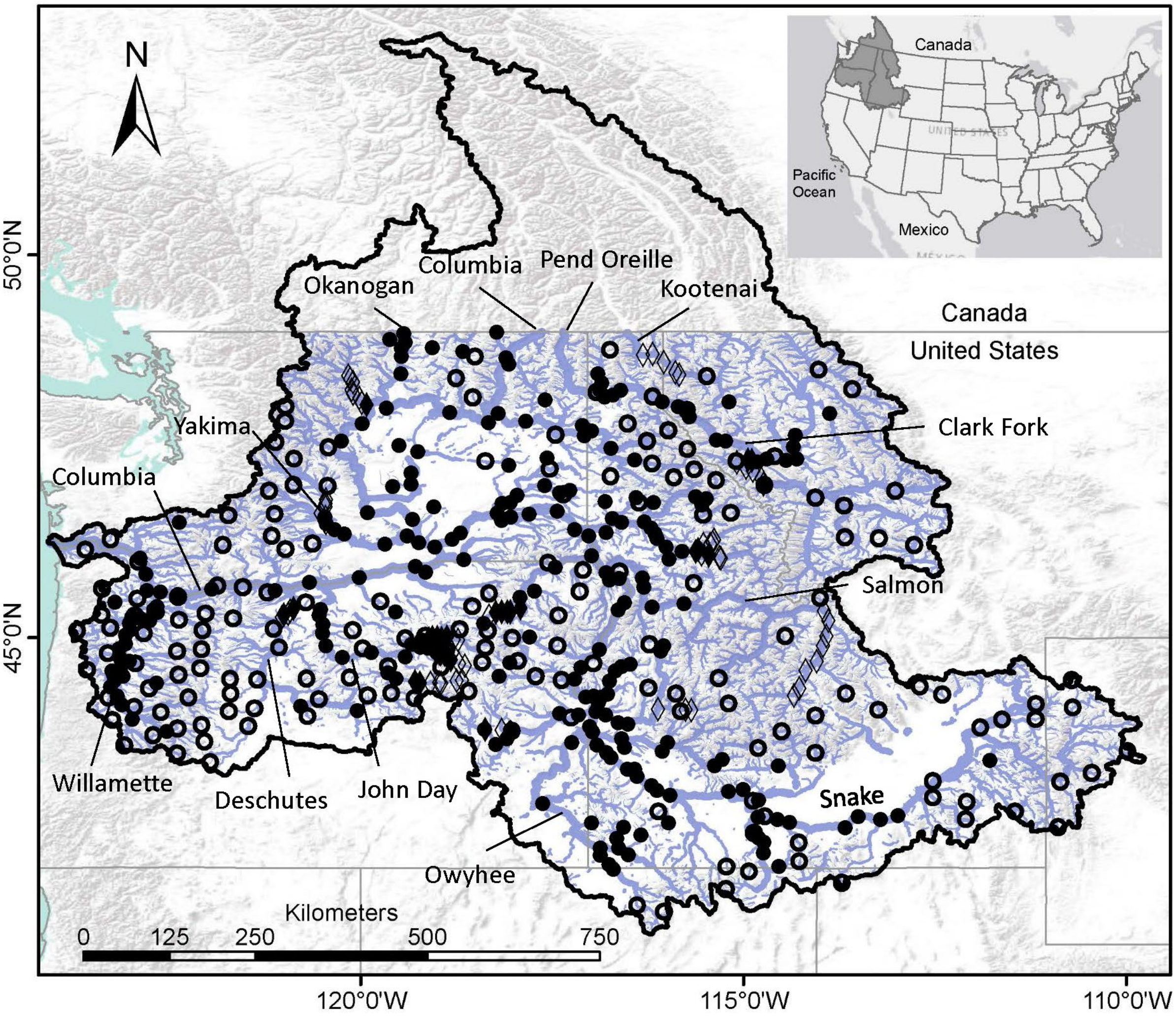

Figure 4.1. The Columbia River Basin and the presence (filled circles) and absence (open circles) of smallmouth bass from the assembled regional database and eDNA presence (filled diamonds) and absence (open diamonds) data. The Canadian portion of the Columbia River Basin was not included in the model although smallmouth bass occurs along the United States border. Stream order 1 not depicted.

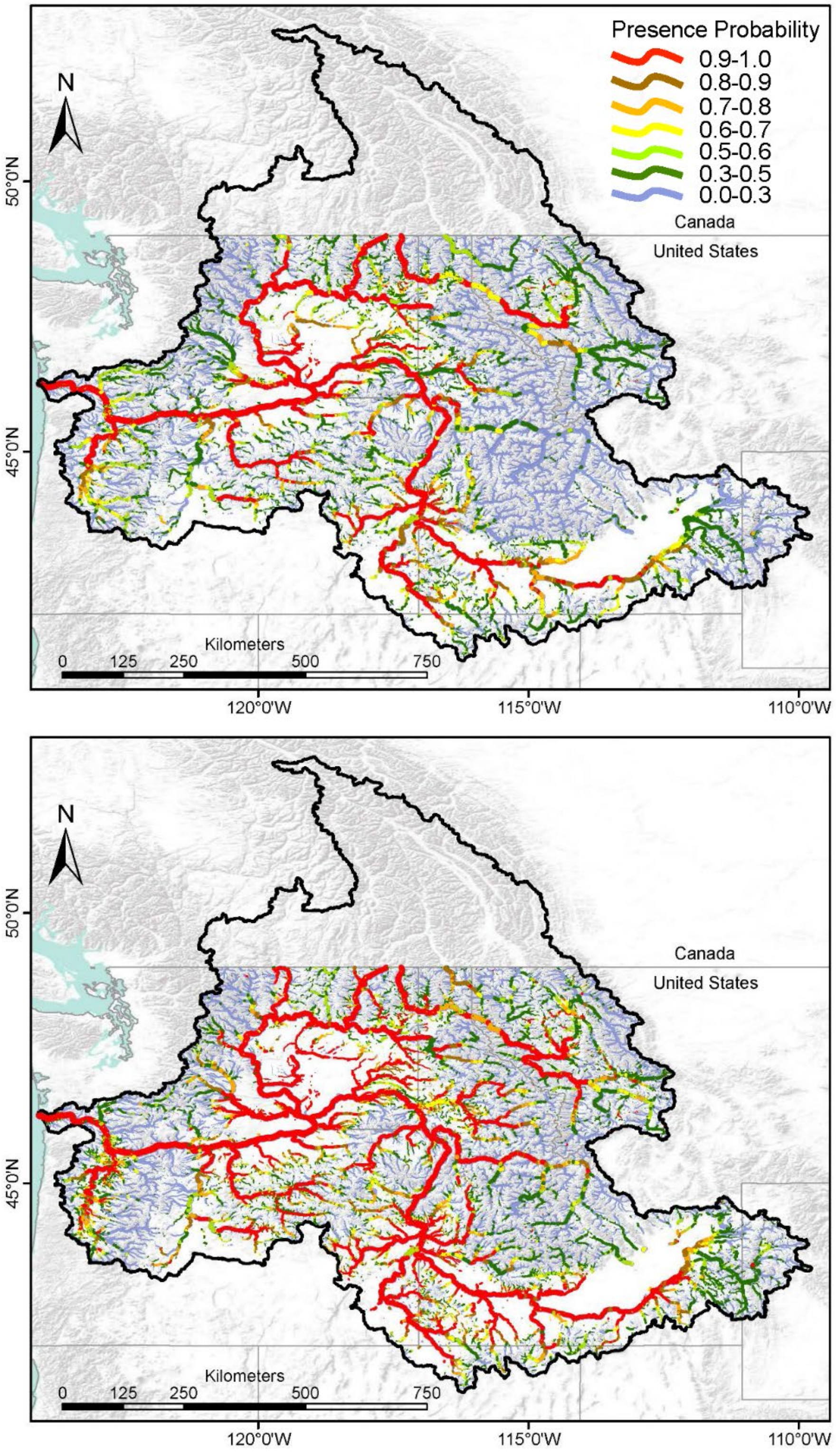

Figure 4.2. Modeled distribution of smallmouth bass for a) current day (1981-2016) and b) projected future (2080). Stream order 1 not depicted.

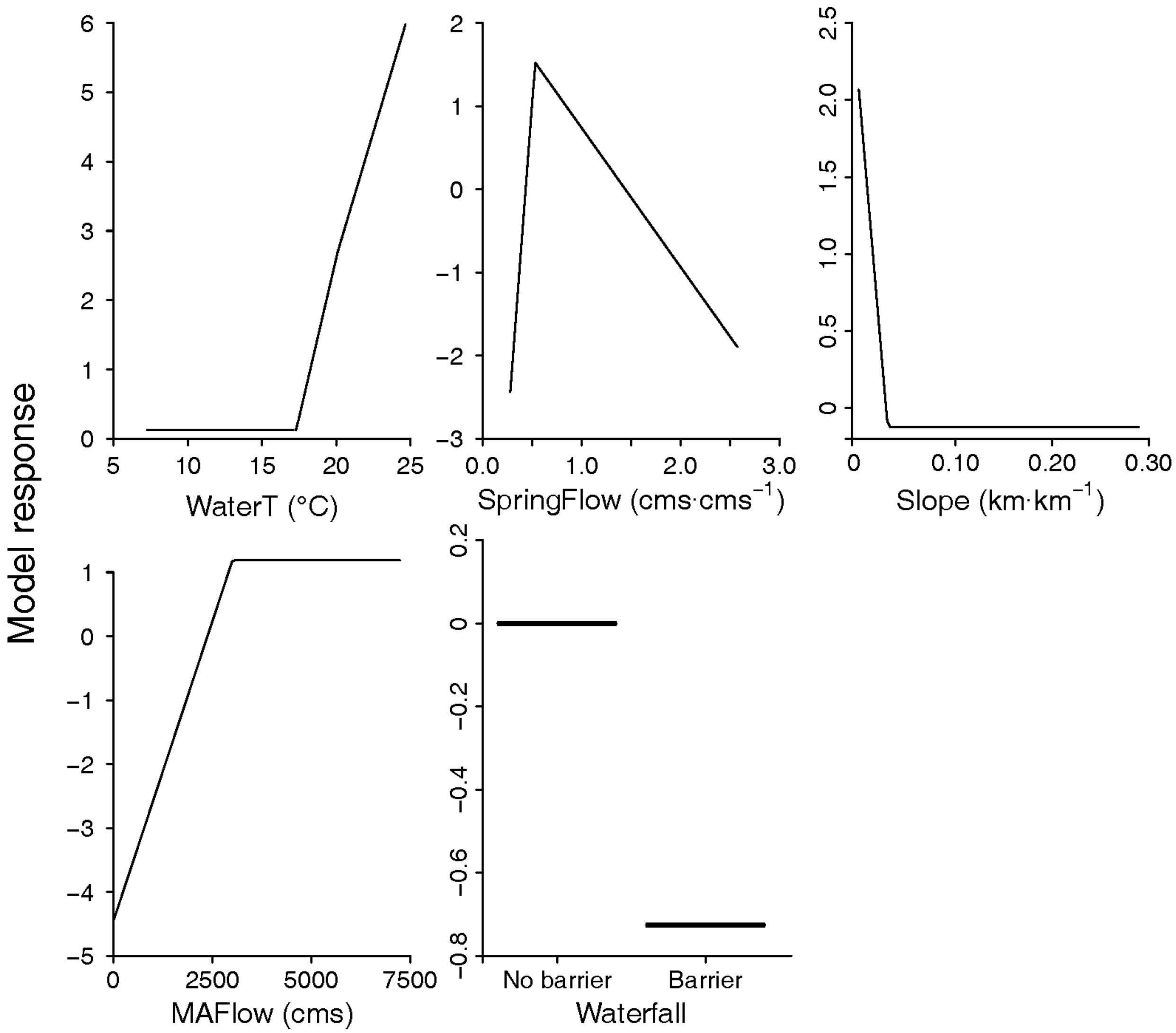

Figure 4.3. Response curves for environmental variables included in the species distribution model (only those with significant contributions to the model are shown). The x-axes represent the range of predicted values for each environmental variable. The y-axes represent each variable's standardized effect on the model response.

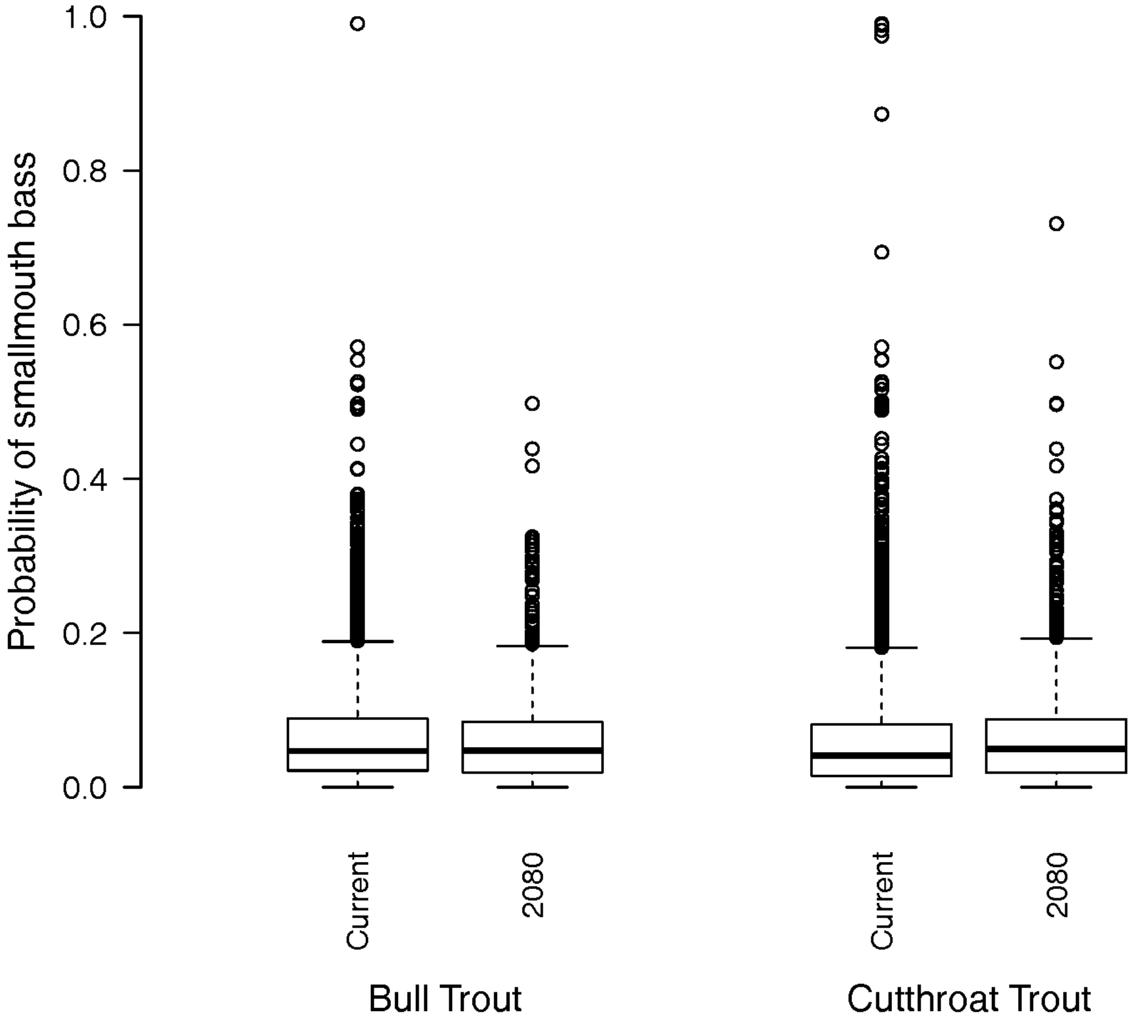

Figure 4.4. The probability of smallmouth bass occurrences in designated climate refugia for bull trout and cutthroat trout under current (1981-2016) and future (2080) climate scenarios. The lines at the top, middle and bottom of each box represent the 75th percentile, median and 25th percentile of the values, respectively, vertical bars (whiskers) represent 95% confidence interval, and points represent all observations outside the confidence interval.

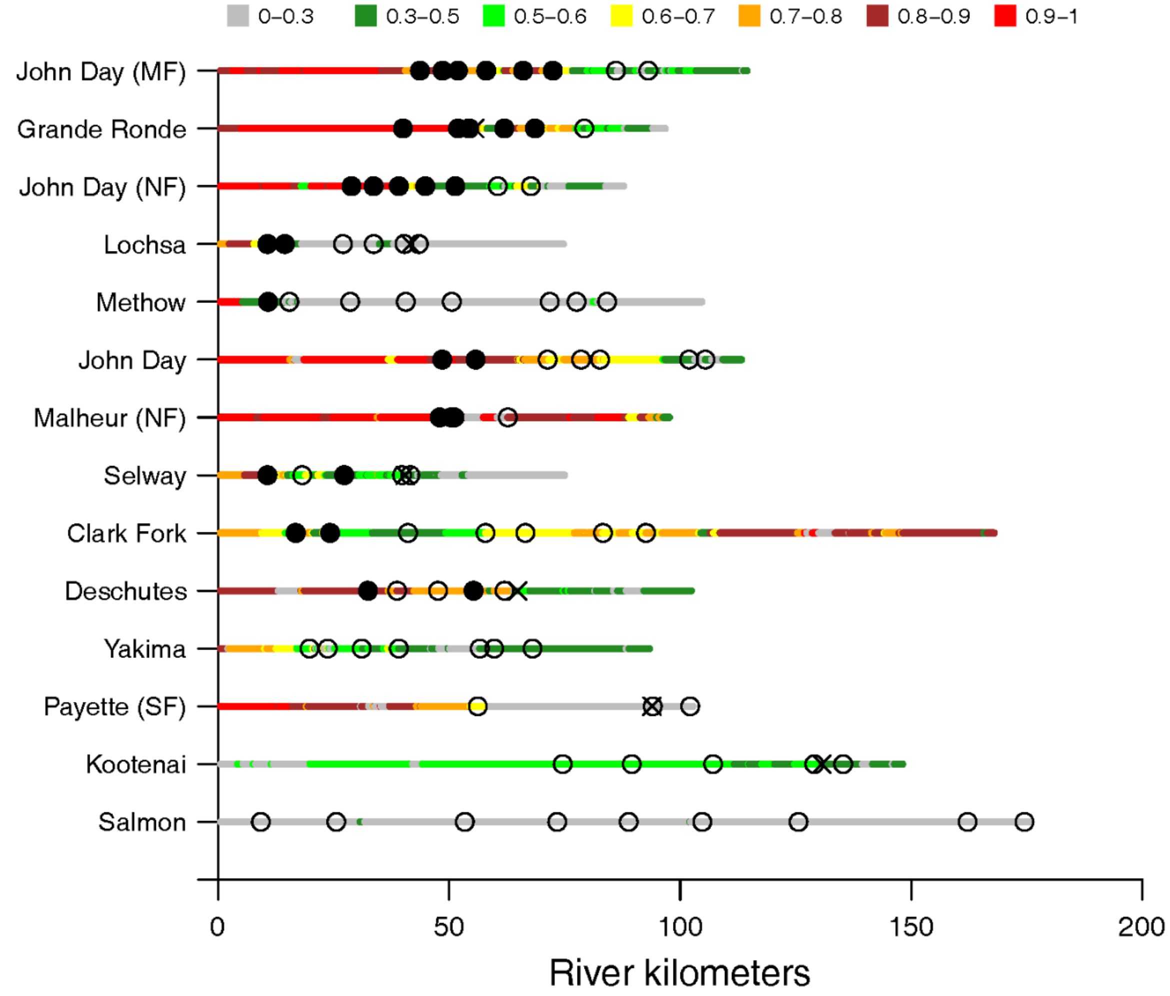

Figure 4.5. The predicted probability of smallmouth bass occurrence (color gradient) in 14 major tributaries of the Columbia River Basin, as well as observed presence (closed circles) and absence (open circles) locations according to eDNA sampling. The x-axis is a measure of relative distance, where 0 indicates the most downstream and 200 the most upstream portions of the sampled rivers. Known waterfalls or cascades thought to be barriers to upstream dispersal are indicated with an ‘X’. SF, MF and NF refer to South, Middle and North Fork, respectively.

4L. Chapter 4 Supplemental Material

Table 4.S1

Publications from which smallmouth bass (*Micropterus dolomieu*) presence data were extracted.

Publication	Location
Rieman et al. 1991	John Day Reservoir, McNary Dam
Zimmerman 1999	The Dalles Dam, McNary Dam
Zimmerman and Ward 1999	Bonneville Dam, John Day Reservoir, Little Goose Dam, Lower Granite Dam, Hell’s Canyon Dam

References:

Rieman, B.E., Beamesderfer, R.C., Vigg, S., and Poe, T.P. 1991. Estimated loss of juvenile salmonids to predation by northern squawfish, walleyes, and smallmouth bass in John Day reservoir, Columbia River. T. Am. Fish. Soc. **120**(4): 448-458. doi: 10.1577/1548-8659(1991)120<0448:ELOJST>2.3.CO;2.

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Table 4.S2

Correlation between predictor variables.

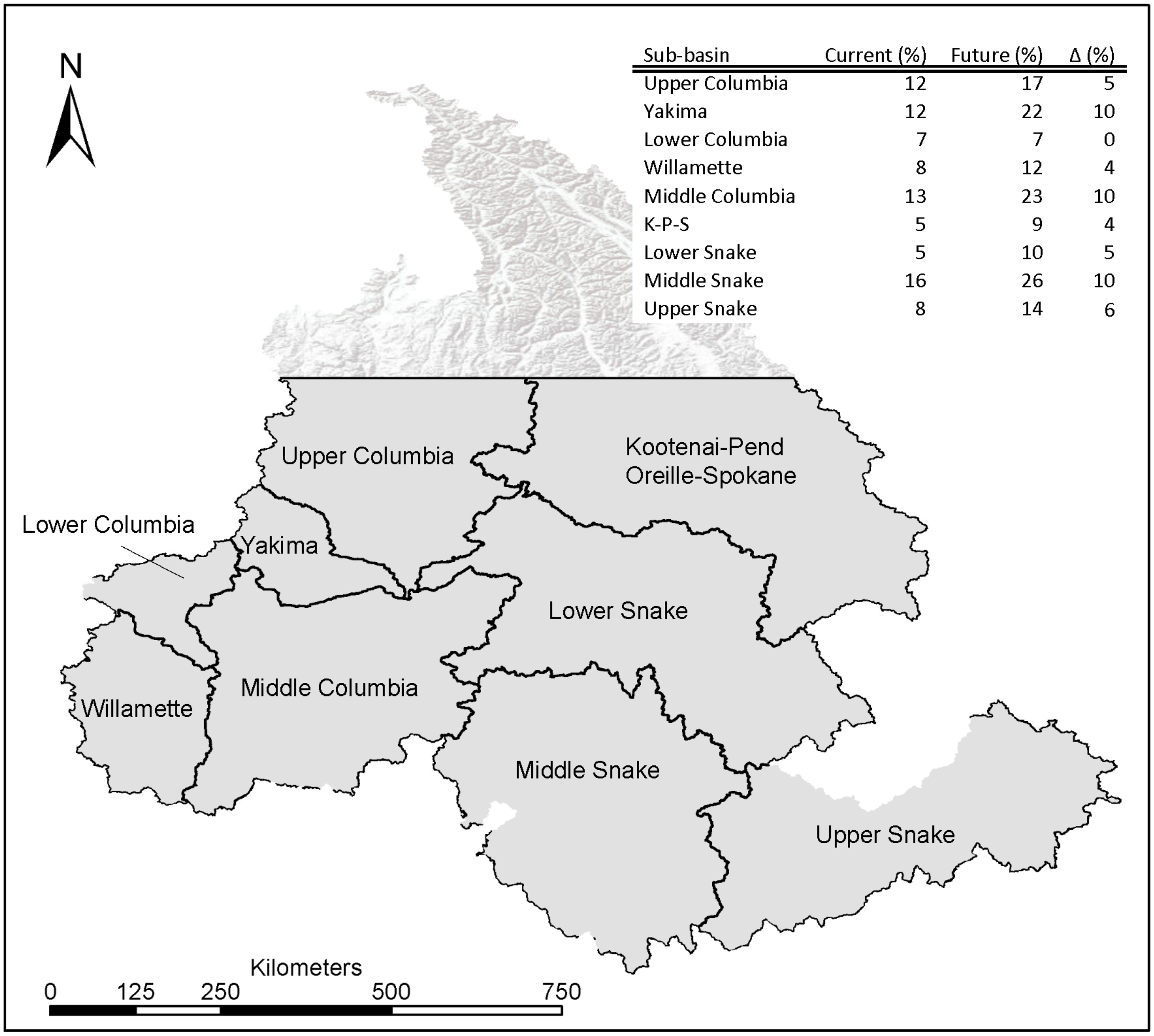
Variable name	WaterT	WinterAirT	SpringFlow	MAFlow	Slope
WaterT					
WinterAirT	0.53				
SpringFlow	-0.16	-0.59			
MAFlow	0.26	0.25	0.12		
Slope	-0.54	-0.24	0.06	-0.11	
DamDensity	0.04	0.11	-0.11	-0.01	0.06

Table 4.S3

The mean, minimum, and maximum values for the predictor variables at all locations where smallmouth bass are present vs. absent.

		Presence	Absence
Variable name	Unit	Mean (Min, Max)	Mean (Min, Max)
WaterT	°C	19.4 (9.6, 24.7)	15.1 (7.3, 21.4)
WaterT2080	°C	22.0 (11.6, 27.6)	17.4 (9.4, 24.2)
WinterAirT	°C	2.5 (-2.6, 7.3)	0.7 (-7.0, 6.9)
WinterAirT2080	°C	5.9 (0.7, 10.1)	4.0 (-3.3, 9.6)
SpringFlow	$cms \cdot cms^{-1}$	0.9 (0.3, 1.9)	1.0 (0.3, 2.6)
SpringFlow2080	$cms \cdot cms^{-1}$	0.6 (0.3, 1.3)	0.6 (0.2, 2.1)
MAFlow	cms	425.6 (7.0×10^{-4}, 7 234)	43.1 (0.02, 627.8)
MAFlow2080	cms	467.7 (7.7×10^{-4}, 7 877)	47.0 (0.02, 692.0)
Slope	$km \cdot km^{-1}$	0.004 (1.0×10^{-5}, 0.2)	0.02 (1.0×10^{-5}, 0.3)
DamDensity	$dams \cdot km^{-2}$	0.003 (0, 0.1)	0.003 (0, 0.1)
Waterfall	NA	NA	NA

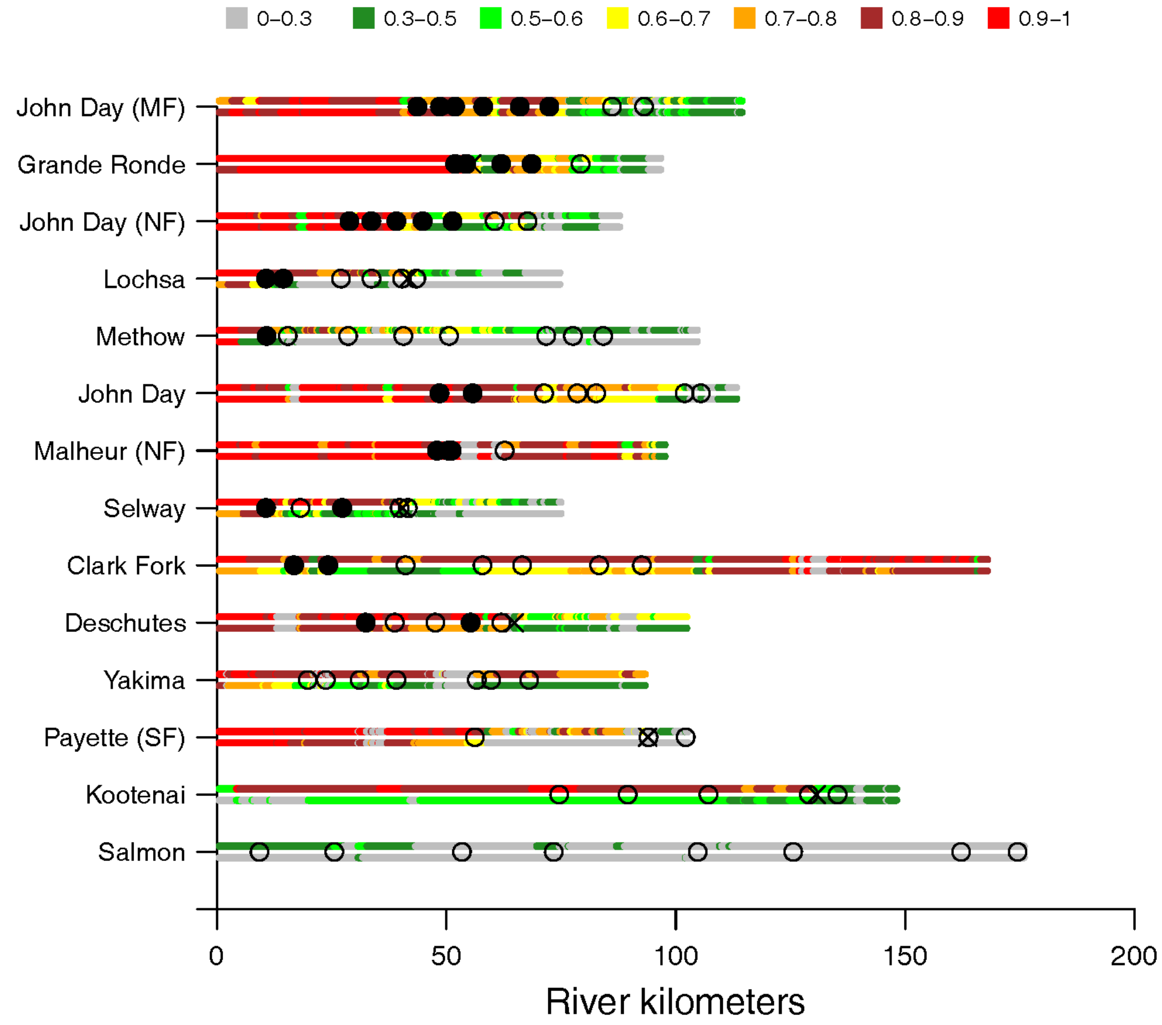
Figure 4.S1



Sub-basin	Current (%)	Future (%)	Δ (%)
Upper Columbia	12	17	5
Yakima	12	22	10
Lower Columbia	7	7	0
Willamette	8	12	4
Middle Columbia	13	23	10
K-P-S	5	9	4
Lower Snake	5	10	5
Middle Snake	16	26	10
Upper Snake	8	14	6

The nine HUC 4 sub-basins of the Columbia River Basin, the associated percent of total riverine habitat in each sub-basin predicted to be occupied by smallmouth bass under current and future (i.e., 2080) climate conditions using the combined historical and eDNA distribution data, and the percent change in predicted occupancy from current to future conditions (Δ).

Figure 4.S2



Paired comparisons between the predicted probability of smallmouth bass occurrence (color gradient) from the initial (top segments) and final (bottom segments) models in 14 major tributaries of the Columbia River Basin, as well as observed presence (closed circles) and absence (open circles) locations according to eDNA sampling. The x-axis is a measure of relative distance, where 0 indicates the most downstream and 200 the most upstream portions of the sampled rivers. Known waterfalls or cascades thought to be barriers to upstream dispersal are indicated with an ‘X’. SF, MF and NF refer to South, Middle and North Fork, respectively.

Chapter 5: Comparing predation and competition threats to rearing juvenile Chinook salmon from native and nonnative predatory fishes

Authors: E.S. Rubenson, D.J. Lawrence, and J.D. Olden

5A. Abstract

The introduction of nonnative predators can dramatically alter food web interactions, potentially threatening the persistence of native fishes. In parts of the North Fork John Day River, nonnative smallmouth bass (*Micropterus dolomieu*) and native northern pikeminnow (*Ptychocheilus oregonensis*) are sympatric with rearing juvenile spring Chinook salmon (*Oncorhynchus tshawytscha*), presenting potential predation and competition threats to juvenile Chinook salmon that are largely unstudied. We examined the trophic ecology and diet requirements of these species using stable isotopes (δ13C and δ15N) and fatty acids. Stable isotope and fatty acid analyses were largely concordant, supporting our overall results. We found that smallmouth bass and northern pikeminnow occupy different niches, increasing overall predator diversity. In addition, we found a disproportionate reliance on autochthonous resources across the entire community and low isotopic uniqueness, suggesting the potential for competition between fish species given resource scarcity. However, we found little evidence of a strong direct predation or competition threat from either predator to juvenile Chinook salmon. The integration of both stable isotope and fatty acid analyses allowed us to better assess the trophic impacts of nonnative predators invading critical salmon rearing habitat in headwater streams.

5B. Keywords

Smallmouth bass, stable isotopes, fatty acids, northern pikeminnow, Chinook salmon

5C. Introduction

A long and rich history exists for the global practice of intentionally introducing nonnative fishes to water bodies, often resulting in dramatically altered species assemblages (Copp et al. 2005). These new assemblages often form novel food web structures, and when the introduced species are high trophic level predators, the impacts on native species can be particularly pronounced (Baxter et al. 2004, Eby et al. 2006). If successfully established in the ecosystem, introduced predators either increase predator species richness or replace native predators (Eby et al. 2006). The implications of these introductions can change predation or competition pressures to native species, which if severe enough, can result in extirpation (Baxter et al. 2004, Eby et al. 2006, Cucherousset and Olden 2011). Juvenile life-history stages of introduced predators can also present a competition threat to small-bodied fishes if habitat and diet requirements overlap (Pilger et al. 2010), potentially further impacting the viability of native populations.

Knowledge of diet requirements and trophic position are critical to understanding both predatory and competitive interactions that arise from the introduction of nonnative species. These data can be obtained by analyzing species' elemental and biochemical compositions, classically done through use of stable isotope (SI) analyses. SI analyses provide a measurement of the relationship between consumers and resources in a community, revealing aspects of trophic structure and basal resources (Layman et al. 2007, Cucherousset and Villéger 2015). This is typically obtained by analyzing carbon and nitrogen ratios, where carbon reveals information about species' predominant energy pathways that support growth of the consumer (i.e., diet) and nitrogen reveals species' trophic levels (i.e., how predatory a species is) (Post 2002). Together, carbon and nitrogen define a species' trophic position. The small number of available biomarkers (typically carbon and nitrogen, and less often, sulfur and hydrogen), however, can leave diets largely unresolved or underdetermined, identifying prey guilds rather than individual prey species. More recently, the addition of fatty acid (FA) analyses have presented new opportunities to analyze trophic structure and diet composition (Iverson et al. 2004, Brett et al. 2016). FAs reveal information about consumer diets because they are both deposited into consumer tissue with relatively little modification and primary producers often have distinctive FA profiles (Brett et al. 2006, O'Donovan et al. 2018). Both SI and FA biomarkers provide insights into what consumers have eaten over ecologically relevant time scales (i.e., weeks to months, depending on biomarker, body size, and metabolism) (Post 2002, Brett et al. 2006), but rely on the availability and accuracy of coefficients that account for trophic modification of dietary tracers (i.e., fractionation rates for SIs, calibration coefficients for FAs) (Iverson et al. 2004, Caut et al. 2009). The longer established use of SIs, however, has provided several generic fractionation rates generated from meta-analyses (Post 2002, Caut et al. 2009, Blanke et al. 2017), whereas the same has yet to be determined for FA calibration coefficients. Developing calibration coefficients generally requires laboratory-based experiments, feeding specific diets to consumers and determining the differential metabolism of individual FAs. Because of difficulties associated with these laboratory experiments, few animals currently have an established set of FA calibration coefficients (Bromaghin et al. 2017). Despite these challenges, examining both SI and FA can help resolve potential competition and predation dynamics amongst consumers of interest.

In the Columbia River Basin (CRB), native salmonids are ecologically, culturally, and socioeconomically important species. Habitat alteration, climate change, overfishing, hatcheries, and nonnative species proliferation, however, have contributed to dramatic declines in salmon abundance, prompting massive recovery efforts costing the region upwards of $300 million annually (Naiman et al. 2012, Rieman et al. 2015, Hand et al. 2018). The current emphasis on habitat restoration, however, has yet to result in significant improvements to salmonid populations. As such, attention has pivoted towards food web approaches (Hand et al. 2018), to include the effects of nonnative species (Naiman et al. 2012). For instance, research shows that introduced predators such as smallmouth bass (*Micropterus dolomieu*), walleye (*Sander vitreus*), and northern pike (*Esox lucius*) have increased predation pressure on juvenile salmonids, especially during their outmigration (Rieman et al. 1991, Vigg et al. 1991, McMahon and Bennett 1996, Zimmerman 1999, Walrath et al. 2015, Erhardt et al. 2018). In addition, studies have shown that the native predator, northern pikeminnow (*Ptychocheilus oregonensis*) is an efficient predator of juvenile salmonids, particularly in the presence of other nonnative predators (Rieman et al. 1991, Tabor et al. 1993, Zimmerman and Ward 1999). This has led to a massive campaign to reduce the number of northern pikeminnow using a sportfish bounty program (Ward and Zimmerman 1999). The vast majority of these studies examines direct predation threats to salmonids and occurs in mainstem or reservoir habitats where the threat to juvenile salmonids is predominantly isolated to outmigration events. Little is known, however, about how native and nonnative piscivores that are sympatric with river-rearing juvenile salmonids might be compromising salmon recovery goals, requiring a food web perspective.

Northern pikeminnow and smallmouth bass are high trophic-level piscivores that have broad distributions throughout the CRB (Wydoski and Whitney 2003, Rubenson and Olden in review). Historically, northern pikeminnow was one of only a few native predators in the Columbia River (Poe et al. 1994). Before the prolific introductions of other piscivores into the CRB, northern pikeminnow predominantly consumed insects, crayfish, and sculpin, with only a small portion of its diet consisting of juvenile salmonids despite seasonal increases of salmonid proportions during outmigration events (Poe et al. 1994). By contrast, smallmouth bass is a popular nonnative sport fish first introduced into the CRB in the 1920s (Carey et al. 2011). Since that time, smallmouth bass has been introduced throughout the region and is still stocked in some headwater reservoirs. Although implicated as a top predator of outmigrating salmon in mainstem or reservoir habitats (Naughton et al. 2004, Fritts and Pearsons 2006, Erhardt et al. 2018), little is known about its potential predation or competition threat to salmonids that spend one year or more rearing in headwater habitats (Lawrence et al. 2012, Rubenson and Olden in review).

The primary objective of this study was to examine potential threats of predation and competition by northern pikeminnow and nonnative smallmouth bass to rearing juvenile spring Chinook salmon (*Oncorhynchus tshawytscha*) in a headwater stream. Our objectives were 1) to compare the trophic positions and diets of adult smallmouth bass and northern pikeminnow to explore potential predation threats to juvenile Chinook salmon and 2) to examine the potential competition effects of juvenile smallmouth bass and juvenile northern pikeminnow on juvenile spring Chinook salmon (*Oncorhynchus tshawytscha*). All three species co-occur at the upstream distribution edge of smallmouth bass in the North Fork John Day River, Oregon.

5D. Methods

Samples were collected at the upstream edge of smallmouth bass distribution in the North Fork John Day River from July 12-16, 2010 (n = 135) with a small number of additional prey samples collected on June 26, 2013 (n = 16) (Fig. 5.1). The North Fork John Day River originates in the Blue Mountains of northeastern Oregon and supports one of the last remaining wild-only spring Chinook salmon populations in the United States. Smallmouth bass was initially introduced in the mainstem John Day River approximately 42 km downstream of the confluence of the mainstem and North Fork John Day River (Shrader and Gray 1999). Since that time, its distribution has spread upstream into the North Fork John Day River, with occurrences observed as far upstream as river kilometer 110, for a total upstream expansion of 152 km (Lawrence et al. 2012, Rubenson and Olden 2016). Juvenile spring Chinook salmon rear in-stream for one year and are vulnerable to both the competitive (juvenile) and predator (adult) effects of resident species during this time. Historically juvenile spring Chinook salmon used much of the North Fork John Day River (river kilometers 0 – 168) during the early summer (May – July) (Lindsay et al. 1986). Recent surveys show that smallmouth bass and juvenile Chinook salmon now share habitat between river kilometers 47 and 110 during this period, with the majority of juvenile Chinook salmon occurring farther upstream (Lawrence et al. 2012, Rubenson and Olden 2017). Northern pikeminnow is also present in this portion of the North Fork John Day River year-round (Torgersen et al. 2006).

In an effort characterize predator diets during maximum period of overlap with rearing juvenile Chinook salmon, we sampled during the early summer and across river kilometers 47-101 (Lawrence et al. 2012). Samples were collected to represent a diverse set of potential prey items, including aquatic and terrestrial invertebrates, small-bodied native fishes, and our species of interest: juvenile Chinook salmon, juvenile and adult smallmouth bass, and juvenile and adult northern pikeminnow. Overall, 151 samples were processed, representing 20 species of fish, aquatic invertebrates, and terrestrial invertebrates (Table S5.1).

Adult smallmouth bass (total length [TL]: 168 – 275 mm) and northern pikeminnow (TL: 207 – 415 mm) were collected using hook and line sampling. Smaller-bodied smallmouth bass and northern pikeminnow were captured using a beach seine (see below). Fish were measured, weighed, and muscle tissue was harvested below the anterior dorsal fin for SI and FA analysis using a 5 mm disposable biopsy punch (Ackerson et al. 2014). Biopsied tissues were transferred to microcentrifuge tubes and placed on ice until they could be frozen. In the lab, tissue samples were freeze-dried for 24 hours in preparation for SI and FA analysis. Fish were returned to the water and released upon recovery.

Potential prey items, including juvenile smallmouth bass, juvenile northern pikeminnow, juvenile Chinook salmon, other small-bodied fish, and crayfish were collected using a beach seine. Macroinvertebrates were either hand collected or obtained by kick-sampling the stream bottom with a 500 µm mesh D-net. Samples were placed on ice until they could be frozen. All prey samples were freeze-dried for 24 hours and then ground into a powder. For analysis, aquatic invertebrates were grouped by functional feeding groups based upon the life-history stage during which they were captured (e.g., larval versus adult). Functional feeding groups were collector-filterer, grazer, collector-gatherer, and predatory invertebrate.

Stable isotopes (SI)

Samples were measured for δ13C and δ15N isotopes by the UC Davis Stable Isotope Facility using a PDZ Europa ANCA-GSL elemental analyzer interfaced to a PDZ Europa 20-20 isotope ratio mass spectrometer. Isotope composition of samples is reported with delta values (δ) in per mil (‰), expressed relative to international standards Vienna PeeDee Belemnite for carbon and atmospheric air for nitrogen.

The isotopic structure of the food web was examined to determine the diversity of basal resources and potential for competitive or predatory interactions between smallmouth bass, northern pikeminnow, and juvenile Chinook salmon. Isotopic diversity was visualized using isotopic biplots and quantified by calculating metrics for isotopic richness and dispersion (Cucherousset and Villéger 2015). These metrics are scaled between 0 and 1 to allow for mathematical independence from each other and to account for differences in sample size. Isotopic richness (IRic) is comparable to convex hull area (Layman et al. 2007), and represents the amount of isotopic space filled by a group of organisms. Isotopic dispersion (IDis) provides a measure of the average degree of trophic diversity in the community, trending towards 0 when all groups of species have similar stable isotope values. Competition potential was estimated by calculating redundancy in isotopic space, represented by an isotopic uniqueness metric (IUni) (Cucherousset and Villéger 2015). Here, values close to 0 indicate that every organism has at least one other organism with the same position in isotopic space, suggesting that paired species likely have similar diets. Potential species interactions were further examined by calculating the overlap in isotopic space, using the metrics isotopic similarity (ISim) and isotopic nestedness (INes) developed by Cucherousset and Villéger (2015). When sample sizes of individuals of each species-group were unequal, individuals were bootstrapped, and the mean and standard deviation of the isotopic metrics were calculated. Overlap was calculated between adult smallmouth bass and adult northern pikeminnow to examine the relationship of the top two predators in the ecosystem. Overlap was also evaluated between juvenile Chinook salmon and juvenile northern pikeminnow and juvenile smallmouth bass to examine potential competition.

Diet preferences were estimated for the species of interest using the Bayesian mixing model MixSIAR (Stock and Semmens 2016). Bayesian mixing models estimate the relative contribution (i.e., proportion) of different prey resources in the diet of a specific consumer based upon the conditional probability of different proportions given the consumer isotopic signature. Because we only had two isotopic tracers, we could only determine a solution of up to three prey resources. As such, all prey species were grouped into one of three categories: prey fish, aquatic invertebrate, or terrestrial invertebrate. Prey fish included juvenile northern pikeminnow, juvenile smallmouth bass, juvenile Chinook salmon, juvenile sucker species (*Catostomus sp.*), sculpin species (*Cottus sp.*), redside shiner (*Richardsonius balteatus*), longnose dace (*Rhinichthys cataractae*), and speckled dace (*Rhinichthys osculus*). We used the average trophic discrimination values of δ13C = 0.4‰ +/- 1.3 and δ15N = 3.4‰ +/- 1.0 from Post (2002), which estimate the change in δ13C and δ15N from prey to predator. Finally, we included a generalist (i.e., uniform) prior for each species. Results of the mixing models were used to compare the likelihood of piscivory in adult smallmouth bass and northern pikeminnow. We also compared diets among juvenile smallmouth bass, northern pikeminnow, and Chinook salmon to assess the potential for competition of resources between these species of interest.

Fatty Acids (FA)

FA samples were processed following Brett et al. 2009. Briefly, FAs were extracted using chloroform/methanol, followed by methylation and subsequent extraction using hexane/diethyl ether. The samples were then evaporated to dryness and re-suspended in gas chromatograph grade hexane. Analysis of FAs was carried out in the University of Washington Lake Lab on a HP 6890 gas chromatograph with a J&W Scientific DB-23 column (30 m x 0.25 mm, 0.15 µm film thickness), a flame ionization detector, and helium was used as the carrier gas. One µL of sample was injected. The following 85-minute temperature program was used: 50ºC held for five minutes, ramp at 10ºC per minute to 100ºC, ramp at 2ºC per minute to 140ºC, ramp at 1ºC per minute to 180ºC, hold for five minutes, ramp at 2ºC per minute to 200ºC and finally ramp at 10ºC per minute to 240ºC. Peaks were identified based on a 37 Supelco standard from Sigma Aldrich and from past data gathered on the instrument and verified on GC-MS.

Each FA was measured as the proportion of the total FA composition of each sample. Non-dietary and extended-dietary FAs were removed from the dataset (as determined by Iverson et al. 2004 and Budge et al. 2012). In addition, only FAs that represented > 0.5% of the total FA compositions for at least one species were retained. The remaining FAs were renormalized to 100%. The FA data were arcsine transformed before statistical analysis (Budge et al. 2012). Individual outliers were assessed using a multivariate outlier test using a Bray-Curtis distance matrix and examining all individuals that were > three standard deviations from the mean. This resulted in two outliers being removed before statistical analyses were conducted.

FA profiles were compared among species to determine the amount of potential diet similarity. FA profiles were first visualized using principal coordinate analysis (PCoA) using a Bray-Curtis distance matrix. Permutational analysis of variance (PERMANOVA) and multivariate homogeneity of group variances were then calculated between each target species-pair to assess the amount of overlap and variance between each pair's FA profiles. All analyses were completed in R version 3.5 (R Foundation for Statistical Computing, Vienna).

5E. Results

We found a relative homogeneity of basal resources, suggesting the potential for broad community impacts from nonnative smallmouth bass. The community had an isotopic richness (IRic) score of 0.562. This indicates a moderate degree of trophic diversity influenced by a few species-groups at the extremes (i.e., northern pikeminnow, terrestrial invertebrates, and aquatic collector-gatherers) (Fig. 5.2). The carbon-isotopes showed some isotopic diversity, ranging from -14.60‰ to -31.23‰, but the majority of the community fell within the narrower carbon-isotope range of -18‰ to -24‰ (Fig. 5.2), indicating a likelihood that the food web predominantly depends upon aquatic basal resources. In addition, we found an isotopic uniqueness (IUni) value of 0.268, which indicates that most species share isotopic space, and therefore diet, with at least one other consumer. Juvenile smallmouth bass, in particular, appears to share isotopic space with other small-bodied fishes, with the exceptions of juvenile northern pikeminnow and juvenile Chinook salmon (Fig. 5.2). This suggests a high potential for direct competition with the native community. By contrast, adult smallmouth bass were more isolated in isotopic space. Overall, the combination of a homogeneous resource base and low isotopic uniqueness suggests the potential for competition between species, with particularly high overlap between juvenile smallmouth bass and other small-bodied native fishes.

We found little evidence for substantial direct predation from either adult northern pikeminnow or adult smallmouth bass on juvenile Chinook salmon, although they did appear as the top trophic predators in the community. The community nitrogen-isotope values ranged from -0.48‰ to 9.77‰, with adult northern pikeminnow and adult smallmouth bass defining the upper values (Fig. 5.2). The isotopic dispersion (IDis) value of 0.426, however, shows that isotopic values among groups are relatively well distributed. This indicates that distinct apex predators and primary consumers do not dominate the community, but instead there is a diversity of species distributed relatively evenly in nitrogen-isotope space. Juvenile Chinook salmon, in particular, had relatively enriched nitrogen-isotope values, especially when compared to other small-bodied prey fishes and adult northern pikeminnow and adult smallmouth bass (Fig. 5.2). This both contributes to a lower isotopic dispersion score, but also suggests that juvenile Chinook salmon do not constitute large portions of the adult species' diets. Specifically, assuming an approximate δN15 fractionation rate of 3.4‰ +/- 1.0, the relative positioning of juvenile Chinook salmon (δN15 = 6.7‰ +/- 0.3) to adult northern pikeminnow (δN15 = 7.6‰

+/- 1.0) and adult smallmouth bass (δN15 = 6.4‰ +/- 0.5) is high for juvenile Chinook salmon to be a substantial prey-item for these predators. This was in contrast to the other small-bodied fish species, which had nitrogen-isotopes closer to the expected profile of prey to northern pikeminnow or smallmouth bass.

Although community-wide competition from smallmouth bass appears likely, there was little evidence to support substantial direct competition between our target species. We found low isotopic niche overlap between adult northern pikeminnow and adult smallmouth bass (ISim = 0.051 +/- 0.005; INes = 0.232 +/- 0.026 [Fig. 5.3a]), and no or low overlap between the prey species-pairs examined (ISim = 0.005; INes = 0.013 for juvenile smallmouth bass-juvenile northern pikeminnow [Fig. 5.3b]; ISim = 0; INes = 0 for juvenile northern pikeminnow-juvenile Chinook salmon [Fig. 5.3c] and juvenile smallmouth bass-juvenile Chinook salmon [Fig. 5.3d]). Adult smallmouth exhibited lower nitrogen-isotope signatures relative to adult northern pikeminnow, suggesting that smallmouth bass relies more on aquatic invertebrates than prey fishes relative to northern pikeminnow. The more enriched nitrogen-isotope signatures of juvenile Chinook salmon differentiate it from other prey fish species (Fig. 5.2). This positioning of juvenile Chinook salmon in isotopic space suggests some level of piscivory and/or a higher reliance on terrestrial invertebrates relative to smallmouth bass.

Bayesian mixing models supported the inferences gleaned from the isotopic plots and metrics. Adult northern pikeminnow was the most piscivorous of the fishes (Fig. 5.4a), whereas adult smallmouth bass appeared to rely more heavily on a mixture of aquatic invertebrates and prey fishes (Fig. 5.4b). Juvenile Chinook salmon (Fig. 5.4e) appears to be slightly differentiated from juvenile northern pikeminnow (Fig. 5.4c) and juvenile smallmouth bass (Fig. 5.4d) by a higher reliance on terrestrial invertebrates.

The FA profile of the functional feeding groups mimicked the results of the stable isotope analysis (Fig. 5.5a,b). In total, 30 total FA were detected among the consumers, and 11 dietary FAs were retained for the analysis (Table 5.1). These dietary FAs were predominantly comprised of highly unsaturated FAs and polyunsaturated FAs. The PCoA ordination illustrates the difference in FA profiles among the groups (Fig. 5.5a). The FAs contributing the most to the first principal component (PC1; 43% variation explained) were docosahexaenoic acid (DHA; 22:6n3), docosapentaenoic acid (DPA; 22:5n3), and alpha-linolenic acid (ALA; 18:3n3), which generally differentiated fishes (higher DHA and DPA; 22:6n3 and 22:5n3) from invertebrates (higher ALA; 18:3n3) (Fig. 5.5a). Similar to the stable isotope analysis, adult northern pikeminnow and smallmouth bass appear at the top of the food web, with more enriched levels of DHA and DPA than prey fishes. Smallmouth bass had a larger variance in FA space, potentially indicating a more diversified diet (Fig. 5.5b, Table 5.2). This is also true of juvenile Chinook salmon, which also had relatively large variance compared to other species (Fig. 5.5b, Table 5.2). The FAs contributing the most to the second principal component (PC2; 15% variation explained) were eicosapentainoic acid (EPA; 20:5n3) and linoleic acid (LIN; 18:2n6). These appear to differentiate more benthic consumers (higher EPA; 20:5n3) from more terrestrial consumers (higher LIN; 18:2n6) (Fig. 5.5a). Here, juvenile Chinook salmon is more enriched with LIN compared to other (more benthic) species, such as dace and sculpin that were more enriched with EPA. Juvenile smallmouth bass trend towards a more benthic profile, plotting relatively high on PC2 of the PCoA ordination, whereas adult smallmouth bass exhibit a diverse diet across aquatic and terrestrial resources. The relatively strong terrestrial resource signal in adult smallmouth bass diet strongly contrasts with the isotopic analysis. Although several of the

FA profiles of our target fish species overlapped (Fig. 5.5b), all FA profiles were significantly different from one another, potentially due to the differences in variances (Table 5.2).

5F. Discussion

We found little evidence that juvenile Chinook salmon is a dominant prey species of either native northern pikeminnow or nonnative smallmouth bass in the headwaters of the North Fork John Day River. Instead, juvenile Chinook salmon exhibited a relatively enriched-nitrogen signal compared to northern pikeminnow and smallmouth bass, indicating either no or low contributions to the predator diets. This is in stark contrast to downstream habitats, where juvenile salmon comprise significant portions of both northern pikeminnow and smallmouth bass diets during outmigration events (Tabor et al. 1993, Poe et al. 1994, Ward and Zimmerman 1999, Naughton et al. 2004, Erhardt et al. 2018). By contrast, the remainder of the prey-fishes fell within the expected nitrogen-signature of prey species to both predators. Although there is not an overwhelming isotopic signal that juvenile Chinook is being consumed by either predator in great quantities, it is unknown whether the piscivory exhibited is sufficient to impose significant predation mortality on rearing Chinook salmon. Multiple diet mixtures that include juvenile Chinook salmon as prey could produce the predator $\delta N15$ profiles we observed, and predation is likely given habitat overlap, opportunity, and general diet preferences observed in other parts of the CRB. The magnitude of this predation potential, however, can only be estimated via more intensive seasonal diet sampling to identify the prey fish species and sizes that actually contribute to the diet of the predators.

Based upon SI and FA signature overlap analyses, adult smallmouth bass appears to occupy a different dietary niche than adult northern pikeminnow in the headwaters of the North

Fork John Day River. This suggests that the two piscivores are able to successfully coexist, increasing the total predator diversity in critical salmon rearing habitat. Congruent with other studies in mainstem and reservoir habitats (Rieman et al. 1991, Tabor et al. 1993, Ward and Zimmerman 1999), we found that native northern pikeminnow is the dominant piscivore in the North Fork John Day River, showing the most enriched SI nitrogen signature and prey fishes comprising > 50% of its diet. By contrast, smallmouth bass appears to be less piscivorous than northern pikeminnow in this system. Instead, adult smallmouth bass predominantly consumes aquatic invertebrates with a smaller proportion of prey fishes (i.e., 10-40% of its diet) relative to northern pikeminnow. This is supported by previous diet studies conducted on smallmouth bass in our study area, which showed that fish made up approximately 28-46% of stomach contents by wet weight (Lawrence et al. 2015). The successful integration of a nonnative predator can have important indirect effects on the growth and survival of juvenile Chinook salmon. For instance, it has been shown that juvenile Chinook salmon suffer higher stress and reduced growth in the presence of smallmouth bass (Kuehne et al. 2012). Furthermore, smallmouth bass has been shown to alter the diets of other predators (Jackson 2002). Poe et al. (1994) hypothesize that northern pikeminnow in the lower Columbia and Snake Rivers may have shifted to its diet to include a higher proportion of juvenile salmonids in response to competition with nonnative predators (including smallmouth bass) for similar prey. It would be informative to examine the diets of northern pikeminnow and growth of juvenile Chinook salmon in habitats with and without smallmouth bass to examine indirect effects that smallmouth bass may present on rearing juvenile Chinook salmon, be that through reduced growth from increased stress, increased predation by northern pikeminnow, or other mechanisms.

The isotopic signatures and FA profiles of the community suggest that autochthonous resources drive the food web (i.e., primarily aquatic as opposed to terrestrial). Here, we found a relatively small difference in the carbon-signatures of fish and aquatic invertebrates, but a large difference in the terrestrial invertebrate carbon-signature. The carbon-depleted terrestrial invertebrate signature increases the isotopic richness of the community, but the positioning of the prey fishes in carbon-isotopic space (i.e., between -18‰ and -24‰ δC13) as well as the results from the Bayesian mixing models suggest that aquatic resources dominate fish diets. FA profiles support that there is homogeneity in basal resources, with most fish species demonstrating similar FA compositions whereas terrestrial resources are distinctly isolated.

The homogeneity of basal resources and the distribution of consumers in stable isotope and FA space suggest a high potential for competitive interactions at a community level. Low isotopic dispersion and uniqueness scores also indicate that many consumers share similar resources, supported by several species with overlapping FA profiles. Based upon SI analyses alone, however, we found little to no direct isotopic overlap (suggesting different diets) between juvenile smallmouth bass, juvenile northern pikeminnow, and juvenile Chinook salmon. This differentiation could be the result of two contrasting mechanisms. First, each of these species may indeed target different resources. Here, resource partitioning or microhabitat differences between the species may protect juvenile Chinook salmon from potential competition with juvenile smallmouth bass. By contrast, the presence of smallmouth bass could also be displacing juvenile Chinook salmon from its preferred diets or altering the basal resource community. This would result in non-overlapping diets, but may present a potential growth and survival cost to juvenile Chinook salmon. It is important to note that we do not have data on the community in the absence of smallmouth bass, so cannot conclusively determine between these mechanisms.

Historically, however, introduced smallmouth bass are renowned for dominating resource consumption, causing significant shifts to prey availability and resource use once established (Power et al. 1985, Vander Zanden et al. 1999, Jackson 2002). In this region, high densities of both adult and juvenile smallmouth bass can be found in shared reaches with juvenile Chinook salmon (Rubenson and Olden, in press), the impact of which is unknown. In the mainstem John Day River, native fish abundances are low where smallmouth bass has an established year-round presence (Hughes and Herlihy 2012). In other rivers, smallmouth bass was directly implicated in the loss of native species diversity (Weyl et al. 2014). Our data demonstrate that smallmouth bass has integrated into the heart of the food web, able to capitalize on aquatic invertebrate resources that are shared among multiple consumers. Although some diet differentiation may provide respite for species such as juvenile Chinook salmon, more research is needed to conclusively determine the competitive effects of smallmouth bass in headwater streams and how food webs may change in its presence

Juvenile Chinook salmon have a surprisingly high nitrogen-isotope signature relative to other small-bodied fish species in the North Fork John Day River. A couple mechanisms may provide insight into this relative enrichment. First, our Bayesian mixing model results and the position of juvenile Chinook salmon in FA space (i.e., in closer proximity to terrestrial resources relative to other fishes) suggest that juvenile Chinook salmon take advantage of terrestrial invertebrate prey more often than other potential competitors. The large variance in juvenile Chinook salmon’s FA profile also suggests diet diversification relative to other fish species. Other studies have shown that juvenile Chinook salmon consume both aquatic and terrestrial invertebrate prey in streams, with a preference for terrestrial invertebrates depending on the season and conditions (Rondorf et al. 1990, Johnson 2007, Macneale et al. 2010). Terrestrial inputs are largely dependent on the composition and extent of the riparian zone. In the North Fork John Day River, the native riparian vegetation is largely intact. In the neighboring Middle Fork John Day River, however, riparian vegetation has been removed in many regions to support grazing and other development (Lawrence et al. 2014). Given the importance of terrestrial inputs to juvenile Chinook salmon in the North Fork John Day River, it would be interesting to examine whether the SI and FA profiles shift in the Middle Fork John Day River where terrestrial inputs may be depleted and smallmouth bass is present. Second, juvenile Chinook salmon may be exhibiting some level of piscivory. We did not collect potential prey resources, such as egg and fry life history stages, that may present a nitrogen-enriched diet to this size-class of juvenile Chinook salmon. Moderate levels of predation on eggs and fry could reasonably produce elevated nitrogen-isotope levels compared to other species.

Although we did not have FA calibration coefficients for our species, the relative positioning of species in both FA and SI space were comparable. Here, our FAs depict trophic relationships that match SI analyses. Specifically, adult northern pikeminnow and smallmouth bass were set apart from the remainder of the community, the benthic-feeding prey species (i.e., longnose dace, speckled dace, sculpin species, and juvenile smallmouth bass) were grouped, and the prey species that typically feed in the water column (i.e., redside shiner, juvenile Chinook salmon, and juvenile northern pikeminnow) were grouped. These multiple lines of evidence provide a clearer window into the food web of a headwater stream community integrated with nonnative smallmouth bass.

Although the results of the SI and FA analyses were predominantly consistent, there were some important differences highlighting some areas of uncertainty. First, the amount of overlap between some species-pairs, or lack thereof, differed between analyses. In general, SI analyses suggest a high level of shared resources, whereas the significant differences between the examined species-pairs of FA profiles suggest resource partitioning. This is particularly true for juvenile smallmouth bass, which shares isotopic space with the remainder of the prey fish in the community, whereas FA analyses depict juvenile smallmouth bass relatively isolated from other species. Or, by contrast, SI analyses suggest no resource overlap between juvenile northern pikeminnow and juvenile Chinook salmon, whereas there appears to be some overlap (albeit not significant) in FA space. We attribute inconsistencies between SI and FA analyses to not knowing the metabolic processes of our species, such that we cannot account for how lipids are processed and retained (i.e., no availability of FA calibration coefficients). Each of these species likely metabolizes FAs differently, and accounting for these differences may change where species fall relative to one another in FA multivariate space. As such, we offer our FA data and analyses as preliminary, purposefully making general statements about the amount of shared resources or specific diets of consumers based on FA alone. Should FA calibration coefficients for our consumers become available, these ambiguities would be better resolved, and thus help elucidate the effects of smallmouth bass on juvenile Chinook salmon and other native fishes. We urge the continued development of calibration coefficients and modeling efforts that can derive coefficients in the absence of diet studies.

5G. Conclusion

Very little is known about the impacts of resident smallmouth bass in headwater streams where juvenile salmonids rear in-stream for extended periods of time. Given that smallmouth bass has historically been an impactful predator and competitor, resolving its influence on these communities is of utmost concern to managers tasked with conserving and preserving native salmonids in the Pacific Northwest. Our preliminary look into these competition and predation dynamics revealed that smallmouth bass is not presenting a clear and overwhelming threat to the native juvenile spring Chinook salmon population. Even small amounts of increased predation mortality, however, may be sufficient to regulate the Chinook salmon population depending on the abundance and consumption rate of the predators relative to the biomass of the Chinook population. Our data present clear evidence that smallmouth bass has added a predation threat to this region which exhibits a disproportionate reliance on autochthonous resources, warranting a deeper look into its direct and indirect impacts on sympatric salmonids in headwater streams.

5H. Acknowledgements

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5J. Tables

Table 5. Mean (standard deviation) percentage of each dietary fatty acid of target for adult northern pikeminnow (PTOR), adult smallmouth bass (MIDO), juvenile northern pikeminnow (jPTOR), juvenile smallmouth bass (jMIDO), and juvenile Chinook salmon (jONTS).

	PTOR	**MIDO**	**jPTOR**	**jMIDO**	**jONTS**
18.2n6	5.5 (1.9)	8.6 (1.8)	17.1 (8.1)	12.5 (4.4)	23.3 (9.4)
18.3n3	4.2 (1.4)	7.3 (4.3)	11.8 (3.8)	13.9 (6.4)	22.0 (8.1)
18.3n6	Trace	0.1 (0.4)	Trace	0.6 (0.4)	0.7 (2.6)
20.1n9	1.2 (0.5)	0.5 (0.6)	1.9 (1.6)	0.8 (0.6)	0.6 (1.0)
20.2n6	1.4 (0.3)	0.3 (0.3)	2.0 (0.6)	0.6 (0.3)	Trace
20.3n3	1.5 (0.3)	0.9 (0.9)	1.9 (0.3)	1.2 (0.6)	0.2 (0.5)
20.4n3	1.5 (1.1)	1.2 (0.9)	1.4 (0.6)	1.8 (0.5)	2.1 (2.3)
20.4n6	14.3 (3.0)	13.0 (2.6)	9.7 (1.5)	8.2 (2.7)	4.1 (1.3)
20.5n3	20.8 (2.5)	16.3 (3.5)	19.0 (4.5)	16.2 (4.0)	16.4 (3.0)
22.5n3	7.2 (0.7)	11.1 (1.6)	5.1 (1.3)	9.6 (1.1)	4.2 (2.3)
22.6n3	42.4 (5.5)	40.7 (6.7)	30.0 (8.4)	34.6 (9.3)	26.3 (11.5)

Table 5.2. Pairwise comparisons represented by F-values with statistical significance (p-value < 0.05) indicted by an asterisk (*) between our target species: adult northern pikeminnow (PTOR), adult smallmouth bass (MIDO), juvenile northern pikeminnow (jPTOR), juvenile smallmouth bass (jMIDO), and juvenile Chinook salmon (jONTS). Below the diagonal are the PERMANOVA results. Above the diagonal is a comparison of variances between species. Statistical significance indicates that species pairs are statistically different from one another.

	PTOR	MIDO	jPTOR	jMIDO	jONTS
PTOR		10.5*	0.02	1.4	22.4*
MIDO	24.3*		3.9	13.4*	8.7*
jPTOR	50.2*	36.2*		0.8	5.9*
jMIDO	63.1*	30.7*	17.3*		9.2*
jONTS	77.5*	42.5*	7.2*	5.2*	

5K. Figures

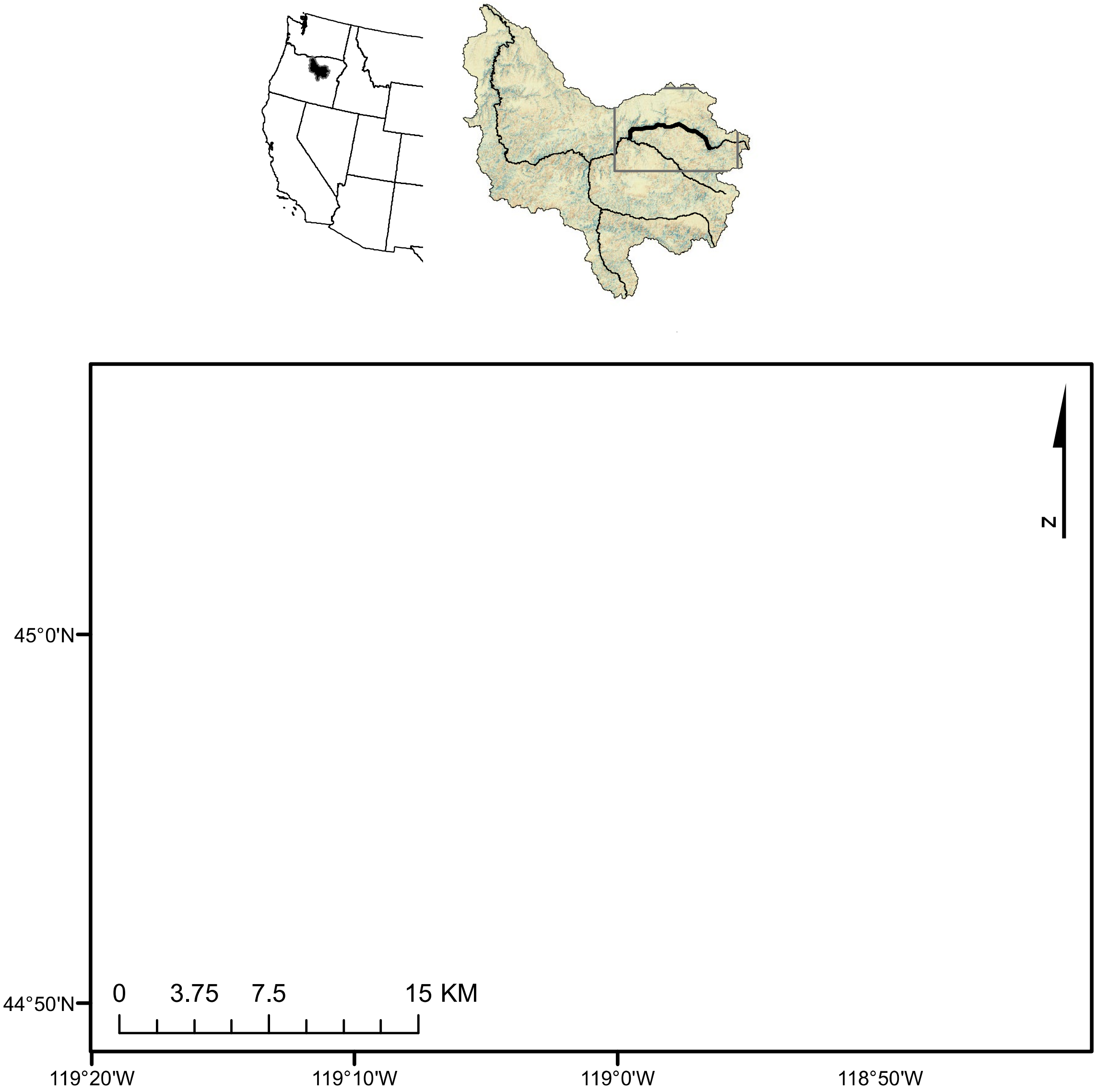

Figure 5.1. Map of the North Fork John Day River (lower) and its relative position Oregon (upper). The study section is delineated by river kilometer (RKM) demarcations.

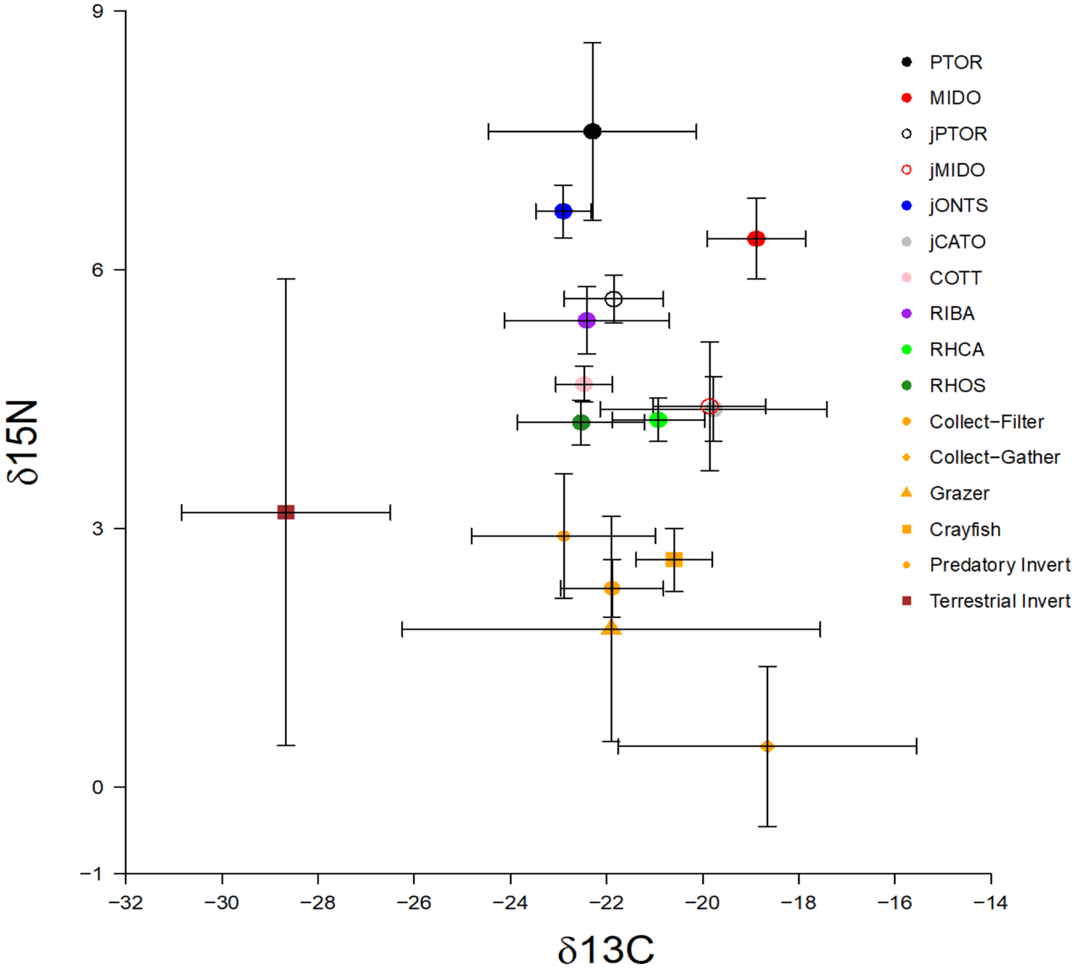

Figure 5.2. The relative position of each species group collected in stable isotope space. Stable isotopes are represented by mean (points) and standard deviations (arrows). Fish species include adult and juvenile northern pikeminnow (PTOR, jPTOR), adult and juvenile smallmouth bass (MIDO, jMIDO), juvenile Chinook salmon (jONTS), juvenile sucker species (jCATO), sculpin species (COTT), redside shiner (RIBA), longnose dace (RHCA), and speckled dace (RHOS). Aquatic insects were grouped by functional feeding groups, to include collector-filterers (Collect-Filter), collector-gatherers (Collect-Gather), grazers, crayfish, and predatory invertebrates.

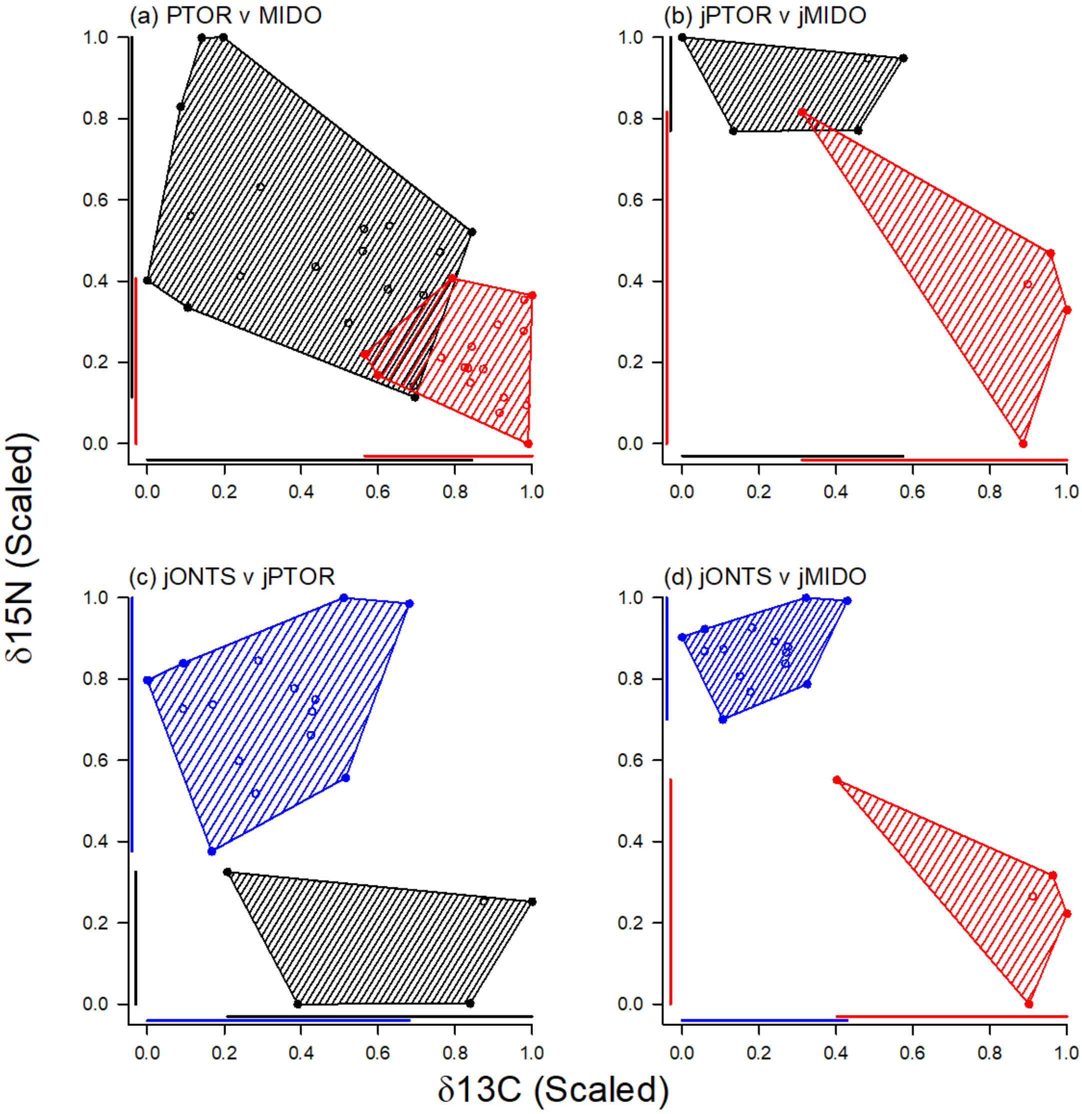

Figure 5.3. Isotopic overlap between a) adult northern pikeminnow (black, PTOR) and adult smallmouth bass (red, MIDO), b) juvenile northern pikeminnow (black, jPTOR) and juvenile smallmouth bass (red, jMIDO), c) juvenile Chinook salmon (blue, jONTS) and juvenile northern pikeminnow (black, jPTOR), and d) juvenile Chinook salmon (blue, jONTS) and juvenile smallmouth bass (red, jMIDO).

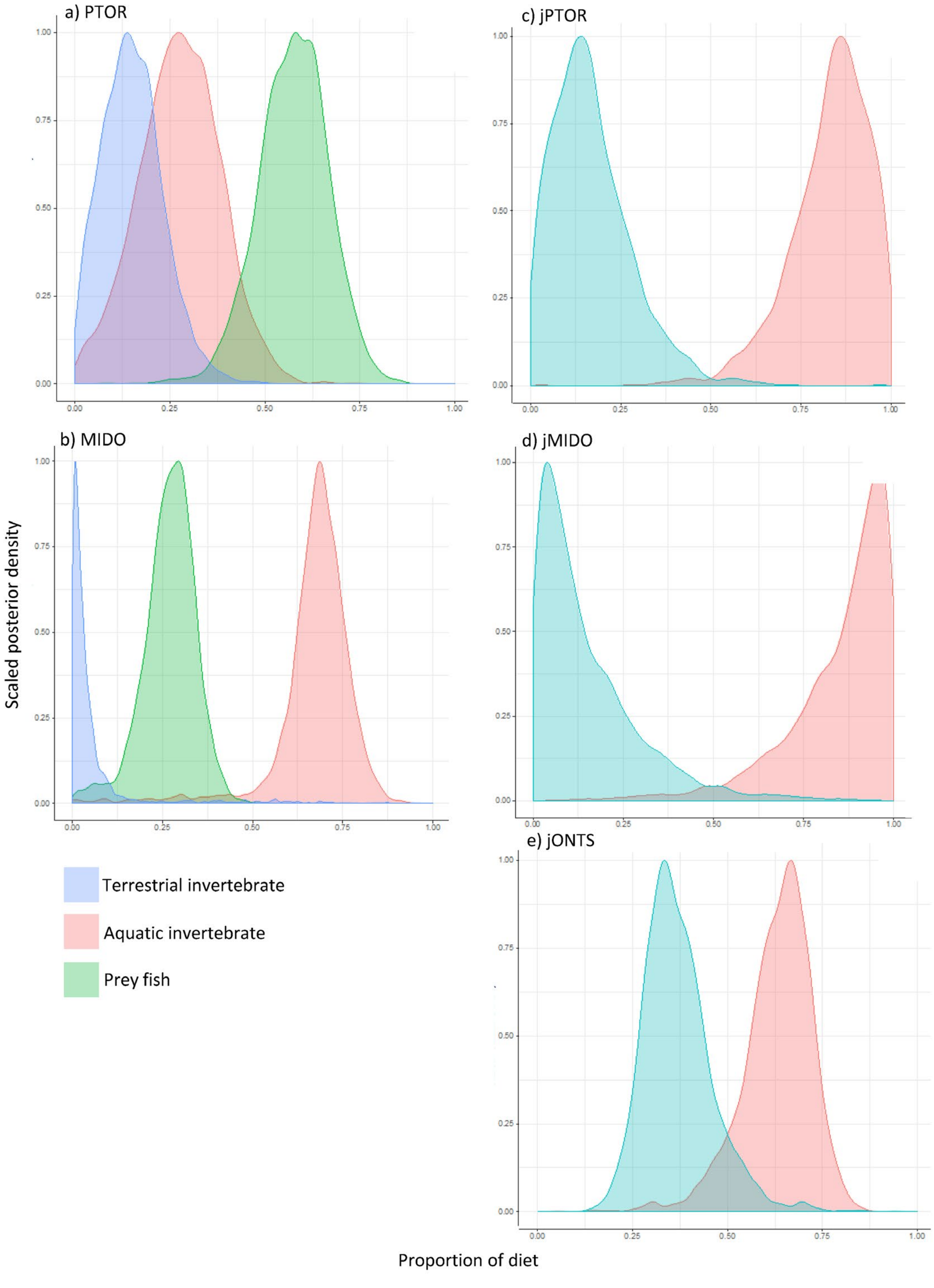

Figure 5.4. Results of diet compositions (colors) produced from Bayesian mixing models for a) adult northern pikeminnow (PTOR), b) adult smallmouth bass (MIDO), c) juvenile northern pikeminnow (jPTOR), d) juvenile smallmouth bass (jMIDO), and e) juvenile Chinook salmon (jONTS).

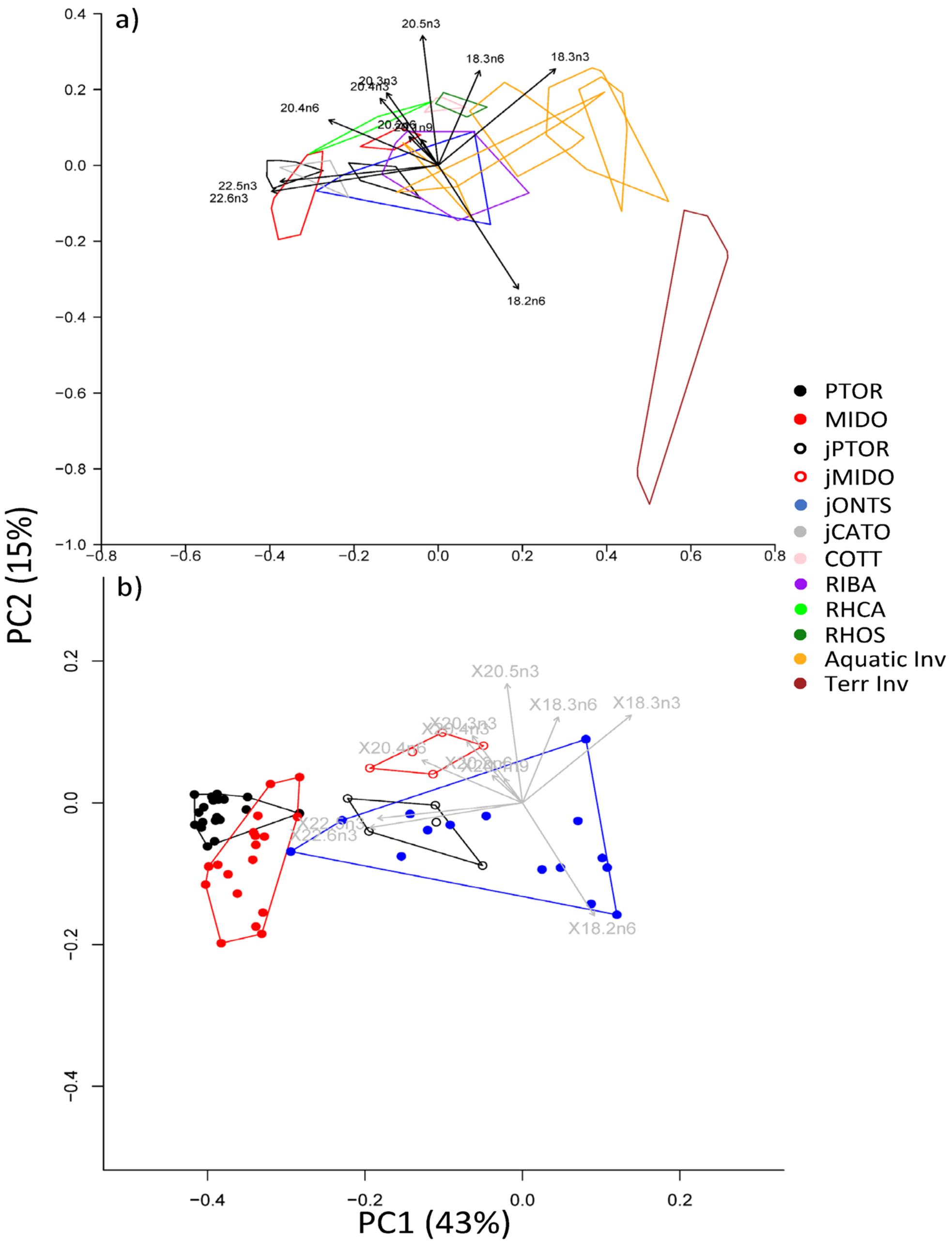

Figure 5.5. The relative position of each species or group collected in fatty acid space as represented by the first 2 principal components of a PCoA ordination for the a) entire community and b) the target species. Convex hulls represent the entire collection of individuals colored by species or group, whereas points represent individual samples (target species only). Species and groups are adult northern pikeminnow (PTOR), adult smallmouth bass (MIDO), juvenile northern pikeminnow (jPTOR), juvenile smallmouth bass (jMIDO), juvenile Chinook salmon (jONTS), juvenile sucker species (jCATO), sculpin species (COTT), redside shiner (RIBA), longnose dace (RHCA), speckled dace (RHOS), aquatic invertebrates, and terrestrial invertebrates.

5L. Chapter 5 Supplemental Information

Table S5.1. A list of collected samples, sample sizes of each species, assigned functional feeding group (FFG; collector gatherer [ColGath], predatory insect [PredInsect], grazer, collector filterer [ColFilt], terrestrial invertebrate [TerrInvert], or abbreviation of fish species), classification in Bayesian mixing model (aquatic invertebrate [AqInv], terrestrial invertebrate [TerrInv], prey fish [PreyFish]), and life history stage of collected species.

Species	Sample Size	FFG	Mixing Model	Life History
Baetidae nymph	1	ColGath	AqInv	nymph
Chironomidae larvae	1	ColGath	AqInv	larvae
Chironomidae pupa	1	ColGath	AqInv	pupa
Coenagrionidae, Coenagrion or Enallagma	1	PredInsect	AqInv	nymph
Ephemerellidae	1	ColGath	AqInv	nymph
Gastropoda	4	Grazer	AqInv	adult
Gomphidae	3	PredInsect	AqInv	nymph
Gomphidae, Ophiogomphus	1	PredInsect	AqInv	nymph
Gonidea angulata, Anodonta, Margaritifera falcata	3	ColFilt	AqInv	adult
Hemiptera	1	TerrInvert	TerrInv	adult
Heptageniidae, Epeorus	1	ColGath	AqInv	nymph
Hydropsychidae, Hydropsyche	2	ColFilt	AqInv	nymph
Hymenoptera, Formicidae	1	TerrInvert	TerrInv	adult
Juvenile sucker species	4	CATO	PreyFish	juvenile
Lepidoptera winged species	3	TerrInvert	TerrInv	adult
Mayfly species	3	ColGath	AqInv	nymph
Micropterus dolomieu	19	MIDO	MIDO	adult
Micropterus dolomieu	5	MIDO_JUV	PreyFish	juvenile
Odonata (unidentified)	3	PredInsect	AqInv	nymph
Odonata (winged adult)	1	PredInsect	TerrInv	adult
Oncorhynchus tshawytscha	15	ONTS	PreyFish	juvenile
Orthoptera species	3	TerrInvert	TerrInv	adult
Pacifastacus leniusculus	10	PALE	AqInv	adult
Perlidae, Claassenia	3	PredInsect	AqInv	nymph
Perlidae, Hesperoperla	1	PredInsect	AqInv	nymph
Plecoptera (unidentified)	1	PredInsect	AqInv	nymph
Pteronarcyidae, Pteronarcys	3	PredInsect	AqInv	nymph
Ptychocheilus oregonensis	20	PTOR	PTOR	adult
Ptychocheilus oregonensis	5	PTOR_JUV	PreyFish	juvenile
Rhinichthys cataractae	6	RHCA	PreyFish	adult
Rhinichthys osculus	4	RHOS	PreyFish	adult
Richardsonius balteatus	15	RIBA	PreyFish	adult
Sculpin species	6	COTT	PreyFish	adult