Multiple invading species and ecological consequences for salmonids in the John Day River, Oregon

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Abstract

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Freshwater ecosystems increasingly face multiple, interacting stressors that threaten the persistence of biodiversity and challenge existing conservation strategies. Among these threats, biological invasions and climate change are becoming more prevalent and can have cumulative effects on imperiled populations of Pacific salmon and steelhead. However, understanding of how invasive species interact with one another and potential responses to climate-driven warming remains limited. My thesis research investigated the ecological impacts of two invasive species, smallmouth bass (*Micropterus dolomieu*) and rusty crayfish (*Faxonius rusticus*), within the John Day River Basin, Oregon, USA, with emphasis on species interactions, dynamic ecological processes, and implications for juvenile steelhead (*Oncorhynchus mykiss*).

In Chapter 1, I developed a spatially explicit individual-based model to compare the interactive effects of smallmouth bass and rusty crayfish under past and future climatic conditions. By comparing single-species and multi-species simulations, I identified emergent patterns in abundance, distribution, and rates of spread across the river network. Smallmouth bass exerted strong top-down control on rusty crayfish populations in reaches where bass were established, reducing crayfish abundance, slowing rates of spread, and limiting range expansion. These findings demonstrate that interactions among invaders can generate disparate estimations that alter invasion trajectories and provide significant value to modeling frameworks.

In Chapter 2, I evaluated the trophic ecology of rusty crayfish along an invasion gradient that spans critical spawning and rearing habitat for a threatened population of steelhead in the South Fork John Day River. Using stable isotope analysis, fatty acid analysis, and assessments of macroinvertebrate community composition, I identified collectively powerful indicators of the ecological impacts of an invasive crayfish on pre-migratory steelhead. Rusty crayfish exhibited substantial variation in trophic position and resource use through the study area, with individuals occupying lower trophic positions upstream and higher trophic positions downstream. Considerable isotopic niche overlap between crayfish and steelhead smolts suggested potential for resource competition during freshwater rearing. Although crayfish density was not associated with reductions in preferred salmonid prey abundance, fatty acid profiles indicated the incorporation of marine-derived nutrients and provided evidence consistent with opportunistic predation on these vulnerable salmonid life stages.

Collectively, this research demonstrates that invasive species can affect freshwater ecosystems through both direct trophic interactions and indirect ecological dynamics mediated by interactions among invaders. The results highlight the importance of accounting for species

interactions when predicting invasion outcomes and suggest that the ecological effects of invasive crayfish may be context dependent across space. As freshwater ecosystems continue to experience warmer temperatures and expanding biological invasions, effective conservation and management of threatened steelhead populations will require an integrated understanding of these complex ecological dynamics.

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DEDICATION

For my friend, Kalena.

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Chapter 1. Predicting climate-driven spread of multiple, interacting species in a river network

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1.1 Abstract

Co-occurring stressors in freshwater ecosystems are becoming increasingly common and threaten the effectiveness of current management strategies for endangered and threatened populations of Pacific salmon and steelhead. The increasing prevalence and growing range of invasive species, paired with rising global water temperatures, presents a novel challenge to understand the potential cumulative impacts. Though the negative effects of individual stressors are well-researched, our understanding of multi-species invasion dynamics and expectations in response to climate change remains limited. To better understand the environmental and biotic context of multi-species invasions, we explored the population metrics (e.g., abundance, distribution) of nonnative smallmouth bass (*Micropterus dolomieu*) and rusty crayfish (*Faxonius rusticus*) in the John Day River, OR, USA. We used individual-based models to represent temperature and density dependencies on a river-reach scale. By comparing single and multi-species scenarios, we identified emergent patterns through disparities in abundance, distribution, and rate of spread. Bass populations exerted strong top-down control of crayfish populations in reaches where bass were well-established, which resulted in reduced rates of spread and lessened range expansion for crayfish. Finally, we identified distinct spatial and temporal patterns of non-additive interactions occurring between the two invaders. Our results affirm the importance of accounting for ecological interactions when modeling population dynamics of invasive species in dendritic systems. As temperatures and invasion rates continue to rise, effective management strategies for threatened and endangered species will rely on a deeper understanding of these dynamics to avoid unexpected outcomes. These results define a case of multispecies interactions which mediate the range expansion of invasive species, particularly as it holds implications for Pacific salmon and steelhead populations in coming decades.

1.2 Keywords

Aquatic ecology, non-native species, crayfish, steelhead trout, John Day River, Oregon, individual-based models

1.3 Introduction

Global growth in the number of nonnative species (Seebens et al., 2017) has underscored the far-ranging ecological (Mack et al., 2000) and economic (Diagne et al., 2021) impacts that threaten biodiversity. Accumulation rates are expected to increase in the coming decades, leading to an unprecedented presence of nonnative species worldwide (Seebens et al., 2021). Complex ecological outcomes arising from multi-species invasions are challenging long-standing management paradigms that often involve population-level suppression of a single non-native species. Although prior research has largely focused on individual-level effects, a comprehensive understanding of a co-occurring invasion's impact on community and ecosystem properties requires inclusion of interactive effects (Johnson et al., 2009; Ricciardi et al., 2013). Failure to account for interactions among multiple invasive species, and the environmental factors that moderate such interactions, can spark unintended consequences that lead to equally or more damaging management outcomes (Brandt et al., 2023; Zavaleta et al., 2001). As such, there is an increasing need to explicitly incorporate species interactions in ecological models that seek to predict the spatiotemporal dynamics of multiple invaders at landscape scales into the future.

Ecological theories address the potential for biotic interactions to modulate the demographic success, defined as sustained growth in abundance on spatial and temporal scales, of introduced species – which is the primary determinant of ecological impact (Mitchell et al., 2006; Parker et al., 1999). Within these theoretical frameworks, demographic success is attributed to one or a combination of present factors: enemies, mutualists, competitors, and abiotic conditions. The outcome for an introduced species, either positive or negative, distinguishes each hypothesis. For example, a pairing of mutualism and favorable environmental conditions create a positive feedback mechanism for what is termed an 'invasional meltdown'

(Simberloff & Von Holle, 1999). Some hypotheses include only one factor but differ based on the predicted outcome; the presence of enemies can increase (enemy release) or decrease (new associations) invasion success (Colautti et al., 2004).

Many ecological theories indicate that interactions are important determinants for understanding the success and overall effects of an invasion (Mitchell et al., 2006). Further evidence suggests that dynamics between co-occurring invasive species play a major role in determining the cumulative impacts to an ecosystem (Jackson et al., 2016). Previous work has classified different interaction types according to (i) the outcome of a species-species interaction, (ii) the effect of species A on species B, or (iii) the effect of species B on species A (Kuebbing & Nuñez, 2015). Based on the outcome or effect, these instances can be sorted into positive, negative, or neutral classes. 'Positive' describes a relationship in which one or both species facilitate invasion success, while 'negative' indicates reduced success. The term 'neutral' defines an inconsequential or non-existent effect between invaders, where interaction outcomes negate one another or are undetectable. (Levine & D'Antonio, 1999). Many combinations are possible when conferring these interaction types at a population or community-scale, though a single type may be a more practical approach at the ecosystem-scale. The occurrence rate of each scenario occurs varies across literature, though documentation of negative relationships is surfacing at an alarming rate (Jackson, 2015; Lopez et al., 2022).

Ecosystems are increasingly subject to multiple species invasions (Ricciardi et al., 2021). The presence or absence of a relationship (e.g., predator-prey, resource competition) between invasive species can transform invasion outcomes when multiple invaders are present (Móréh et al., 2024; Rodriguez, 2006). As additional invasive species are introduced to a system, the novel interaction can facilitate (positive) or impede (negative) the invasion success of either the

primary or secondary species. For example, interspecies predation reduced the establishment probability of various salmon species where non-native trout were already present along the Chilean coast (Arismendi et al., 2014). This and other factors, like competitive abilities of co-invaders (Narayanan et al., 2025), can affect the range expansion of another invader. Griffen et al. (2008) found negative interactions between two invasive crab species through respective predatory behavior and response to environmental variables. The introduction of a secondary invader (*Hemigrapsus sanguineus*) resulted in an inverted density gradient of species occupancy, suggesting potential displacement of the previously introduced species (*Carcinus maenas*). Interactions between invasive species have also been shown to alter expected ecosystem-wide impacts, including threats to native species. A global analysis of bullfrogs (*Lithobates spp.*) and red swamp crayfish (*Procambarus clarkii*) revealed reduced consumption of native anuran prey by bullfrogs where high densities of crayfish were present, suggesting that dynamics between the two invaders vary as a function of density (Liu et al., 2018). Biological invasions generate a wide-array of impacts to ecosystems, acutely seen with declining diversity across aquatic communities (Gallardo et al., 2016).

Freshwater ecosystems are becoming increasingly subject to novel threats, from interacting invasive species (Rahel et al., 2008) to rapidly changing climates (Strayer, 2010; Woodward et al., 2010). Mounting evidence highlights the importance of understanding the increasingly intense and unpredictable effects of multiple stressors across freshwater ecosystems (Reid et al., 2019). Rivers, in particular, present a unique set of challenges for anticipating the implications of climate change for the spread of invasive species given the dendritic nature of these systems that shape the speed and direction of expansion (Giometto et al., 2017). As systematic conservation efforts increasingly incorporate adaptive strategies to address climate

change, consideration must be given to how riverscape heterogeneity facilitates species distributions (Hodgson et al., 2009). The natural heterogeneity of riverscapes exerts strong controls on interactions between multiple stressors, consequently altering rates of spread (Jin et al., 2014) and population abundance of invasive species (Haubrock et al., 2023). Spatial gradients produce the most variation in classification (i.e., sign) and strength of interspecific relationships relative to other context-dependent gradients, species interaction class, location, and ecosystem type (Chamberlain et al., 2014). These strong environmental (e.g., temperature) and spatial gradients (e.g., density) are major factors that control high-level outcomes (Pellissier et al., 2018).

Numerous modeling frameworks offer predictive insight into the spread and population growth of invasive species (Dominguez Almela et al., 2022; Thompson et al., 2021). A variety of these mechanistic modeling approaches can functionally represent the spatial and temporal variability of species-level interactions, including direction and magnitude in response to environmental factors. Individual-based models (IBMs), which rely on complex decision rules and represent spatiotemporal variability, are particularly useful to recreate population dynamics and inform strategies for conservation management. These investigations become increasingly difficult in response to climate change because environmental conditions influence biotic interactions (e.g., interaction strength, relative abundance of interacting species) that can manifest in broader patterns of abundance and distribution (Kordas et al., 2011). In cases with complex dynamics and a suite of potential outcomes, the inclusion of stochastic variables improves the accuracy and informative power of predictive models (Hastings & Wysham, 2010). Furthermore, such efforts aim to increase the robustness of models by ensuring they can

effectively anticipate the growing abundance and range of multiple invaders in response to changing environmental regimes.

In the Columbia River basin, habitat alteration, climate change, overfishing, hatcheries, and nonnative species have contributed to dramatic declines in salmon abundance, prompting massive recovery efforts (Rieman et al., 2015). The inability to meet goals for sufficient salmonid recovery (Bilby et al., 2024) has prompted management to shift and focus on food web approaches to address the effects of nonnative species (Hand et al., 2018; Naiman et al., 2012). These challenges are well evident in the John Day River Basin (21,000 km^2), a major tributary of Columbia River, where a notable multi-species invasion is unfolding with concerning consequences for endangered Pacific salmon populations. Here, nonnative smallmouth bass (*Micropterus dolomieu*; hereafter SMB) – a globally invasive species (Abreo et al., 2026) – were intentionally introduced as a sportfish to the middle reaches of the mainstem and spread upstream (Carey et al., 2011), whereas rusty crayfish (*Faxonius rusticus*; hereafter RC) were believed to be incidentally introduced as part of a school classroom release to the upper mainstem and rapidly dispersed downstream (Olden et al., 2009). Meeting the longstanding management challenges in the Columbia River Basin will necessitate approaches that take multiple species interactions into account when developing models to predict the future distribution and effects of invasive species.

SMB and RC overlap in their native ranges and now are widely sympatric across the John Day River Basin. SMB are known as prolific predators of crayfish, where consumption rates are positively correlated with crayfish abundance (Stein, 1977; Wolf et al., 2022) and choice of individuals is solely dependent on gape-width limitation (Schake et al., 2014). Despite adult crayfish providing energetic benefits, this added food source can simultaneously act as a predator

of early life stage SMB. This phenomenon, known as predator-prey reversal, describes biological interactions where a species that is usually considered prey instead acts as predator at particular ages or phase of life (Barkai & McQuaid, 1988). Such relationships can vary in strength as a function of the density of the species involved (Sánchez-Garduño et al., 2014). In this case, high densities of RC may cause recruitment failure within the SMB population by inducing nest abandonment and enabling complete consumption of eggs (Baldridge & Lodge, 2013). The nutrient-dense eggs produced by SMB are an extremely favorable prey item that RC are known to extort, in addition to those from other substrate-spawning fishes (Ellrott et al., 2007; Morse et al., 2013). Anticipated changes (e.g., rising water temperatures, reduced suitable habitat for cold-water species) throughout the John Day River (Ruesch et al., 2012) will influence interactive effects like consumption rates of SMB (Pease & Paukert, 2014) and RC (Mundahl & Benton, 1990), and modify interaction dynamics like predator-prey reversal (Dorn et al., 1999). These shifting environmental conditions similarly determine species distributions, even on fine seasonal scales, which hold direct implications for species native to the river (Lawrence et al., 2012).

Effective management necessitates moving beyond single species approaches to understand the population dynamics that result from a combination of interacting invaders and a changing climate (LaForgia et al., 2020). Concurrent invasions are further complicated by climate-induced changes to stream temperature, particularly in riverine systems. Spatially explicit, individual-based modeling (SEIBM) can facilitate deeper understanding of the influences, direct and indirect, among these factors and how the relationship defines population-level outcomes (Thompson et al., 2021). Here, we integrate autecological knowledge of SMB and RC into an SEIBM to advance a mechanistic integrated assessment of multiple invaders in a

riverine network. Our study aimed to address the following objectives: (1) how does accounting for species interactions influence predicted spatial patterns in the distribution and abundance of SMB and RC over time? and (2) how might climate-induced stream warming affect multiple invader populations in the future? Within these research goals, we sought to retrospectively explore how biotic interactions and changing stream temperatures shape the population-level response of each invader across a dendritic network demonstrating marked heterogeneity in species density and environmental conditions. Our findings have implications for guiding ongoing monitoring of multiple interacting invasive species, and informing any future management strategies that seek to control population spread and ecological impacts in salmon-bearing rivers.

1.4 Materials and Methods

1.4.1 Study system

This study was conducted in the John Day River (JDR), which originates in the Blue Mountains of northeastern Oregon and extends 450 km to the Columbia River (Figure 1.1). Undammed along its entire length, the river is the fourth longest free-flowing river in the contiguous United States. Precipitation predominantly occurs in the form of snow and rain, with snowmelt leading to high flows in the late spring and baseflow conditions extending into the summer period. Air temperatures within the study area range from winter lows of 18˚C to summer highs of 30˚C. In the North and Middle Forks, the headwaters support the last remaining, wild-only Chinook Salmon Evolutionary Significant Units south of the Canadian border. Across the watershed, two populations of invasive species are rapidly expanding and, consequently, raising concern among managers.

In 1971, the Oregon Department of Fish and Wildlife (ODFW) introduced approximately 100 adult SMB to two locations above Tumwater Falls in the lower Mainstem with the goal of establishing a small, recreational fishery (Shrader & Gray, 1999). In the half-century since, SMB expanded their range and now extend into the middle Mainstem, North Fork and Middle Fork. This fishery has been highlighted in recent research due to high predation and competition effects on salmon and Steelhead (Carey et al., 2011; Rubenson et al., 2020). Simultaneously, RC have been rapidly spreading and increasing in abundance across the basin (Messager & Olden, 2018). The ability of invasive RC to reach extremely high densities (Messager & Olden, 2019) and disrupt ecological processes through polytrophic feeding habits (Twardochleb et al., 2013) has incited concern among management agencies. The presence of RC is very likely the result of an intentional release of a classroom organism (used in science curricula) around the turn of the century and in proximity of Mount Vernon, Oregon (Olden et al., 2009). In the approximate 25 years since their presumed introduction, rusty crayfish have rapidly spread throughout the JDR catchment at rates exceeding 15 km/year, raising concerns that the mainstem of the Columbia River may soon be reached (Messager & Olden, 2018). Both SMB and RC populations have and continue to grow their respective ranges into critical spawning and rearing habitat for native Chinook salmon and Steelhead populations (Lawrence et al., 2012).

1.4.2 Modeling approach

We use an individual-based modeling framework, HexSim, to evaluate the dynamics and spread of multiple invasive species across a network (Schumaker & Brookes, 2018). HexSim was originally developed for terrestrial systems, but here we leverage a recently developed 'network version' that facilitates the modeling of river reaches in dendritic landscapes (HexSim 4.0.20 available at http://www.hexsim.net/). HexSim simulates a sequence of life events (e.g.,

reproduction, movement, survival) of each species that vary both across space and time in relation to environmental conditions. We chose this adaptable framework to maximize available data; poorly understood processes can be accommodated through omission, sensitivity analyses, or probabilistic representation. Within the software, we simulated population dynamics of SMB and RC that functionally vary in response to reach-scale attributes (e.g., intra-/interspecific density, habitat). Each iteration, or step, of the event sequence represents a month (Figure 1.2). Throughout the sequence, individuals within a population retain or update accumulator values as specified by or calculated during events. These accumulated values represent the state of an individual and dictate subsequent processes. For example, the outcome of a propagation event is dependent on prior events that record individual (e.g., average fecundity), population (e.g., sexually mature proportion), and network (e.g., temperature range) values. To reduce the model's computational load, we applied a female-only structure to both populations. A super-individual approach was implemented to further manage the volume of age-0 SMB and RC, where 1 individual represents 4. Initial calibration testing confirmed that this ratio (1:4) did not reduce the predictive capabilities of the model.

The HexSim network is composed of 'reaches' that retain static (e.g., slope) and dynamic (e.g., temperature) habitat characteristics. The network structure of the John Day River was built from the National Hydrography Dataset Plus Version 2 (NHDPlusV2), representing 2217 perennial reaches or segments. We selected reach-level attributes, contained in NHDPlusV2, that were applicable to modeled processes including total reach length (km), width (m), channel gradient (%), and Strahler stream order. These environmental attributes were used to generate the HexSim workspace (.grid) and network overlay (.snx) files during initial model construction, then integrated into events (i.e., movement, reproduction, survival) as known influential factors

for both species. For example, we used width to represent density-dependent processes that affect SMB fecundity. We elected to exclude headwater reaches with intermittent hydrology as neither species routinely occupy these habitats. Potential barriers to movement are small and infrequently occur in the system thus were not represented in the model.

Average daily stream temperatures from May 1999 to May 2030 were estimated using a statistical modeling framework developed by Siegel et al. (2023). Representative of mechanistic processes, this Generalized Additive Model framework predicts water temperature of all free-flowing river and stream reaches across Water Region 17. Approximately 20 years (1993–2013) of daily water temperature data were collected, processed, and made available to the public by the USDA Forest Service as part of the NorWeST project. Model fitting centered around antecedent air temperature (*Tl*), defined as "flow-dependent flexible-lag air temperature" and developed by Siegel et al. (2022). Climate data was compiled from the PRISM Climate Group at Oregon State University (e.g., air temperature), National Snow and Ice Data Center (e.g., snowpack), and National Water Model (e.g., flow predictions). Spatial data defining landscape characteristics were sourced from EPA StreamCat (e.g., 30-year mean annual precipitation), NHDPlusV2 (e.g., flow direction), and the National Land Cover Dataset (e.g., canopy cover). Continuous form data (e.g., air temperature, snowpack) were summarized to produce local and regional averages. Effects of climate covariates on air temperature were parameterized with a GAM tensor product smoothers and interactions fit. Spatial landscape covariates were modeled with linear effects. For a complete list of covariates that were used, see Table A.1 (Appendix 1.1).

This modeling framework supported the spatial (reach-scale) and temporal (daily average stream temperature) scales to be used as input for the HexSim model, but the original source

code required modifications to account for a reduced spatial extent and limited data (Version 1, V1). First, we developed Version 2 (V2) by reducing the extent of records included in the response dataset from a subregion (HUC-4) to watershed-level (HUC-6). The watershed of interest (170702) largely comprises reaches that are classified as transitional. Other watersheds from the Middle Columbia subregion (1707), which have proportionally more rain- and snow-dominated reaches, were excluded to reduce potential influence during model fitting and shorten the processing time. Second, we excluded two predictor variables: snowpack (*SWE*) and flow (*Q*). Future climate projections did not contain these variables at the needed spatiotemporal scale at the time of this study. Consequently, both factors (*SWE*, *Q*) were removed to make Version 3 (V3). Model validation was performed following the methods used by Siegel et al. (2023), including root mean squared error and mean absolute error statistics. These results, in addition to temporal cross-validation, demonstrated a similar degree of accuracy and precision to output from V1. Statistics of model fit were calculated across a longer, historical period (1990 – 2020) to evaluate differences between V2 and V3. More details can be found in Appendix 1.1 (Supporting Information 1.10.1.1).

For application across the future period (2025–2030), we used an additive delta approach to estimate average daily air temperatures from projected average monthly temperatures. Past and future datasets containing air temperature were sourced from the PRISM Climate Group (Daly et al., 1994) and ClimateNA (desktop application, Version 7.0), respectively. PRISM files were resampled in ArcGIS Pro (Esri Inc. (2024), Version 3.5) to match the resolution of ClimateNA files prior to downscaling. Each raster file (n = 5693) represented downscaled predictions of average air temperatures for each day in the 15-year period. For additional information, see Appendix 1.1 (Supporting Information 1.10.1.2).

1.4.3 Smallmouth bass life history

To simulate population dynamics of SMB, we varied the structure and parameters of model scenarios that represented a basic form of their life history. Most aspects of SMB life history, within and beyond native ranges, are well-documented in literature as demonstrated by our systematic review that returned over 500 peer-reviewed publications (See Appendix 1.3). All model scenarios began in May 1999, the approximate time that RC were introduced, with the initialization of female individuals (~15,000) that vary randomly by age. Each run randomly places the starting population into reaches throughout the lower and mid-mainstem based on 1990–2000 presence-absence reports from monitoring reports (Appendix 1.10.3.2). Using this range approximation, we extrapolated the starting population from a conservative estimate of age 1+ fish across the recreational fishery area managed by ODFW (Shrader & Gray, 1999). This population estimate was chosen because the survey period (1990–1994) had the closest proximity to our model start date. The SMB population is defined by a 6-year lifespan that comprises young-of-year (age-0), juvenile (age-1 – age 2), and adult (age-3 – age-6) stages. Following initialization, individuals begin monthly iterations of the event sequence during which a portion will move, reproduce, and or die. Each event group (e.g., movement, recruitment, survival) integrates a unique combination of biotic and abiotic variables to represent ecological processes throughout the model's 30-year duration. Monthly survival rates are described with a piecewise function spanning 0˚C and 35˚C, with optimal ranges differing between adults (18-26˚C) and juveniles (25-29˚C), that defines the proportion of SMB that die each month. Major factors that affect population persistence were monitored using segment managers, a separate population that is functionally unique to HexSim and allows users to monitor attributes of the entire network by maintaining one 'manager' per reach. See Appendix 1.2 (Supporting

Information 1.10.2.3) for additional SMB model details and Appendix 1.3 (Supporting Information 1.10.3.1) for literature that informed model parameters.

1.4.4 Rusty crayfish life history

Population dynamics of RC were simulated following the approach used for SMB (See Section 1.4.3). Prior to developing the RC population, we identified a publication containing a systematic literature review on rusty crayfish and other freshwater crayfish species (Messager & Olden, 2018). This comprehensive collection was supplemented with a scoping update, where we searched for and reviewed additional papers published since December 2018 using the described criteria (See Appendix 1.3.3). To maintain comparability between the two populations and model versions (i.e., single-species, multi-species), we replicated the basic life history structure parallel to SMB. The overall structure, which included temperature and density dependencies, was preserved but model parameters were replaced according to species-specific metrics. We used the presumed time and location of introduction from Olden et al. (2009) to initialize a small number of individuals (n = 10) into the two reaches of the upper mainstem that abut a municipal park in Mount Vernon, Oregon. Individual RC follow a 4-year life span that includes juvenile (age-0) to adult (age-1 to age-3) stage classes. Each month, a proportion of randomly selected individuals move and a proportion of individuals, dependent on age and reach temperature, die. Though many factors affecting mortality rates exist, we represented the influence of temperature on survival by using constant rates within an optimal range (17–21˚C) and reduced rates following a linear relationship (-5% maximum) outside this optimum. Recruitment occurs each year using a 12-step trigger. Each May, new propagules were introduced to the network by a fixed, age-dependent proportion of adults whose mean fecundity varied according to age and intraspecific density. To manage the computational intensity of the model, age-0 individuals were represented

by super-individuals in a 1:4 ratio. Common among individual-based models, this strategy reduced the high volume of propagules following recruitment and the overall duration of each model run. See Appendix 1.2 (Supporting Information 1.10.2.2) for additional details on the RC model structure and Appendix 1.3 (Supporting Information 1.10.3.3) for literature that informed model parameters.

1.4.5 Species interactions

To predict population-level outcomes across the river network and a multi-decade time span, we simulated invader interactions using well-informed areas of impacts. There is limited information regarding interactions between bass and crayfish. A literature review guided how the interactive effects of multiple invaders and climate change were represented within the model's architecture (See Appendix 1.4). Sub models within interaction event groups were informed by existing models to minimize complexity and represent select processes with the fewest uncertainties. The strength of interactions was determined by dynamic variables (e.g., temperature, density) where each reach and step produced a distinct combination. Final versions of the single-species models were combined to create the initial multi-species model by alternating analogous event groups. See Section 1.4.6 for details regarding model validation for separate scenarios. Due to the complexity of ecological interactions and limitations of individual-based models, direct effects were limited to cross-predation where crayfish consume bass and bass consume crayfish. Simulated predation was integrated into the event sequence following movement and recruitment, but prior to survival, to maximize our ability to detect emergent patterns.

The first interaction event group simulates RC predating upon SMB, hereafter referred to as RC-SMB predation. Evidence indicates that SMB are highly susceptible to predation from RC prior to reaching the post-larval stage, where nest abandonment rates and guarding behavior

increase alongside crayfish density (Baldridge & Lodge 2013). Among the many factors affecting recruitment, high densities of RC have been shown to directly impact SMB survival rates in this early stage of life. Here, we define the period during which SMB are vulnerable to predation as the first two months of life (i.e., age-0 individuals, within month-0 to month-1). The exponential model developed by Baldridge & Lodge (2013) provides a logistic regression (r^2 = 0.70) estimating proportion of nests abandoned as a function of crayfish density (crayfish m^{-2}). To represent this relationship in the HexSim model, we created a survival matrix that uses binned values of RC density to define the number of SMB prey according to fixed proportion values. First, bins were divided by intervals of five to define predatory pressure which ranged from low (1–5) to high (>25). Second, the average proportion (of nests abandoned, effectively vulnerable SMB individuals) for each bin was calculated using values that were extrapolated from the exponential regression. The inverse of these averages defined the survival rates for SMB prey. For example, if the predatory pressure within a co-occupied reach is moderately high (15–20 RC/m^2) then 20% of SMB prey (age-0, month-0 to month-1) are removed from that reach within the network. Because new propagules were not tied to a particular nest and abandonment implies guaranteed mortality, this approach was effective in representing predator-induced nest abandonment and subsequent "consumption" of SMB on a reach-scale. RC-SMB predation occurs only in reaches where both species are present and the average monthly temperature is above a fixed threshold (≥ 6˚C).

To simulate SMB predating upon RC, we built a second interaction event group (hereafter referred to as SMB-RC predation). Like many Centrarchids, SMB are known to consume crayfish but feeding behaviors and total contribution to diet can vary by population. Prior research in the Columbia River revealed that crayfish are frequently the second most

important food source for introduced SMB, comprising 21-57% of their diets (Beamesderfer & Rieman, 1991; Tabor et al., 1993). We applied pre-existing attributes parameters to represent the complex biological processes (e.g., temperature, prey density) that control feeding behavior and determine dietary composition (See Appendix 1.2, Section 1.11.2.3). In the multi-species model, SMB-RC predation was informed by diet estimates, bioenergetic requirements, and physiological limitations of both species. Each month, individual SMB are sorted into consumption classes (i.e., null, light, moderate, heavy, extreme) based on average stream temperature, age class, and prey density. The number of individual SMB in each consumption class determines the number of RC prey to be "consumed" (i.e., removed from the network) in co-occupied reaches. We used estimates of average gape width (SMB) and average carapace width (RC) to accurately represent consumption across age classes, effectively limiting unrealistic consumption of large RC by small SMB (i.e., juvenile (age-1 to age-2) SMB were restricted to young (age-1 to age-2) RC). Following this size selection event, RC prey were then individually marked and subject to definitive (0/1 = 0/100% mortality rate) survival event. Like the first interaction event, SMB-RC predation occurs only in reaches where both species are present and the average monthly temperature is above a fixed threshold (≥ 10˚C).

1.4.6 Model validation

The single-species models were developed through a pattern-oriented approach as described by Grimm et. al. (2006) to validate predictive ability. Specifically, we tested modeled distribution against observations that provided sufficient detail (e.g., location within the watershed) and were temporally relevant (e.g., a survey done in 2015 could be ascribed to the 2016 reference extent). These data were derived from system-specific documentation ranging from state reports with non-target fish counts at traps to research publications with extensive

snorkel surveys (Appendix 1.3). In ArcGIS, we populated a point-based vector layer with generalized metadata (reference year, confirmed presence or absence) and overlayed the line-based vector layer copied from the model workspace to extrapolate the expected range of either species for model calibration.

Calibration of single-species models relied on data-rich time intervals and thus were different for SMB (2012, 2016, 2020) and RC (2010, 2016, 2020). The SMB reference data included presence-absence records for 2012 (9-6) (DeHart et. al., 2012; Wilson et. al., 2006), 2016 (39-12) (Bare et al., 2016; Rubenson & Olden, 2019), and 2020 (4-0) (Bare et al., 2019). Estimates of density in the North Fork and Middle Fork were included for the corresponding years: 2012 (Lawrence et al., 2012), 2016 (Rubenson & Olden, 2016), and 2020 (Franklin et al., 2019). Additionally, we compared census reports to empirical estimates of age class structure (Shrader, 1999) to confirm model functionality following the burn-in period. The RC reference data contained presence-absence records and invasion extent estimates for 2010 (22-27) (Adams, 2005; Olden et al., 2009; Sorenson, 2012), 2016 (48-11) (Messager & Olden, 2019), and 2020 (23-16) (E. Blevins, per. com. July 2023).

Single-species model performance was evaluated with model fit statistics including correct classification rate, where overall accuracy is defined as the percentage of all observations the model predicted correctly. Further, we measured how well the models detected presences (sensitivity) and how well each model detected absences (specificity) where ability is described by the proportion of correctly predicted presence (true positive rate) or absence (true negative rate). SMB and RC models were assessed in singularity to mitigate potential errors when we merged these populations into a single network workspace and began developing interaction events. To determine the location of each interaction event group in the event sequence, we

added fixed-rate survival events for SMB and RC. Prey survival rates were reviewed as complexity of interactions was increased incrementally (e.g., incorporating density dependence). Further analyses were conducted to ensure reach-level mortality rates appropriately corresponded with predatory pressure. For example, we queried the number of SMB individuals assigned to each consumption class and compared these to manual calculations based on RC density, reach temperature, and individuals per age class. Observations from state and tribal agencies provided approximate convergence timing between SMB and RC populations (2010) to inform our evaluation of distribution. Because adaptive behavior was represented in predator-prey interaction events, multi-species model results were considered robust.

1.4.7 Evaluating population trends and emergent patterns

The locations of simulated individuals were recorded for each time step in all model scenarios, serving as the primary data for subsequent analyses. The SMB population reached a baseline equilibrium after 8 to 10 years following model initialization. Because the start of the time period was many decades after the actual introduction event and initial population size was an interpolated estimate, we expected SMB abundance to be highly variable throughout the first decade (1999-2009). The complex structure of the reproduction and survival event groups likely contributed to the different durations observed across replicates. To mitigate potential influence, we limited our analyses to model output after SMB had definitively reached equilibrium (May 2010, 132 steps). The RC population did not exhibit this growth because we the simulation begins at the assumed time of introduction and comprised a small number of individuals.

To detect population-level outcomes in response to biotic interactions, we quantified changes in abundance, distribution, and rate of spread. These metrics were used to assess emergent properties of simulation results. Individuals across the modeled system were

representative of shifting thermal conditions, species-specific limitations, and ecological interactions. These output data were averaged across 10 replicates in a standardized data processing pipeline (accessible at www.github.com/cvaage/hexsim-results) for application in subsequent analyses (e.g., modeled mean population size). Interaction effects were evaluated by comparing single-species and multi-species model results, allowing us to detect emergent trends across spatial (e.g., watershed, section) and temporal (e.g., monthly, yearly) scales.

1.5 Results

1.5.1 Stream temperature model

The final (V3) stream temperature model predicted values during the 30-year period with reasonable accuracy and precision on daily (MAE = 1.43, RMSE = 1.84, Table 1.A2) and monthly scales (MAE = 1.21, RMSE = 1.54, Table 1.A2). As expected, model performance was lower after removing snowpack (*SWE*) and flow (*Q*) covariates. However, prediction error was marginally higher and nearly identical to the reported fit statistics of Siegel et al. (2023): MAE = 1.32 (-0.08%) and RMSE = 1.76 (-0.04%). Air temperature metrics produced variation that sufficiently aligned with observed temperatures at the reach-level throughout the watershed. At the larger scale of Water Region 17, we found a notable loss in predictive ability when relying on only air temperature metrics for daily (MAE = 1.53, RMSE = 1.95, Table 1.A2) and monthly (MAE = 1.29, RMSE = 1.65, Table 1.A2) estimates. The final model, which relied on data within the John Day watershed, demonstrated lower error in both statistical tests (-0.06%) compared to the original spatial extent.

1.5.2 Species model performance and sensitivity

Modeled distribution of SMB largely matched the observational data used for reference across the lower and upper mainstem, including dispersal into larger tributaries like Thirty-Mile Creek. Overall model classification confirmed satisfactory performance with accuracies of 79% (2012) and 80% (2016). The SMB model produced specificities of 78% (2012) and 80% (2016), and sensitivities of 84% (2012) and 80% (2016). We did identify discrepancies between observed and modeled distribution in specific areas along the upstream invasion front. Extensive testing was performed to produce upstream colonization and spawning behaviors observed in the North and Middle Fork. Despite well-informed dispersal patterns (Rubenson & Olden, 2017), these seasonal and individual-level behaviors could not be accurately represented within the constraints of the network movement event structure.

Predicted distribution of RC aligned well with observed patterns of range expansion. Model classifications were highly successful with accuracies of 88% (2010), 88% (2016), and 83% (2020). The RC model produced specificities of 91% (2010), 86% (2016), and 81% (2020), and sensitivities of 98% (2010), 98% (2016), and 96% (2020). Because model initialization coincided with their introduction, RC did not require a burn-in period unlike the SMB model. The modeled occupancy of RC reproduced empirical distributions, but specificity consistently declined through time. Accuracy and sensitivity was generally constant over the evaluated timespan and declined slightly at the final reference interval (2020). Across all major river sections, modeled range expansion of RC accurately reflected upstream spread.

1.5.3 Multispecies model outcomes

Evaluation of SMB and RC abundance estimates revealed that cross-species interactions affect model outcomes. For both model scenarios (single-species, multi-species), we observed a

gradual decline in SMB population size and logistic growth in RC population size of the John Day River. Following the burn-in period, relative abundance of SMB (Figure 1.3A) was increasingly negative and less variable through time and evident at decade intervals: 2010 (-2.1 to -7.5%), 2020 (-29.5 to -31.0%), and 2030 (-46.0 to -47.2%). We saw more variation, including positive trends, in the relative abundance of RC (Figure 1.3A) across the same intervals: 2010 (-0.1 to 23.5%), 2020 (141.1 to 155.4%), and 2030 (-23.1 to 21.9%). Change in population size, or difference between scenarios (MS-SS) at each monthly time step, for SMB (Figure 1.3B) and RC (Figure 1.3C) further reflected the aforementioned patterns. After 2010, we observed disparities in population size for SMB which did not occur for RC until about 2019. The differences in modeled abundance of SMB gradually became increasingly negative, while RC exhibited increasingly positive growth before reaching the maximum ($\Delta = \sim 4.9^{e+6}$) around 2023-2024. We identified a sharp decline following this peak for RC, where effect size reached 0 in 2028 and became increasingly negative through the modeled duration.

Comparing temporal differences in distribution (i.e., occupied extent in RKM) between modeled scenarios showed spatial affects resulting from invader interactions. Over the 30-years, SMB exhibited a negative trend indicating range contraction that was present in both the single-species and multi-species model (Figure 1.4A). Occupied range declined modestly through time in both scenarios but was consistently higher under baseline conditions (single-species scenario, sans interactions). We found a significant relationship in the fitted linear model, indicating scenarios change differently through time ($p < 0.01$). Coefficient values revealed that the SMB single-species scenario had a lower rate of contraction (-8.35 RKM / year) than the multi-species scenario (-8.85 RKM / year). These patterns were evidenced more broadly with a lower net range contraction in the multi-species scenario (524.8 RKM) compared to the single-species scenario

(634.8 RKM). For RC, predicted occupancy showed a positive trend representing expansion that progressed through the duration of the modeled time period (Figure 1.4B). We found a relatively limited difference in approximated annual trend for multi-species (+1.68 RKM / year) and single-species (+1.76 RKM / year) scenarios. However, RC exhibited a greater difference in total range contraction than SM lower in the multi-species model (1,016.6 RKM) than the single-species species model (1,286.7 RKM).

1.6 Discussion

Through the development of a SEIBM involving multiple invasive species and spanning several decades, we showed that cross-species interactions influence important metrics of invasion success within a mechanistic model. We also discovered that such effects are likely to vary across space and time, producing unique outcomes during invasion. Comparison of abundance, distribution, and rate of spread exposed patterns of asymmetry across spatiotemporal extents and between aquatic invasive species. Together, these results contribute to a growing understanding of invasive species dynamics and the influential role of ecological interactions, driving invasion outcomes in aquatic ecosystems.

Ecological relationships (i.e., biotic interactions) fundamentally alter projected abundances. Relative abundance trajectories revealed different responses of the study species, where smallmouth bass showed consistent decline and rusty crayfish showed dramatic increases. The long-term reduction in abundance of smallmouth bass suggests that interactive effects can further suppress population growth, becoming more pronounced as a simultaneous invasions progress. This growing divergence in population size suggests that multiple mechanisms are occurring, demonstrating the value of accounting for predation. Other ecological mechanisms

(e.g., competitive pressure, feedback amplification) may also be manifesting and can produce dynamic outcomes (reduced recovery capacity driven by mechanisms of competition, habitat displacement, or indirect effects). Changes in juvenile recruitment rates can exert strong control of population dynamics of riverine smallmouth bass and impact overall resiliency to competing invasions (Keplinger & Rota, 2024).

For abundance of rusty crayfish, there was a large positive effect size during the initial convergence with smallmouth bass that continued through 2023 where rusty crayfish initially benefit from simulated conditions. These outcomes are likely reflective of growing resource subsidy from eggs to RC that outweigh any larger negative effect of mortality from SMB predation. As the downstream invasion of RC continued into river reaches with high SMB abundances, we expect RC population size to decrease starting in the mid- to late 2020s. This pattern is demonstrative of many ecological concepts: overshoot-and-collapse dynamic, delayed density dependence, and niche expansion. Specifically, the transition from initial benefit to later reversal may be a result of mechanisms like competitive release (from bass decline), increased prey and resource availability, and intraspecific competition. Strong asymmetry between species provides evidence for interactions acting in non-additive ways (e.g., synergistic, antagonistic).

Interactions between invasive species fundamentally alter invasion dynamics and spatial distribution but may do so in asymmetrical ways. Spatial trajectories of both invaders were nonlinear through time and substantially different relative to projections from the single-species scenarios. Though both SMB scenarios predicted substantial range contraction through time, the rate was constantly higher (~0.5 RKM / year faster) with the inclusion of interspecific interactions. Conversely, RC were largely unaffected (~0.08 RKM / year) and continued to expand despite increasing overlap with SMB. These findings suggest that cross-species

interactions result in more rapid predicted contraction and intensify losses of overall range. In ecosystems with multiple invaders, biotic interactions may exacerbate range loss by reducing persistence or altering habitat suitability. Across the modeled period, biotic interactions caused a notable contraction in the predicted range of SMB and a marginal effect on that of RC. Larger contraction for both populations may suggest differences in starting conditions, temporal trajectories, and possibly nonlinear dynamics. The RC population expanded by nearly 1,300 RKM in the single-species scenario and inclusion of interactions reduced expansion to approximately 1,000 RKM. This difference indicates that cross-species interactions can constrain modeled expansion of rusty crayfish. Collectively, these analyses of distribution showed various outcomes including direction (contraction, expansion) and interaction effects (altered temporal dynamics, reduced expansion).

Cross-species interactions altered not only the magnitude of invasion success, but also the spatial configuration of occupancy within the network. Modeled interactions between invasive species generated spatially heterogenous effects where changes (loss/gain) in predicted distribution were evident on many scales, from relatively localized reaches to extended invasion fronts. Inclusion of interactions with crayfish generally reduced bass occupancy relative to the single-species scenario and were sporadic in nature. These patterns imply that invasion mitigation is context dependent and likely mediated by local habitat characteristics. Losses concentrated in tributaries and small peripheral reaches offer potential mechanisms such as habitat-specific competitive suppression or lower dispersal success. For crayfish, interactions with bass changed distribution projections in both positive and negative ways depending on the area of the network. Reaches throughout the upper mainstem were increasingly occupied and suggest that crayfish redistributed in response to competitive pressures downstream.

Mechanistically, these patterns may be the result of competitive displacement, reduced intraspecific density, and altered prey dynamics. Observed clusters indicate that river topology influence invasion outcomes, resulting from simulated environmental gradients or connectivity constraints, and particularly valuable for informing management practices (e.g., dispersal bottlenecks, tributaries as thermal refugia, and invasion barriers).

In the John Day River, climate warming is predicted to reduce suitable habitat for Chinook salmon (Lawrence et al., 2012) and other salmonid species (Ruesch et al., 2012), and here we demonstrate short-term predicted outcomes for multiple invasive species that threaten freshwater biodiversity Climate-induced changes in thermal regimes of rivers can promote expansion of invasive species (Battin et al., 2007; Crozier et al., 2021; Isaak et al., 2015; Rubenson et al., 2020). Warming stream temperatures and increasing variability promotes secondary spread of invasive species (Comte et al., 2013) and further alters interactions between invaders, and their effects on native species (Rahel et al., 2008). By representing known interactions in this case, we reveal outcomes that were otherwise obscured and apply these within evolving management frameworks. A vast majority of management strategies do not address how multiple invasive species may interact in complex ways and respond to climate change (Beaury et al., 2020). Developing predictions with increasingly comprehensive models promotes our ability to address deficiencies invasion rates (abundance, distribution), (2) impact to juvenile salmon (stronger or weaker), (3) efficacy of climate adaption strategies

The present study demonstrates the utility of multi-species individual-based models for capturing the role of both abiotic and biotic factors sharing the population trajectories of invasive species. As introductions and expansion rates continue to climb, modeling approaches should explicitly and collectively address effects of such interactions. Complex interactions between

abiotic and biotic conditions may result in ecological surprises, or unanticipated changes that result from previously unsuspected processes. The broader field of ecological sciences has previously classified these instances by their respective bases: dynamic, pattern, or intervention (Doak et al., 2008). In the case of the John Day River, these surprises could be manifesting through interactions between the multiple invasive species that are present. The divergence between the single and multispecies models, shown in SMB upstream dispersal patterns, support the inclusion of biotic interactions and various abiotic dependencies in these types of models and akin frameworks. Though difficult to ascertain, speculation can be made about the response of smallmouth bass to the downstream wave of crayfish. Previous work, within the North and Middle Forks, documented the continually expanding range of smallmouth bass upstream and presence of satellite individuals that facilitate the leading edge of invasion (Lawrence et al., 2012; Rubenson et al., 2019). Both smallmouth bass (Carey et al., 2011; Rubenson et al., 2020; Sanderson et al., 2009) and rusty crayfish (Edmonds et al., 2011; Griffiths et al., 2004) exert predation and competitive pressures on juvenile salmon, prompting a critical need for research on these novel dynamics and improved understanding of future patterns.

Ecological models are valuable tools for studying a wide array of processes and have been proven to enhance management strategies, but such methodologies are limited by a variety of factors (i.e., data availability, system specific applicability) that hinder their practical application. Preceding work by Messager & Olden (2018) assessed how this population of rusty crayfish respond to various control strategies. There were greater similarities between the management and multi-species model scenarios for comparable metrics (e.g., distribution) in contrast to the single-species scenario, suggesting that co-occurring invaders may facilitate preferred management outcomes. As such, we caution managers and other parties that may use

ancillary results from ecological research, with particular emphasis towards conclusions that represent a subset of a system. System characteristics are critically important to consider as part of any ecological study. Future work, within the scope of invasion models, should emphasize the inherent complexities of representing ecologically accurate scenarios.

In this study, we evaluated the role of cross-species interactions and warming water temperature affects the abundance and distribution of multiple invasive populations. Our SEIBM simulated the basic life history of and biotic interactions between smallmouth bass and rusty crayfish populations in the John Day River. Results from analyses on abundance, distribution, and spread rate over a 30-year period enabled us to investigate emergent patterns and contextualize our findings within theoretical frameworks for invasion ecology. These research goals informed a joint retrospective and prospective assessment concerning the ability of biotic interactions and changing climactic conditions to shape the population-level responses of invasive species across a dendritic network.

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1.8 References

Abreo, N. A., Tibo, J., Nogueira, A. B., Nikolaou, A., Kaya, C., Tarkan, A. S., Kurtul, I., Katsanevakis, S., Sousa, R., Everts, T., García-Berthou, E., Hargrove, J. S., Franco, A. C. S., Herder, J. E., Olden, J. D., Woodford, D. J., Kouba, A., Britton, J. R., Soto, I., & Haubrock, P. J. (2026). Worldwide Invasions of Centrarchidae: The Dark Side of the Sunfish Family. *Fish and Fisheries*, faf.70083. https://doi.org/10.1111/faf.70083

Arismendi, I., Penaluna, B. E., Dunham, J. B., García De Leaniz, C., Soto, D., Fleming, I. A., Gomez-Uchida, D., Gajardo, G., Vargas, P. V., & León-Muñoz, J. (2014). Differential invasion success of salmonids in southern Chile: Patterns and hypotheses. *Reviews in Fish Biology and Fisheries*, *24*(3), 919–941. https://doi.org/10.1007/s11160-014-9351-0

Baldridge, A. K., & Lodge, D. M. (2013). Intraguild predation between spawning smallmouth bass (Micropterus dolomieu) and nest-raiding crayfish (Orconectes rusticus): Implications for bass nesting success. *Freshwater Biology*, *58*(11), 2355–2365. https://doi.org/10.1111/fwb.12215

Barkai, A., & McQuaid, C. (1988). Predator-Prey Role Reversal in a Marine Benthic Ecosystem. *Science*, *242*(4875), 62–64.

Battin, J., Wiley, M. W., Ruckelshaus, M. H., Palmer, R. N., Korb, E., Bartz, K. K., & Imaki, H. (2007). Projected impacts of climate change on salmon habitat restoration. *Proceedings of the National Academy of Sciences*, *104*(16), 6720–6725. https://doi.org/10.1073/pnas.0701685104

Beamesderfer, R. C., & Rieman, B. E. (1991). Abundance and Distribution of Northern Squawfish, Walleyes, and Smallmouth Bass in John Day Reservoir, Columbia River. *Transactions of the American Fisheries Society*, *120*(4), 439–447. https://doi.org/10.1577/1548-8659(1991)120%3C0439:AADONS%3E2.3.CO;2

Beaury, E. M., Fusco, E. J., Jackson, M. R., Laginhas, B. B., Morelli, T. L., Allen, J. M., Pasquarella, V. J., & Bradley, B. A. (2020). Incorporating climate change into invasive species management: Insights from managers. *Biological Invasions*, *22*(2), 233–252. https://doi.org/10.1007/s10530-019-02087-6

Bilby, R. E., Currens, K. P., Fresh, K. L., Booth, D. B., Fuerstenberg, R. R., & Lucchetti, G. L. (2024). Why Aren't Salmon Responding to Habitat Restoration in the Pacific Northwest? *Fisheries*, *49*(1), 16–27. https://doi.org/10.1002/fsh.10991

Brandt, A. J., Png, G. K., Jo, I., McGrannachan, C., Allen, K., Peltzer, D. A., D'Antonio, C., Dickie, I. A., French, K., Leishman, M. R., Ostertag, R., Parker, I. M., Stanley, M. C., Suding, K. N., & Bellingham, P. J. (2023). Managing multi-species plant invasions when interactions influence their impact. *Frontiers in Ecology and the Environment*, *21*(8), 370–379. https://doi.org/10.1002/fee.2658

Carey, M. P., Sanderson, B. L., Friesen, T. A., Barnas, K. A., & Olden, J. D. (2011). Smallmouth Bass in the Pacific Northwest: A Threat to Native Species; a Benefit for Anglers. *Reviews in Fisheries Science*, *19*(3), 305–315. https://doi.org/10.1080/10641262.2011.598584

Chamberlain, S. A., Bronstein, J. L., & Rudgers, J. A. (2014). How context dependent are species interactions? *Ecology Letters*, *17*(7), 881–890. https://doi.org/10.1111/ele.12279

Colautti, R. I., Ricciardi, A., Grigorovich, I. A., & MacIsaac, H. J. (2004). Is invasion success explained by the enemy release hypothesis? *Ecology Letters*, *7*(8), 721–733. https://doi.org/10.1111/j.1461-0248.2004.00616.x

Comte, L., Buisson, L., Daufresne, M., & Grenouillet, G. (2013). Climate-induced changes in the distribution of freshwater fish: Observed and predicted trends. *Freshwater Biology*, *58*(4), 625–639. https://doi.org/10.1111/fwb.12081

Crozier, L. G., Burke, B. J., Chasco, B. E., Widener, D. L., & Zabel, R. W. (2021). Climate change threatens Chinook salmon throughout their life cycle. *Communications Biology*, *4*(1), 222. https://doi.org/10.1038/s42003-021-01734-w

Daly, C., Neilson, R. P., & Phillips, D. L. (1994). A Statistical-Topographic Model for Mapping Climatological Precipitation over Mountainous Terrain. *Journal of Applied Meteorology*, *33*(2), 140–158. https://doi.org/10.1175/1520-0450(1994)033%3C0140:ASTMFM%3E2.0.CO;2

Diagne, C., Leroy, B., Vaissière, A.-C., Gozlan, R. E., Roiz, D., Jarić, I., Salles, J.-M., Bradshaw, C. J. A., & Courchamp, F. (2021). High and rising economic costs of biological invasions worldwide. *Nature*, *592*(7855), 571–576. https://doi.org/10.1038/s41586-021-03405-6

Doak, D. F., Estes, J. A., Halpern, B. S., Jacob, U., Lindberg, D. R., Lovvorn, J., Monson, D. H., Tinker, M. T., Williams, T. M., Wootton, J. T., Carroll, I., Emmerson, M., Micheli, F., & Novak, M. (2008). Understanding and Predicting Ecological Dynamics: Are Major Surprises Inevitable. *Ecology*, *89*(4), 952–961. https://doi.org/10.1890/07-0965.1

Dominguez Almela, V., Palmer, S. C. F., Andreou, D., Gillingham, P. K., Travis, J. M. J., & Britton, J. R. (2022). Predicting the influence of river network configuration, biological traits and habitat quality interactions on riverine fish invasions. *Diversity and Distributions*, *28*(2), 257–270. https://doi.org/10.1111/ddi.13459

Dorn, N. J., Mittelbach, G. G., & Kellogg, W. K. (1999). More than predator and prey: A review of interactions between fish and crayfish. *Vie et Milieu/Life & Environment*, 229–237.

Edmonds, N. J., Riley, W. D., & Maxwell, D. L. (2011). Predation by *Pacifastacus leniusculus* on the intra-gravel embryos and emerging fry of *Salmo salar*. *Fisheries Management and Ecology*, *18*(6), 521–524. https://doi.org/10.1111/j.1365-2400.2011.00797.x

Ellrott, B. J., Marsden, J. E., Fitzsimons, J. D., Jonas, J. L., & Claramunt, R. M. (2007). Effects of Temperature and Density on Consumption of Trout Eggs by Orconectes propinquus and O. rusticus. *Journal of Great Lakes Research*, *33*(1), 7–14. https://doi.org/10.3394/0380-1330(2007)33%5B7:EOTADO%5D2.0.CO;2

Gallardo, B., Clavero, M., Sánchez, M. I., & Vilà, M. (2016). Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology*, *22*(1), 151–163. https://doi.org/10.1111/gcb.13004

Giometto, A., Altermatt, F., & Rinaldo, A. (2017). Demographic stochasticity and resource autocorrelation control biological invasions in heterogeneous landscapes. *Oikos*, *126*(11), 1554–1563. https://doi.org/10.1111/oik.04330

Griffen, B. D., Guy, T., & Buck, J. C. (2008). Inhibition between invasives: A newly introduced predator moderates the impacts of a previously established invasive predator. *Journal of Animal Ecology*, *77*(1), 32–40. https://doi.org/10.1111/j.1365-2656.2007.01304.x

Griffiths, S. W., Collen, P., & Armstrong, J. D. (2004). Competition for shelter among over-wintering signal crayfish and juvenile Atlantic salmon. *Journal of Fish Biology*, *65*(2), 436–447. https://doi.org/10.1111/j.0022-1112.2004.00460.x

Hamlet, A. F., Elsner, M. M., Mauger, G. S., Lee, S.-Y., Tohver, I., & Norheim, R. A. (2013). An Overview of the Columbia Basin Climate Change Scenarios Project: Approach, Methods, and Summary of Key Results. *Atmosphere-Ocean*, *51*(4), 392–415. https://doi.org/10.1080/07055900.2013.819555

Hand, B. K., Flint, C. G., Frissell, C. A., Muhlfeld, C. C., Devlin, S. P., Kennedy, B. P., Crabtree, R. L., McKee, W. A., Luikart, G., & Stanford, J. A. (2018). A social–ecological perspective for riverscape management in the Columbia River Basin. *Frontiers in Ecology and the Environment*, *16*(S1), S23–S33. https://doi.org/10.1002/fee.1752

Hastings, A., & Wysham, D. B. (2010). Regime shifts in ecological systems can occur with no warning. *Ecology Letters*, *13*(4), 464–472. https://doi.org/10.1111/j.1461-0248.2010.01439.x

Haubrock, P. J., Cuthbert, R. N., & Haase, P. (2023). Long-term trends and drivers of biological invasion in Central European streams. *Science of The Total Environment*, *876*, 162817. https://doi.org/10.1016/j.scitotenv.2023.162817

Hewett, S. W., & Johnson, B. L. (1987). *A Generalized Bioenergetics Model of Fish Growth for Microcomputers*.

Hodgson, J. A., Thomas, C. D., Wintle, B. A., & Moilanen, A. (2009). Climate change, connectivity and conservation decision making: Back to basics. *Journal of Applied Ecology*, *46*(5), 964–969. https://doi.org/10.1111/j.1365-2664.2009.01695.x

Humston, R., Doss, S. S., Wass, C., Hollenbeck, C., Thorrold, S. R., Smith, S., & Bataille, C. P. (2017). Isotope geochemistry reveals ontogeny of dispersal and exchange between main-river and tributary habitats in smallmouth bass Micropterus dolomieu. *Journal of Fish Biology*, *90*(2), 528–548. https://doi.org/10.1111/jfb.13073

Isaak, D. J., Young, M. K., Nagel, D. E., Horan, D. L., & Groce, M. C. (2015). The cold-water climate shield: Delineating refugia for preserving salmonid fishes through the 21st century. *Global Change Biology*, *21*(7), 2540–2553. https://doi.org/10.1111/gcb.12879

Jackson, M. C. (2015). Interactions among multiple invasive animals. *Ecology*, *96*(8), 2035–2041. https://doi.org/10.1890/15-0171.1

Jackson, M. C., Loewen, C. J. G., Vinebrooke, R. D., & Chimimba, C. T. (2016). Net effects of multiple stressors in freshwater ecosystems: A meta-analysis. *Global Change Biology*, *22*(1), 180–189. https://doi.org/10.1111/gcb.13028

Jin, Y., Hilker, F. M., Steffler, P. M., & Lewis, M. A. (2014). Seasonal Invasion Dynamics in a Spatially Heterogeneous River with Fluctuating Flows. *Bulletin of Mathematical Biology*, *76*(7), 1522–1565. https://doi.org/10.1007/s11538-014-9957-3

Johnson, P. T. J., Olden, J. D., Solomon, C. T., & Vander Zanden, M. J. (2009). Interactions among invaders: Community and ecosystem effects of multiple invasive species in an experimental aquatic system. *Oecologia*, *159*(1), 161–170. https://doi.org/10.1007/s00442-008-1176-x

Keplinger, B., & Rota, C. T. (2024). Effects of poor recruitment on riverine Smallmouth Bass population dynamics. *North American Journal of Fisheries Management*, *44*(2), 247–261. https://doi.org/10.1002/nafm.10994

Kordas, R. L., Harley, C. D. G., & O'Connor, M. I. (2011). Community ecology in a warming world: The influence of temperature on interspecific interactions in marine systems. *Journal of Experimental Marine Biology and Ecology*, *400*(1–2), 218–226. https://doi.org/10.1016/j.jembe.2011.02.029

Kuebbing, S. E., & Nuñez, M. A. (2015). Negative, neutral, and positive interactions among nonnative plants: Patterns, processes, and management implications. *Global Change Biology*, *21*(2), 926–934. https://doi.org/10.1111/gcb.12711

LaForgia, M. L., Harrison, S. P., & Latimer, A. M. (2020). Invasive species interact with climatic variability to reduce success of natives. *Ecology*, *101*(6), e03022. https://doi.org/10.1002/ecy.3022

Lawrence, D. J., Olden, J. D., & Torgersen, C. E. (2012). Spatiotemporal patterns and habitat associations of smallmouth bass (Micropterus dolomieu) invading salmon-rearing habitat. *Freshwater Biology*, *57*(9), 1929–1946. https://doi.org/10.1111/j.1365-2427.2012.02847.x

Levine, J. M., & D'Antonio, C. M. (1999). Elton Revisited: A Review of Evidence Linking Diversity and Invasibility. *Oikos*, *87*(1), 15–26. https://doi.org/10.2307/3546992

Liu, X., Wang, S., Ke, Z., Cheng, C., Wang, Y., Zhang, F., Xu, F., Li, X., Gao, X., Jin, C., Zhu, W., Yan, S., & Li, Y. (2018). More invaders do not result in heavier impacts: The effects of non-native bullfrogs on native anurans are mitigated by high densities of non-native crayfish. *Journal of Animal Ecology*, *87*(3), 850–862. https://doi.org/10.1111/1365-2656.12793

Lopez, B. E., Allen, J. M., Dukes, J. S., Lenoir, J., Vilà, M., Blumenthal, D. M., Beaury, E. M., Fusco, E. J., Laginhas, B. B., Morelli, T. L., O'Neill, M. W., Sorte, C. J. B., Maceda-Veiga, A., Whitlock, R., & Bradley, B. A. (2022). Global environmental changes more frequently offset than intensify detrimental effects of biological invasions. *Proceedings of the National Academy of Sciences*, *119*(22). https://doi.org/10.1073/pnas.2117389119

Mack, R. N., Simberloff, D., Mark Lonsdale, W., Evans, H., Clout, M., & Bazzaz, F. A. (2000). Biotic Invasions: Causes, Epidemiology, Global Consequences, and Control. *Ecological Applications*, *10*(3), 689–710. https://doi.org/10.1890/1051-0761(2000)010%5B0689:BICEGC%5D2.0.CO;2

Messager, M. L., & Olden, J. D. (2018). Individual-based models forecast the spread and inform the management of an emerging riverine invader. *Diversity and Distributions*, *24*(12), 1816–1829. https://doi.org/10.1111/ddi.12829

Messager, M. L., & Olden, J. D. (2019). Phenotypic variability of rusty crayfish (Faxonius rusticus) at the leading edge of its riverine invasion. *Freshwater Biology*, *64*(6), 1196–1209. https://doi.org/10.1111/fwb.13295

Mitchell, C. E., Agrawal, A. A., Bever, J. D., Gilbert, G. S., Hufbauer, R. A., Klironomos, J. N., Maron, J. L., Morris, W. F., Parker, I. M., Power, A. G., Seabloom, E. W., Torchin, M. E., & Vázquez, D. P. (2006). Biotic interactions and plant invasions. *Ecology Letters*, *9*(6), 726–740. https://doi.org/10.1111/j.1461-0248.2006.00908.x

Móréh, Á., Jordán, F., & Scheuring, I. (2024). Effects of joint invasion: How co-invaders affect each other's success in model food webs? *Ecological Modelling*, *492*, 110735. https://doi.org/10.1016/j.ecolmodel.2024.110735

Morse, J. W., Elgin, A., & Reisinger, L. (2013). Invasive crayfish Orconectes Rusticus (Decapoda, Cambaridae) is a more effective predator of substrate nesting fish eggs than native crayfish (O. VIRILIS). *Crustaceana*, *86*, 387–402. https://doi.org/10.1163/15685403-00003187

Mundahl, N. D., & Benton, M. J. (1990). Aspects of the Thermal Ecology of the Rusty Crayfish Orconectes rusticus (Girard). *Oecologia*, *82*(2), 210–216.

Naiman, R. J., Alldredge, J. R., Beauchamp, D. A., Bisson, P. A., Congleton, J., Henny, C. J., Huntly, N., Lamberson, R., Levings, C., Merrill, E. N., Pearcy, W. G., Rieman, B. E., Ruggerone, G. T., Scarnecchia, D., Smouse, P. E., & Wood, C. C. (2012). Developing a broader scientific foundation for river restoration: Columbia River food webs.

Proceedings of the National Academy of Sciences, *109*(52), 21201–21207. https://doi.org/10.1073/pnas.1213408109

Narayanan, N., Lutz, P., & Shaw, A. K. (2025). Coexistence of coinvading species with mutualism and competition. *Ecology*, *106*(2), e70039. https://doi.org/10.1002/ecy.70039

Olden, J. D., Larson, E. R., & Mims, M. C. (2009). Home-field advantage: Native signal crayfish (Pacifastacus leniusculus) out consume newly introduced crayfishes for invasive Chinese mystery snail (Bellamya chinensis). *Aquatic Ecology*, *43*(4), 1073–1084. https://doi.org/10.1007/s10452-009-9244-9

Parker, I. M., Simberloff, D., Lonsdale, W. M., Goodell, K., Wonham, M., Kareiva, P. M., Williamson, M. H., Holle, B. V., Moyle, P. B., Byers, J. E., & Goldwasser, L. (1999). *Impact: Toward a Framework for Understanding the Ecological Effects of Invaders*.

Pease, A. A., & Paukert, C. P. (2014). Potential impacts of climate change on growth and prey consumption of stream-dwelling smallmouth bass in the central U nited S tates. *Ecology of Freshwater Fish*, *23*(3), 336–346. https://doi.org/10.1111/eff.12084

Pellissier, L., Albouy, C., Bascompte, J., Farwig, N., Graham, C., Loreau, M., Maglianesi, M. A., Melián, C. J., Pitteloud, C., Roslin, T., Rohr, R., Saavedra, S., Thuiller, W., Woodward, G., Zimmermann, N. E., & Gravel, D. (2018). Comparing species interaction networks along environmental gradients. *Biological Reviews*, *93*(2), 785–800. https://doi.org/10.1111/brv.12366

Rahel, F. J., Bierwagen, B., & Taniguchi, Y. (2008). Managing Aquatic Species of Conservation Concern in the Face of Climate Change and Invasive Species. *Conservation Biology*, *22*(3), 551–561. https://doi.org/10.1111/j.1523-1739.2008.00953.x

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, *94*(3), 849–873. https://doi.org/10.1111/brv.12480

Ricciardi, A., Hoopes, M. F., Marchetti, M. P., & Lockwood, J. L. (2013). Progress toward understanding the ecological impacts of nonnative species. *Ecological Monographs*, *83*(3), 263–282. https://doi.org/10.1890/13-0183.1

Ricciardi, A., Iacarella, J. C., Aldridge, D. C., Blackburn, T. M., Carlton, J. T., Catford, J. A., Dick, J. T. A., Hulme, P. E., Jeschke, J. M., Liebhold, A. M., Lockwood, J. L., MacIsaac, H. J., Meyerson, L. A., Pyšek, P., Richardson, D. M., Ruiz, G. M., Simberloff, D., Vilà, M., & Wardle, D. A. (2021). Four priority areas to advance invasion science in the face of rapid environmental change. *Environmental Reviews*, *29*(2), 119–141. https://doi.org/10.1139/er-2020-0088

Ridgway, M., Shuter, B., Middel, T., & Gross, M. (2002). Spatial Ecology and Density-Dependent Processes in Smallmouth Bass: The Juvenile Transition Hypothesis. *American Fisheries Society Symposium*, *2002*.

Rieman, B. E., Smith, C. L., Naiman, R. J., Ruggerone, G. T., Wood, C. C., Huntly, N., Merrill, E. N., Alldredge, J. R., Bisson, P. A., Congleton, J., Fausch, K. D., Levings, C., Pearcy, W., Scarnecchia, D., & Smouse, P. (2015). A Comprehensive Approach for Habitat Restoration in the Columbia Basin. *Fisheries*, *40*(3), 124–135. https://doi.org/10.1080/03632415.2015.1007205

Rodriguez, L. F. (2006). Can Invasive Species Facilitate Native Species? Evidence of How, When, and Why These Impacts Occur. *Biological Invasions*, *8*(4), 927–939. https://doi.org/10.1007/s10530-005-5103-3

Rubenson, E. S., Lawrence, D. J., & Olden, J. D. (2020). Threats to Rearing Juvenile Chinook Salmon from Nonnative Smallmouth Bass Inferred from Stable Isotope and Fatty Acid Biomarkers. *Transactions of the American Fisheries Society*, *149*(3), 350–363. https://doi.org/10.1002/tafs.10237

Rubenson, E. S., & Olden, J. D. (2017). Dynamism in the upstream invasion edge of a freshwater fish exposes range boundary constraints. *Oecologia*, *184*(2), 453–467. https://doi.org/10.1007/s00442-017-3885-5

Ruesch, A. S., Torgersen, C. E., Lawler, J. J., Olden, J. D., Peterson, E. E., Volk, C. J., & Lawrence, D. J. (2012). Projected Climate-Induced Habitat Loss for Salmonids in the John Day River Network, Oregon, U.S.A. *Conservation Biology*, *26*(5), 873–882. https://doi.org/10.1111/j.1523-1739.2012.01897.x

Sánchez-Garduño, F., Miramontes, P., & Marquez-Lago, T. T. (2014). Role reversal in a predator–prey interaction. *Royal Society Open Science*, *1*(2), 140186. https://doi.org/10.1098/rsos.140186

Sanderson, B. L., Tran, C. D., Coe, H. J., Pelekis, V., Steel, E. A., & Reichert, W. L. (2009). Nonlethal Sampling of Fish Caudal Fins Yields Valuable Stable Isotope Data for Threatened and Endangered Fishes. *Transactions of the American Fisheries Society*, *138*(5), 1166–1177. https://doi.org/10.1577/T08-086.1

Schake, C. L., Dembkowski, D. J., & Wuellner, M. R. (2014). *Gape:Body Size Relationship for Smallmouth Bass*.

Schumaker, N. H., & Brookes, A. (2018). HexSim: A modeling environment for ecology and conservation. *Landscape Ecology*, *33*(2), 197–211. https://doi.org/10.1007/s10980-017-0605-9

Seebens, H., Bacher, S., Blackburn, T. M., Capinha, C., Dawson, W., Dullinger, S., Genovesi, P., Hulme, P. E., van Kleunen, M., Kühn, I., Jeschke, J. M., Lenzner, B., Liebhold, A. M., Pattison, Z., Pergl, J., Pyšek, P., Winter, M., & Essl, F. (2021). Projecting the continental accumulation of alien species through to 2050. *Global Change Biology*, *27*(5), 970–982. https://doi.org/10.1111/gcb.15333

Seebens, H., Blackburn, T. M., Dyer, E. E., Genovesi, P., Hulme, P. E., Jeschke, J. M., Pagad, S., Pyšek, P., Winter, M., Arianoutsou, M., Bacher, S., Blasius, B., Brundu, G., Capinha, C., Celesti-Grapow, L., Dawson, W., Dullinger, S., Fuentes, N., Jäger, H., … Essl, F. (2017). No saturation in the accumulation of alien species worldwide. *Nature Communications*, *8*(1), 14435. https://doi.org/10.1038/ncomms14435

Shrader, T., & Gray, M. E. (1999). *Biology and management of John Day River smallmouth bass*. Fish Division, Oregon Department of Fish and Wildlife.

Siegel, J. E., Fullerton, A. H., FitzGerald, A. M., Holzer, D., & Jordan, C. E. (2023). Daily stream temperature predictions for free-flowing streams in the Pacific Northwest, USA. *PLOS Water*, *2*(8), e0000119. https://doi.org/10.1371/journal.pwat.0000119

Simberloff, D., & Von Holle, B. (1999). Positive Interactions of Nonindigenous Species: Invasional Meltdown? *Biological Invasions*, *1*(1), 21–32. https://doi.org/10.1023/A:1010086329619

Stein, R. A. (1977). Selective Predation, Optimal Foraging, and the Predator-Prey Interaction Between Fish and Crayfish. *Ecology*, *58*(6), 1237–1253. https://doi.org/10.2307/1935078

Strayer, D. L. (2010). Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology*, *55*(s1), 152–174. https://doi.org/10.1111/j.1365-2427.2009.02380.x

Tabor, R. A., Shively, R. S., & Poe, T. P. (1993). Predation on Juvenile Salmonids by Smallmouth Bass and Northern Squawfish in the Columbia River near Richland, Washington. *North American Journal of Fisheries Management*, *13*(4), 831–838. https://doi.org/10.1577/1548-8675(1993)013%3C0831:POJSBS%3E2.3.CO;2

Thompson, B. K., Olden, J. D., & Converse, S. J. (2021). Mechanistic invasive species management models and their application in conservation. *Conservation Science and Practice*, *3*(11), e533. https://doi.org/10.1111/csp2.533

Twardochleb, L. A., Olden, J. D., & Larson, E. R. (2013). A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science*, *32*(4), 1367–1382. https://doi.org/10.1899/12-203.1

Wang, T., Hamann, A., Spittlehouse, D., & Carroll, C. (2016). Locally Downscaled and Spatially Customizable Climate Data for Historical and Future Periods for North America. *PLOS ONE*, *11*(6), e0156720. https://doi.org/10.1371/journal.pone.0156720

Whitledge, G. W., Hayward, R. S., Zweifel, R. D., & Rabeni, C. F. (2003). Development and Laboratory Evaluation of a Bioenergetics Model for Subadult and Adult Smallmouth Bass. *Transactions of the American Fisheries Society*, *132*(2), 316–325. https://doi.org/10.1577/1548-8659(2003)132%3C0316:DALEOA%3E2.0.CO;2

Wolf, S. L., Boren, N., Vasquez, B., Dudding, J., & Shields, R. (2022). Population Structure, Diet, and Bioenergetics of Introduced Smallmouth Bass in an Intermountain West Reservoir. *Western North American Naturalist*, *82*(3). https://doi.org/10.3398/064.082.0306

Woodward, G., Perkins, D. M., & Brown, L. E. (2010). Climate change and freshwater ecosystems: Impacts across multiple levels of organization. *Philosophical Transactions of the Royal Society B: Biological Sciences*, *365*(1549), 2093–2106. https://doi.org/10.1098/rstb.2010.0055

Zavaleta, E. S., Hobbs, R. J., & Mooney, H. A. (2001). Viewing invasive species removal in a whole-ecosystem context. *Trends in Ecology & Evolution*, *16*(8), 454–459. https://doi.org/10.1016/S0169-5347(01)02194-2

Zweifel, R. D., Hayward, R. S., & Rabeni, C. F. (1999). Bioenergetics Insight into Black Bass Distribution Shifts in Ozark Border Region Streams. *North American Journal of Fisheries Management*, *19*(1), 192–197. https://doi.org/10.1577/1548-8675(1999)019%3C0192:BIIBBD%3E2.0.CO;2

1.9 Figures

Figure 1.1. Major segments and perennial tributaries of the John Day River. Introduction points of SMB (fish icon) and RC (alien icon).

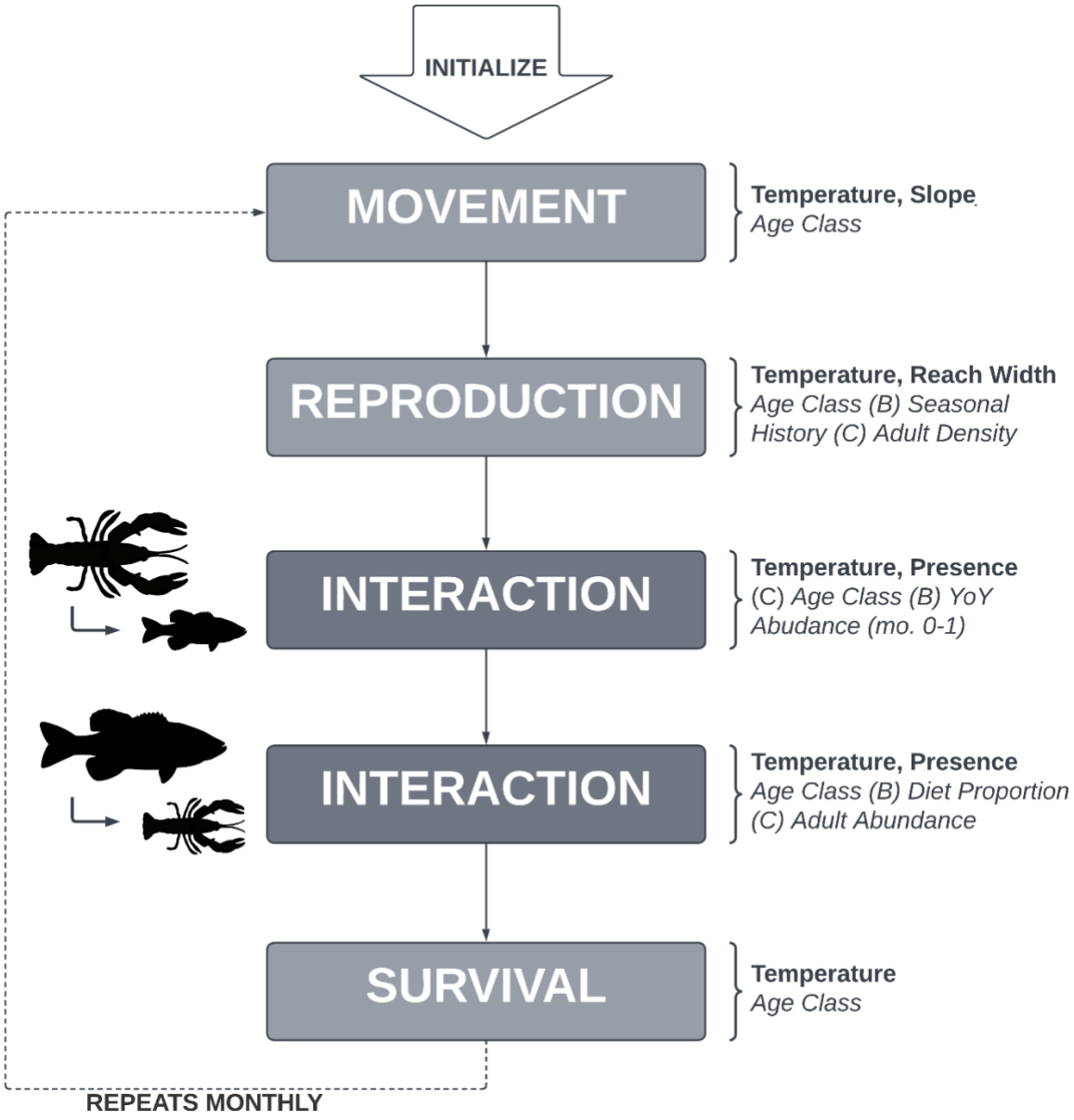


Figure 1.2. Basic sequence of events represented in HexSim framework. Each event references reach-specific (bolded), floater-specific (italicized), and species-specific (lettered) parameters.

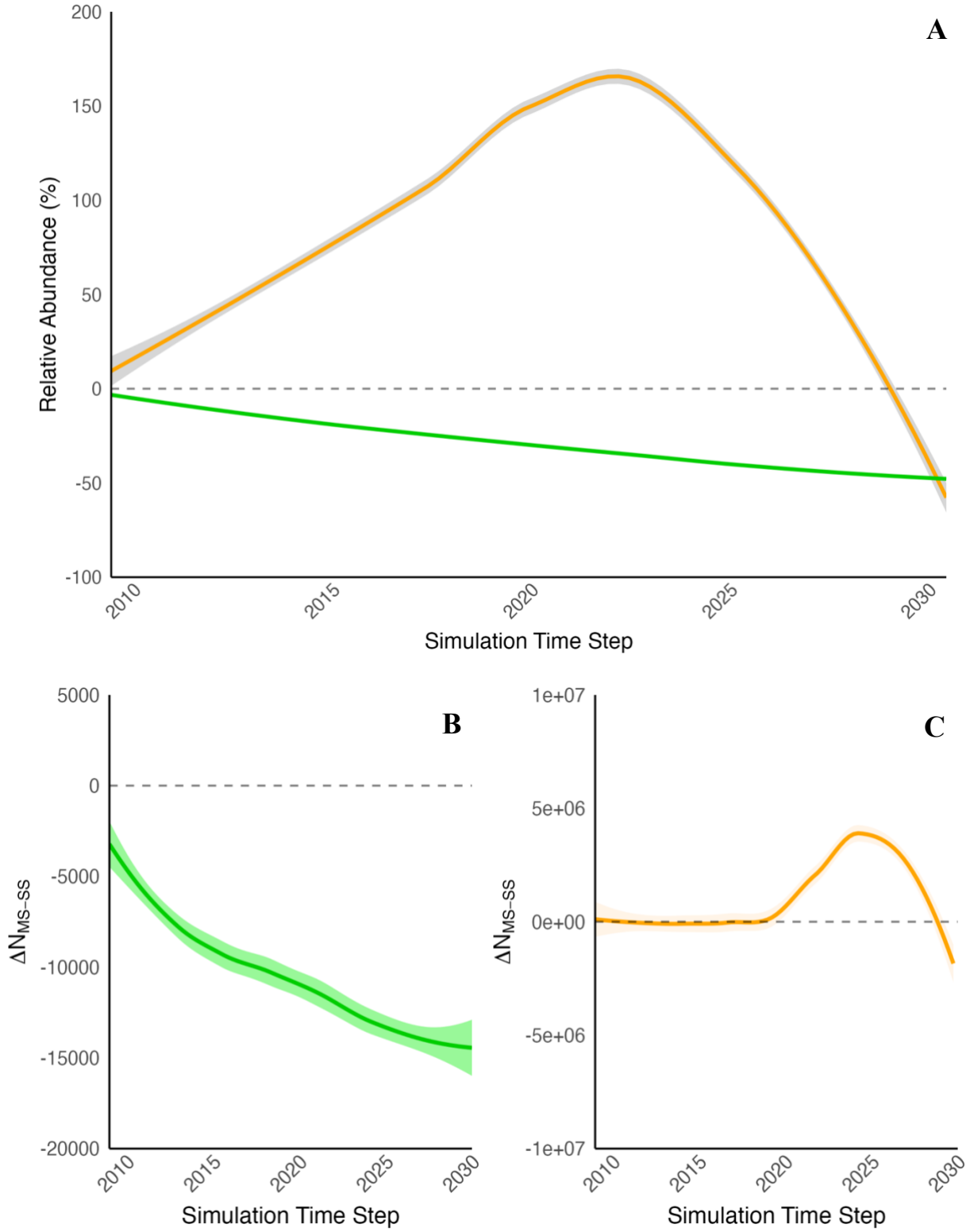


Figure 1.3. Interaction effect on abundance predictions of smallmouth bass (green) and rusty crayfish (orange) shown through relative abundance (A) and population size (B, C).

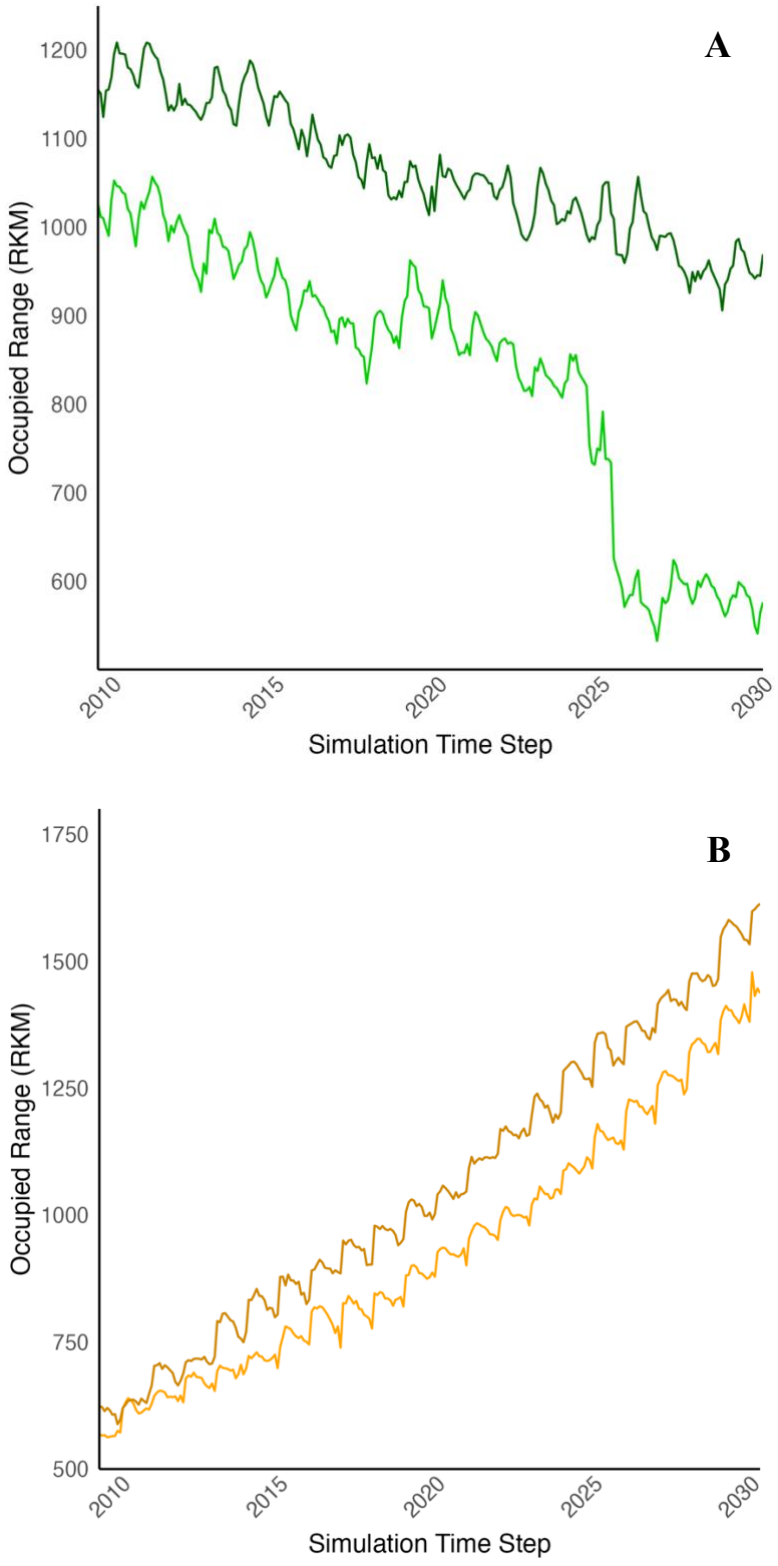


Figure 1.4 Distribution (RKM = river kilometers) of modeled scenarios (light = multi-species, dark = single-species) for smallmouth bass (A) and rusty crayfish (B) populations from 2010 to 2030.

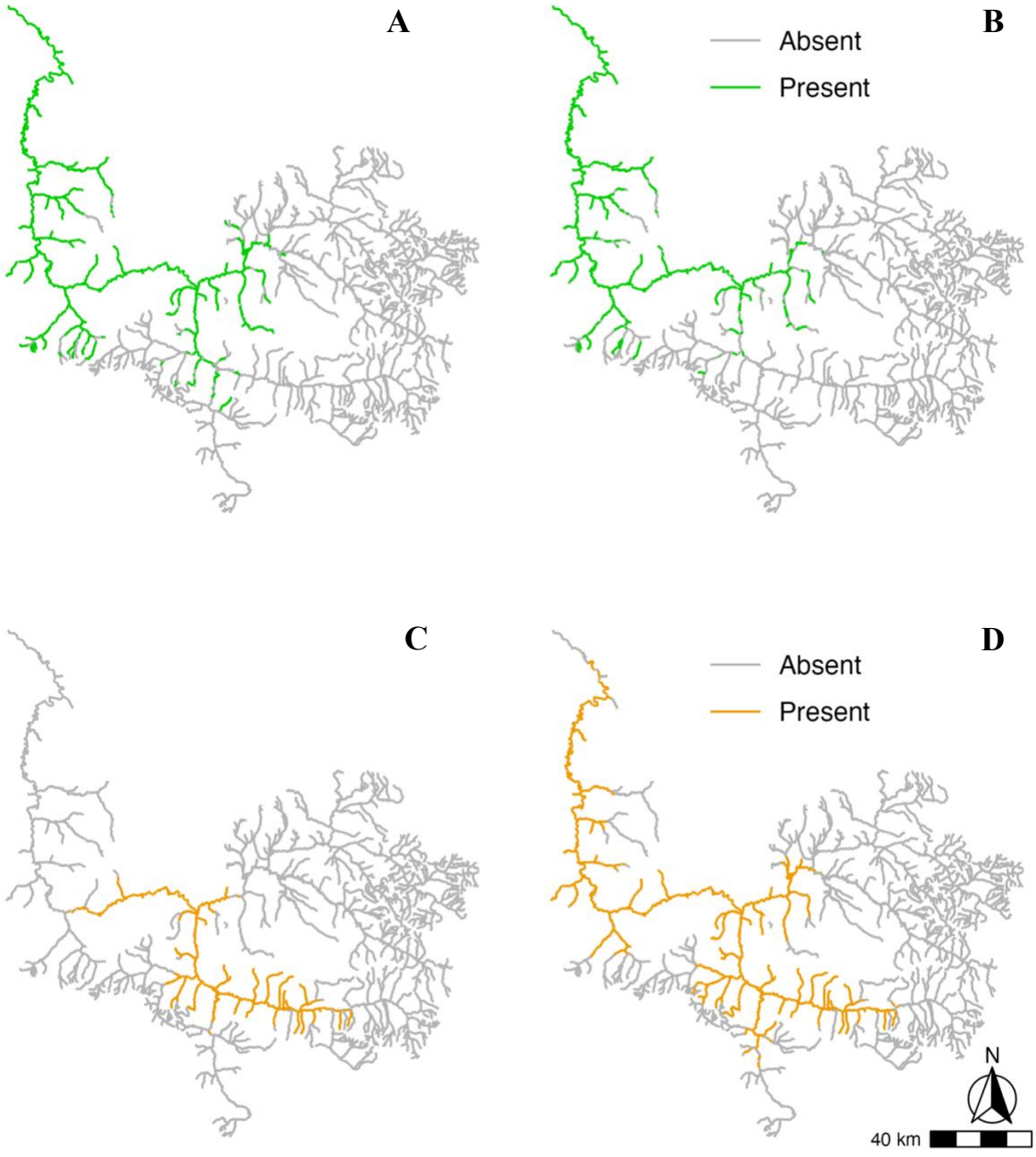


Figure 1.5 Predicted spatial distribution of multi-species model containing SMB (green, top row) and RC (orange, bottom row) in 2010 (left column) and 2030 (right column).

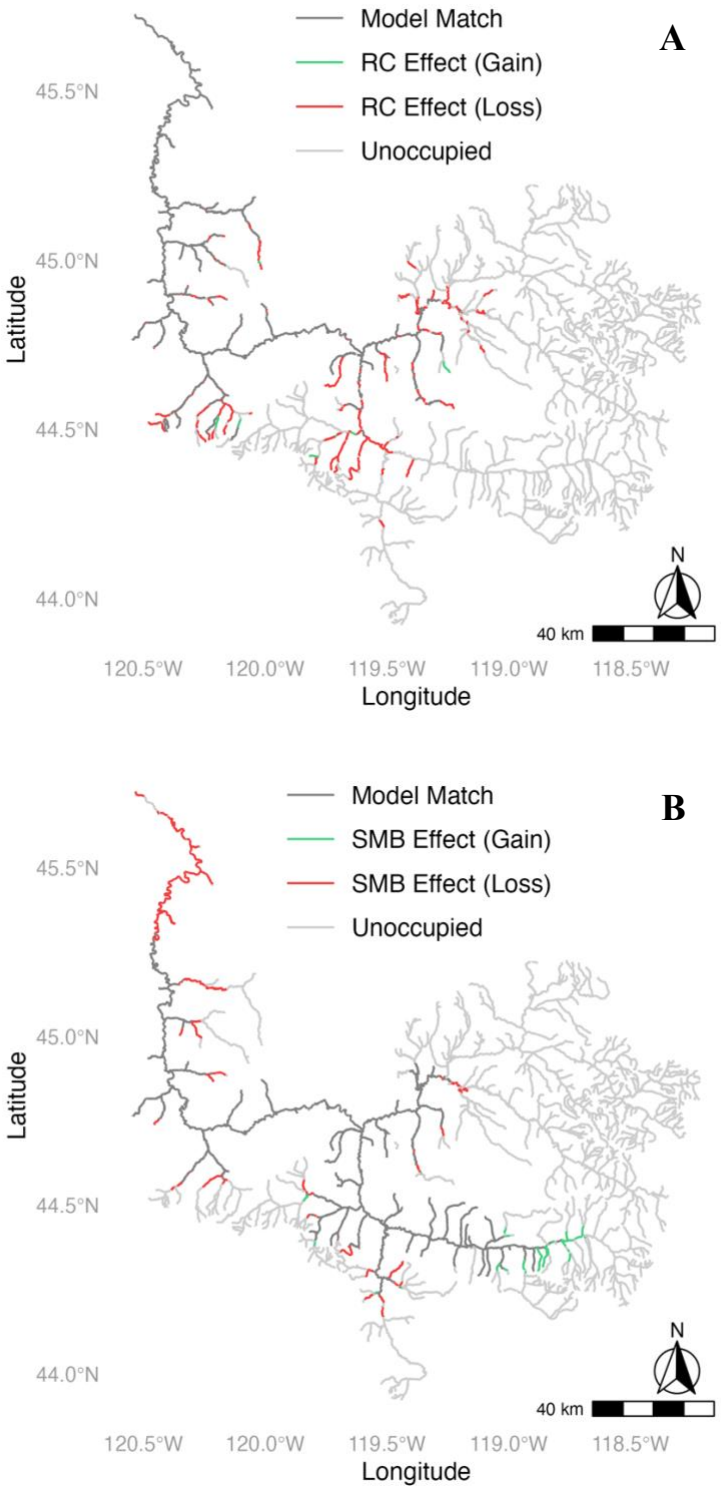


Figure 1.6 Spatial differences in estimated distribution of SMB (A) and RC (B) in 2030. Interaction effects (gain = green, loss = red, SMB and dark grey = match) are depicted throughout the modeled stream network, including absence (light grey) and occupancy is defined by reach (>= 1 individual present).

1.10 Tables

Table 1.1 Fit statistics applied for evaluating single-species model performance for separately developed populations. Accuracy, sensitivity, and specificity listed by reference year for RC (n = 3) and SMB (n = 2) were derived from confusion matrix values (TP = true positive, TN = true negative, FP = false positive, FN = false negative).

Population	Year	TP	TN	FP	FN	Accuracy (%)	Sensitivity (%)	Specificity (%)
RC	2010	121	1907	189	0	87.6	97.7	91
	2016	259	1684	268	6	87.6	97.7	86.3
	2020	306	1532	365	14	82.9	95.6	80.8
SMB	2012	308	1444	408	57	79	84.4	78
	2016	308	1464	369	76	79.9	80.2	79.9

1.11 Supporting information

1.11.1 Appendix 1.1: Stream temperature model development

1.11.1.1 Approach and adaptations

Average daily stream temperatures from May 1999 to May 2040 were estimated using a statistical modeling framework developed by Siegel et al. (2023). Representative of mechanistic processes, this Generalized Additive Model framework predicts water temperature of all free-flowing river and stream reaches across Water Region 17. Approximately 20 years (1993–2013) of daily water temperature data were collected, processed, and made available to the public by the USDA Forest Service as part of the NorWeST project. These records contain daily metrics and can be accessed by downloading the "All Days Daily Summary" files (https://www.fs.fed.us/rm/boise/AWAE/projects/NorWeST/StreamTemperatureDataSummaries.shtml). Climate data was compiled from the PRISM Climate Group at Oregon State University (i.e., air temperature), National Snow and Ice Data Center (i.e., snowpack), and National Water Model (i.e., flow predictions). Spatial data defining landscape characteristics were sourced from EPA StreamCat (i.e., 30-year mean annual precipitation), NHDPlusV2 (i.e., flow direction), and the National Land Cover Dataset (i.e., canopy cover).

Model fitting centered around antecedent air temperature (*Tl*), defined as "flow-dependent flexible-lag air temperature" and developed by Siegel et al. (2022). Continuous form data (i.e., air temperature, snowpack) were summarized to produce local and regional averages. Effects of climate covariates on air temperature were parameterized with a GAM tensor product

smoothers and interactions fit. Spatial landscape covariates (Table 1.A1) were modeled with linear effects:

$$T_w = ti(Tl) + ti(Tws) + ti(DL) + ti(Qr) + ti(Sws) + ti(Tws, Tl) + ti(DL, tl) + ti(Qr, Tl) + ti(Sws, Tws) + Tl * (Lat + E + A + BFI + Ed + S + O + W + I + F + C + U + V + P + R) + SA1 * D + E * A$$

This modeling framework supported the spatial (i.e., reach-scale) and temporal (i.e., daily average stream temperature) scale of the HexSim model input but required modifications to the original source code (Version 1, V1). First, we made Version 2 (V2) by reducing the extent of records included in the response dataset from a subregion (HUC-4) to watershed-level (HUC-6). The watershed of interest (170702) largely comprises reaches that are classified as transitional. Other watersheds from the Middle Columbia subregion (1707), which have proportionally more rain- and snow-dominated reaches, were excluded to reduce potential influence during model fitting and shorten the processing time. Second, we excluded two predictor variables: snowpack (*SWE*) and flow (*Q*). Future climate projections did not contain these variables at the needed spatiotemporal scale at the time of this study. Consequently, both factors (*SWE*, *Q*) were removed to make Version 3 (V3). Model validation was performed following the methods used by Siegel et al. (2023), including root mean squared error and mean absolute error statistics. These results, in addition to temporal cross-validation, demonstrated a similar degree of accuracy and precision to output from V1. Statistics of model fit were calculated across a longer, historical period (1990 – 2020) to evaluate differences between V2 and V3.

1.11.1.2 Downscaling methods

To use this modeling framework across the future time period (2025–2040), we applied a downscaling approach to projected climate data. We sourced average monthly air temperatures at a 1-km resolution using an ensemble of 13 global change models (GCMs) under scenario 2.6 using ClimateNA software version 7.0 (Wang et al., 2016). Historical average daily temperatures (T_H) within the John Day River Basin were obtained from the PRISM Climate Group at Oregon State University (Daly et al., 1994). PRISM provided retrospective data as 4-km gridded cells and calculated using a climate-elevation regression paired with station-specific factors. Pre-processing was necessary to match the resolution between files from PRISM (1-km) and ClimateNA (4-km) prior to temporal downscaling. Thus, all raster files from PRISM (1-km) were resampled using the "aggregate" tool in ArcGIS Pro (Version 3.5). We specified the aggregation technique for continuous values (mean) and the cell factor for matching scales (4).

We used downscaling methodology to estimate average daily air temperatures from projected average monthly temperatures. Widely used in hydrological sciences and ecological modeling, the additive delta approach represents absolute change and can be used for many climate variables (e.g., precipitation, temperature) (Hamlet et al., 2013). Delta values, also referred to as change factors, are defined as the difference or ratio between future and baseline a given climate value. First, we calculated average air temperature of each day and month across the 25-year historical period (Equations 1-2). Equation 1 and 2 result in 366 daily (leap years included) and 12 monthly means, respectively. Next, we obtained a delta value for each unique month and year across the 15-year period by subtracting historical averages from projected averages (Equation 3). These delta values were then added to the average daily air temperature values of the historical, or baseline, period (Equation 4). For example, the estimated average air

temperatures of January 1st, 2030, equals the sum of baseline ($\bar{T}_{H(d,m)}$) and delta values ($\Delta_{F(m,y)}$) for each corresponding cell across the gridded watershed. A total of 5,693 raster files were generated and used as input in the modeling framework (See 1.10.1.1) for predicting average daily stream temperature through the future period. The equations for downscaling were:

$$\bar{T}_{H(d,m)} = \frac{\left(T_{d(m,y1)} + T_{d(m,y2)} \ldots + T_{d(m,y14)}\right)}{25} \quad (1)$$

$$\bar{T}_{H(m)} = \frac{\left(T_{H(m,y1)} + T_{H(m,y2)} \ldots + T_{H(m,y14)}\right)}{25} \quad (2)$$

$$\Delta_{F(m,y)} = T_{F(m,y)} - \bar{T}_{H(m)} \quad (3)$$

$$T_{F(d,m,y)} = \bar{T}_{H(d,m)} + \Delta_{F(m,y)} \quad (4)$$

Table 1.A1 Climate and landscape covariates used in stream temperature modeling framework. Prediction factors that were excluded or applied with modification are bolded.

Symbol	Description	Unit	Source
Tl	Antecedent air temperature	˚C	Siegel et al., 2023
Tws	Mean daily air temperature in upstream watershed	˚C	PRISM
Sws	**Mean daily snowpack (SWE) in upstream watershed**	**mm**	**NSID**
DL	Day length	hours	DayMet
D	Day of calendar year	Julian	-
Qr	**Mean area-standardized flow**	**log(m3/s)**	**NWM; Siegel et al., 2023**
SA1	**April 1st watershed SWE**	**mm**	**NSID**
Lat	Reach latitude	Latitude	NHDPlusV2
E	Mean reach elevation	m	NHDPlusV2
Ed	Difference between reach and mean watershed elevation	m	NHDPlusV2
A	Watershed area	log(km2)	NHDPlusV2
BFI	Percentage of mean flow from base flow	%	NHDPlusV2
S	Slope (gradient)	proportion	NHDPlusV2
O	Percent of watershed in open water	%	StreamCat
W	Percent of watershed covered in wetlands	%	StreamCat
I	Percent of watershed covered in ice	%	StreamCat
F	Percent of 100-m riparian buffer of local catchment forested	%	StreamCat
C	Percent canopy cover over reach streamline	%	NLDC
U	Percent of watershed covered in urban land use	%	StreamCat
V	Percent of watershed covered by extrusive volcanic rock	%	StreamCat
P	Mean annual reach-contributing-area precipitation	mm	StreamCat
R	Mean annual reach-contributing-area air temperature range	˚C	StreamCat

Table 1.A2 Stream temperature model fit statistics (RMSE = root mean squared error, MAE = mean absolute error) and temporal cross-validation predictions between whole and seasonal datasets (m = monthly average predictions, d = daily average predictions).

	Version 1		Version 2		Version 3	
	Full	Temporal	Full	Temporal	Full	Temporal
RMSE (m)	1.76	1.70	1.82	1.85	1.82	1.85
MAE (m)	1.32	1.27	1.42	1.44	1.42	1.45
RMSE (d)	1.56	1.52	1.52	1.55	1.51	1.54
MAE (d)	1.16	1.12	1.18	1.21	1.18	1.21

1.11.2 Appendix 1.2: Model structure and parameters

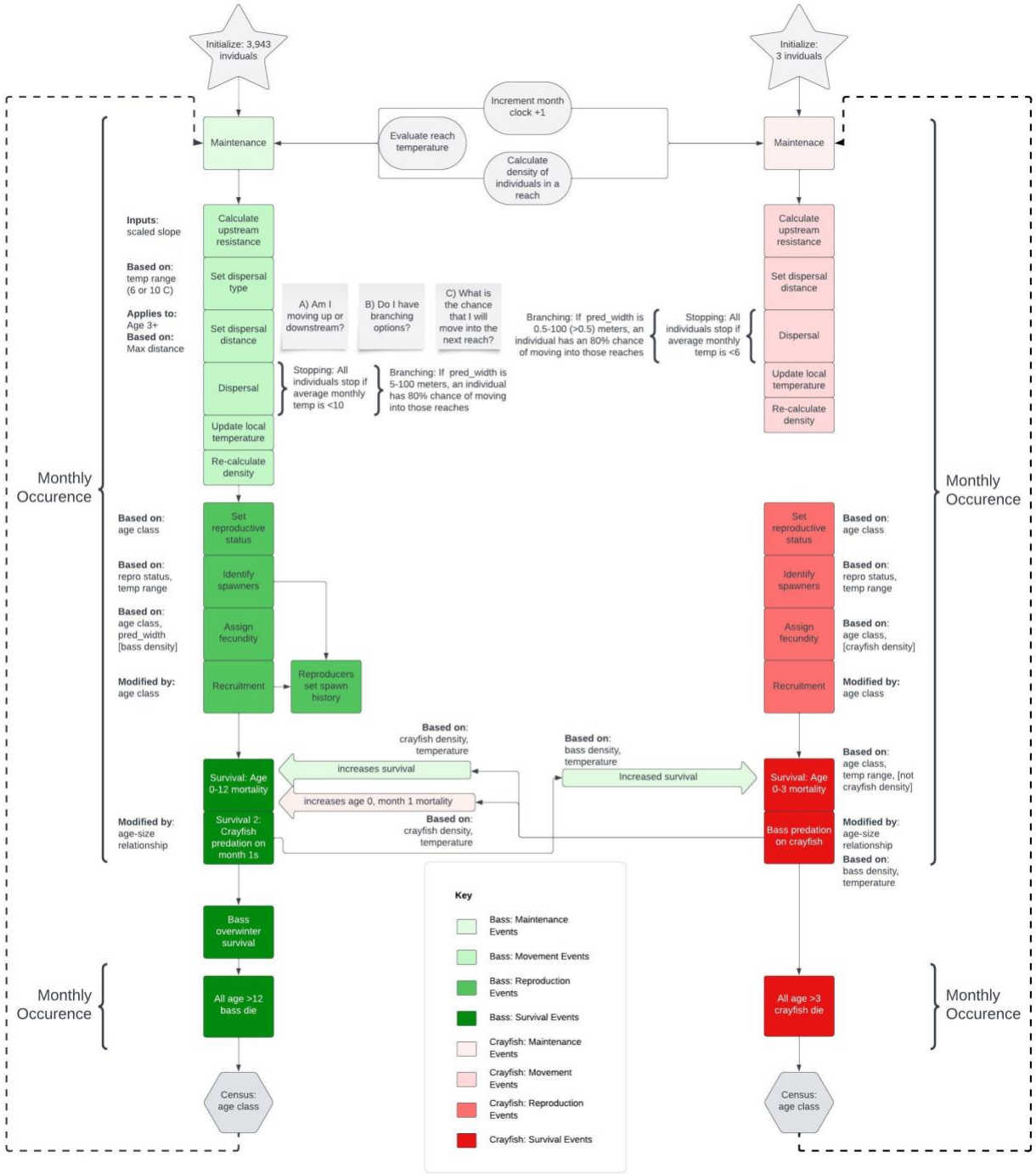


Figure 1.A1 Simulated annual cycle of events of smallmouth bass and rusty crayfish responding to environmental and cross-species interactions in the John Day River, Oregon, USA.

1.11.2.1 Smallmouth bass model structure and parameters

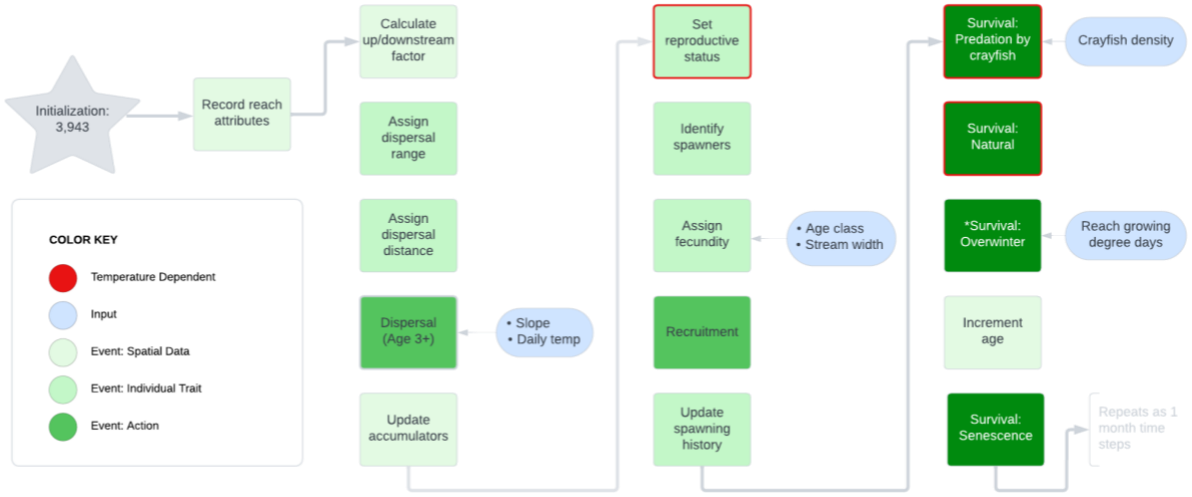


Figure 1.A2 Simulated monthly cycle of events as represented in modeled SMB population.

Movement. Within the movement event group, Movement occurred each month following a series of decision rules. Both dynamic and static values determined downstream and upstream factors that modified the total distance individuals traveled. Past studies indicate that SMB are generally active at temperatures 10˚C and above (Shuter & Post 1990) and demonstrate slower upstream dispersal due to flow shear stress experience in high-gradient channels (Lawrence et al., 2012). Accordingly, upstream resistance was calculated as a multiplicative function of transformed slope and the proportion of days in a month greater than 10˚C. Downstream resistance was a product of only temperature-based proportion. Dispersal ability and distance range were dependent on age-class according to estimates informed by telemetry data from the basin (Rubenson & Olden, 2017). Based on the assigned range, individuals randomly drew a dispersal distance from a normal distribution that was inputted into the subsequent network movement event. In the first year of life, Age 0 SMB exhibit limited movement from the nest of natal origin (Ridgway et al., 2002). Given this pattern and the average reach lengths, all young-

of-year SMB were assigned a dispersal distance of 0. Knowledge on the dispersal patterns of age 1 and 2 SMB is limited. However, recent work demonstrated that approximately 62% to 80% of individuals move from natal spawning grounds during these early life stages (Humston et al., 2017). The observed patterns are hypothesized to be a density dependent response to overcrowding in tributaries to large river systems. The presence of this mechanism is unlikely in the JDR as SMB nesting sites have been documented solely on main channels (i.e., Middle Fork). Thus, all young-of-year SMB were assigned to the smallest dispersal distance bin. A fixed proportion of juveniles and adults (ages 3-6) followed a fixed proportion that placed individuals into one of the five dispersal distance bins. Intensive tests were performed to understand the strength of mechanisms within the network movement event, and for the purpose of resolving these discrepancies. We found that individuals, across all dispersal outcomes, were responding correctly to their assigned values (e.g., distance, direction, stopping). Given the complexity of actual SMB movement, it is likely that this inaccuracy is a result of modeling limitations.

Recruitment. Reproduction of SMB is simulated within an event group comprised of accumulate, transition, and propagation events. Throughout the sequence, individual and reach-level characteristics determine the number of SMB that successfully "spawn" each month. Spawning eligibility, a trait held by individuals, changes according to a series of transition events. The first transition precedes the recruitment event group and randomly assigns a proportion of individuals, based on age class, into two categories: reproductively active and inactive. Individuals retain this status for the remainder of the year (May – April) until the event is triggered again. Within the recruitment event group, the second transition uses the births accumulator to prevent individuals from spawning more than once per year. If the number of births (propagules produced) is greater than zero, this transition assigns the individual to a

subgroup termed “already spawned.” Alternatively, if births are equal to zero then the individual joins the “waiting” subgroup. The third and last transition represents the temperature dependent nature of SMB spawning. This event functionally allows or prevents spawning based on the average monthly stream temperature of each reach, where global variables define the minimum (15˚C) and maximum (28˚C) bounds. If the average monthly temperature of a reach falls within the acceptable range (15-28˚C), all mature occupants are eligible to spawn. Maturity is determined by an annually triggered event that assigns a fixed proportion, based on age class, of randomly selected individuals to the affirmative (mature) category. Within the event group, there are two additional transition events that are iterated monthly. First, individuals who are reproductively active and have not spawned within the current year are reallocated to the eligible category. Second, the monthly average temperature of the reach is evaluated. If the temperature is greater than 15˚C and less than 26˚C, then the previous subset of individuals remains in the eligible category. If any condition is not met (i.e., age, history, temperature), then the individual is assigned to a descriptive category (i.e., too young, already spawned, too cold/warm). These assignments are then reviewed through a census event to ensure proportions are accurate in respect to stream temperatures and empirical data. Fecundity is assigned on an individual basis, with rates defined by age class and reach width. Spawning adults are divided into four categories so that ages three, four, five, and six are assigned unique values. These groups are then further split based on the classifying width of the stream segment individuals are occupying. This approach defines reaches as narrow, moderate, and wide, where fecundity positively correlates with stream width. Because fecundity values are known to vary in response to various biotic and abiotic factors, we found this method to be the most effective in representing density-dependence and carrying capacity. Further, variation among supplied averages is facilitated by supplying a

standard deviation value of either 89 or 156 depending on age class. Next, we supplied the previously calculated values and eligible spawners to a propagation event that introduces young-of-year bass (month 0) to the stream network. Each spawner records the number of new floaters that are produced as births. Any individual with birth counts greater than one is marked as having spawned and the total number of spawners is recorded with a census event. The categorization of eligibility per individual SMB is then updated and saved on a continuous monthly basis until the final step of each year (April).

Survival. Survival rates of SMB are represented in the model in four ways and recur monthly. First, natural mortality is variable in response to age class and average monthly temperature. Drawing on empirical rates of riverine population survival, we set a maximum proportion of individuals that will survive each month. This value is implemented for individuals within the optimal temperature range relative to each age class. For example, the maximum monthly survival rate of juvenile SMB aged 1-2 is 0.96. That rate is applied to individuals occupying reaches with an average monthly temperature between 15 to 27˚C. To integrate temperature-dependent factors, we set a lower threshold of 0.91 (-0.05) at the lowest and highest average monthly stream temperatures. Survival rates for individuals occupying reaches above or below their thermal optimum were interpolated from a linear regression between the high and low survival rates. This structured calculation is not applied to young-of-year SMB (age 0, months 0-11) because mortality is accounted for with constant values, which is addressed shortly. Senescence is also applied monthly, where individuals aged 72 months, or will be 7 years of age at the following time step, are removed from the model. Because overwinter survival of young-of-year smallmouth bass, hereafter YOY, is a well-studied area of the species, we chose to represent their survival with a combination of nest-centric failure rates and latent growing degree

days (GDD). GDD are known as the highest predictor of YOY survival across SMB populations. Using predicted stream temperatures, we calculated the number of GDD each YOY would accumulate once they are initialized in the model. YOY SMB acquire their respective number of GDD at month 4. This value is queried by a survival event that is annually triggered in April, or the last month of yearly intervals. For individuals that persistent, this event is the final determinant for transitioning to age 1 individuals. A pre-calculated approach was feasible in that YOY are not known to move beyond the nest site, particularly in the first two months of life and mortality is largely observed at the culmination of the winter season. Additionally, the modeled interactions with RC relied on the inclusion of egg and larval stages of SMB. However, the high fecundity rates and large population of SMB facilitated a computationally high load. This structure enabled us to simultaneously include the required life stages for RC predation and avoid unnecessary retention of YOY individuals. While GDD are a powerful factor in predicting persistence, we set a three-month sequence of fixed monthly mortality rates to align with empirical survival rates across larval stages. This 3-tier survival event addresses a myriad of natural mortality factors, such as resource competition.

1.11.2.2 Rusty crayfish model structure and parameters

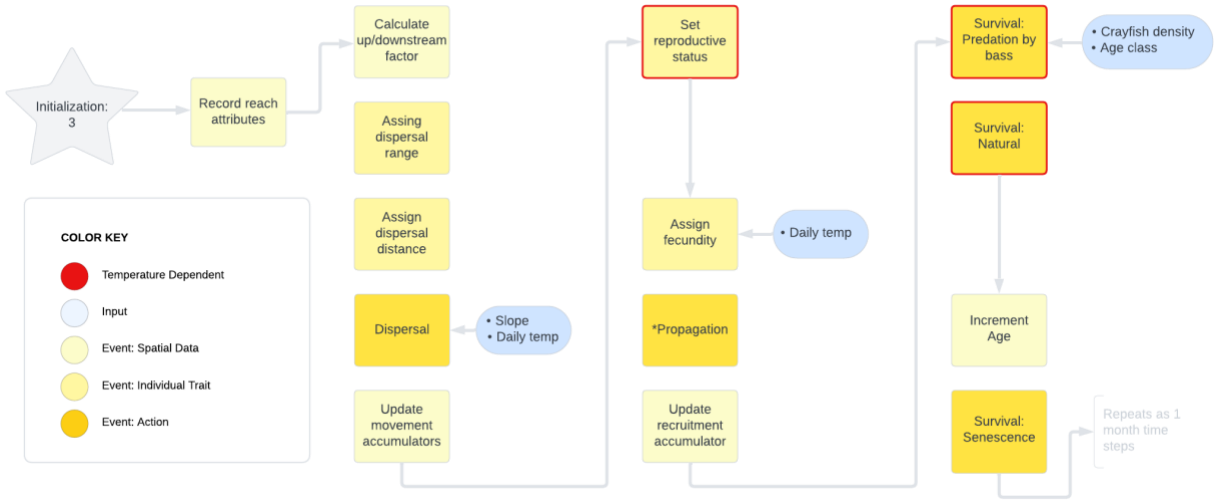


Figure 1.A3 Simulated monthly cycle of events as represented in modeled RC population.

Movement. To begin the movement event group, the upstream and downstream factors are calculated and stored as a network-based property. The upstream factor is the product of the proportion of days above 6 ˚C and a fixed value corresponding to the stream reach gradient. This value translates to the resistance felt by individuals who elect to move upstream. Conversely, the downstream value is solely based on the proportion of days above 10 ˚C. This contrast is representative of the passive movement ability of crayfish which manifests in biased movement downstream. Next, dispersal distances are assigned to individuals. These distances range from 0 to 8,000 meters, where one bin corresponds to a 500-meter span. Age 0 to 3 crayfish are allocated to a new bin each month in reference to a fixed proportion. Individuals then draw a randomized distance from within their bin based on a uniform distribution. Network movement is then implemented, where there is an even probability that a floater will move upstream or downstream. The individual references the assigned distance to move but modifies the value according to the direction. Additional stopping and branching factors apply. An individual will not move the prospective distance if there are no days above the 6 ˚C threshold in their current

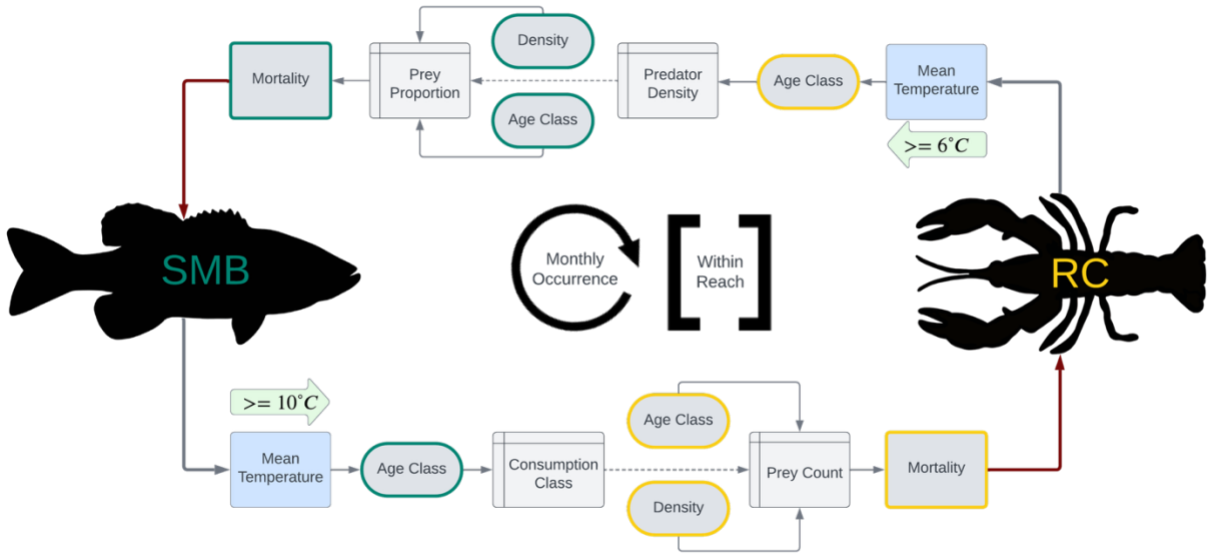
reach. This condition also prevents movement into reaches with no days above 6˚C, forcing the individual to stop at the node between their current and desired reach. For branching, or deciding between multiple reaches in which to enter, individuals are given an 80% likelihood of selecting a reach where daily water temperatures are all above 6˚C. These rules are representative of the predominantly temperature-based movement of crayfish in riverine systems. Total distance moved on both month and annual scales are stored within individual-level accumulators.

Recruitment. Reproduction of RC is represented by an event group that is triggered once annually. In alignment with observed patterns in spawning across the JDR, propagation of individuals likely occurs only in May. Additionally, we supplied fecundity values that are representative of the number of free-floating juveniles produced by each female. This prior calculation addresses natural mortality of crayfish propagules prior to reaching the juvenile stage. At the start of the event group, a proportion of individuals are deemed sexually mature based on age class. This subset of able individuals is then reassigned based on the average monthly temperature of their occupied reach. For example, if an aged 2 floater is sexually mature and occupies a reach above 5˚C, they can draw a fecundity value and are used in the following propagation event. All eligible individuals draw a fecundity value based on age class, which is then modified in response to the density of crayfish in the reach. Density-dependent fecundity and fixed standard deviation values are supplied to the propagation event that introduces age 0 RC. The outcomes of individual-level recruitment are tracked, providing reasons for cases of ineligibility (i.e., too young, too hot/cold).

Survival. Survival for RC mirrors the structure of bass survival in that rates are modified when individuals are outside the ideal temperature ranges. Fixed rates of 0.97, 0.94, and 0.72 are applied to ages 1, 2, and 3 adults respectively. When adult RC are in a reach above 26˚C or

below 18˚C, the rate declines to a maximum of 0.05 from the fixed values. These modified values were calculated using the maximum and minimum temperatures of the time series and extrapolating from a linear regression. Juveniles, aged 0, follow the same structure but call on a maximum survival rate of 0.91 in temperatures between 16 to 22˚C. Crayfish above age 3 are rarely observed in the JDR, thus individuals above the 36-month limit are removed via a survival event. At the end of each step, individuals update their age by one-month increments. At the end of each year (April), annual distance and other accumulated values are cleared. To manage the potential die-off prior to the first reproduction event, survival events are triggered at time step 13 which is the beginning of year 2.

1.11.2.3 Interaction events



Like those representing life history of each species, interaction event groups operated on the monthly cycle. SMB were subject to predation by RC each month where a proportion of SMB prey was consumed by adult RC. We limited RC predators to adults (i.e., age-1 to age-3) whose average carapace length and weight represents the minimum size of documented individuals. Baldridge & Lodge (2013) estimated daily consumption by collecting individual chelae within nesting sites then calculated the carapace length *(L)* and mass *(B)* of each RC consumed by: $L = 4.0708*(c^{0.632})$ and $B = 0.000138*(L^{0.28})$.

A bioenergetics model was used to estimate how many RC prey were consumed by SMB as a function of reach-scale factors: RC density, predator weight, stream temperature. Following the approach used by Whitledge et. al. (2003), we determined specific consumption rates (C, grams consumed per gram of body weight per day) across the four adult age classes of SMB (age-3/4/5/6) as:

$$C = C_{max} \cdot P \cdot f(T)$$

where C_{max} is the maximum specific consumption rate, P is defined by a proportionality constant, and $f(T)$ represents a function of temperature dependency (Hewett & Johnson, 1987). Maximum specific consumption rate (C_{max})

$$C_{max} = CA \cdot W^{CB}$$

$$f(T) = V^{X} \cdot CQ^{(X \cdot (1-V))}$$

To estimate weight (W; grams) of each age class, we calculated mean length-at-age (L) from a proxy population (Zweifel et al., 1999) and applied these averages to a weight-at-length equation from Beamesderfer (1994):

$$W = (1.01 \cdot 10^{-5}) \cdot L^{3.07}$$

(Whitledge et al., 2003).

$$W = Ln(CQ) \cdot (CTM - CTO)$$

$$Y = Ln(CQ) \cdot (CTM - CTO + 2)$$

$$X = \frac{W^2 \cdot (1 + (1 + 40/Y)^{0.5})^2}{400}$$

$$V = (CTM - T)/(CTM - CTO)$$

Drawing on these class assignments, eligible SMB are defined through a network generated property that stores the number of consumers by reach. The occupancy of consumers is then multiplied by the expected prey count to define the number of RC adults, by reach, that could be consumed that month. Using an accumulator expression, we marked individuals by a binary outcome where individuals with a value of 1 will be removed from the network. A survival event represents the predation outcome for that subset of individuals.

1.11.3 Appendix 1.3: Literature informing model parameters

1.11.3.1 Smallmouth bass life history

	Attribute	Parameter	Source
Life History	Population	*Initialization*	Shrader, 1999
	Range	*Native*	Iguchi et al., 2004; Adey et al., 2009
		Invasive	Quinn & Paukert, 2009
	Growth	*Size correlated with density*	DeAngelis, 1991
		Length by age class	Bennett, 1991
		Length frequency distributions	Shrader, 1999
		YOY lengths + adult density	Rubenson, 2019
		Length-frequency distribution	Beasmederfer, 1994
		Size of predatory individuals	Pribyl, 2005
Population	Age Distribution	*Age structure*	ODFW, 2016
		Population composition by age	Rubenson, 2019
		Age-frequency proportion	Shrader, 1999
		Population composition by age	Shuter, 2002
		Year-class strength index	Beasmesderfer, 1994
	Abundance	*Population estimate, range*	Shrader & Gray, 1999
Movement & Range	Features/Types	*Proportion of dispersers*	Humston, 2017
		Seasonality	Shrader, 1999
		Barrier to upstream expansion	Lawrence, 2015
		Seasonal growth	Lawrence, 2015
		Distribution retention	Rubenson, 2019
		Site fidelity	Rubenson, 2019
		Proximal population	Munther, 1970
		Distance ranges, proportions	Funk, 1957
		Up & downstream estimates	Todd, 1989
		Distance ranges, proportions	McClure, 2020
Demography	Reproductive Success	*Nest success*	Cooke, 2003
		Nest success	Rubenson, 2019
		Spawning, flow fluctuations	Beasmesderfer, 1994
	Survival	*Juvenile*	Loppnow, 2014
		Survival proportions	Barthel, 2008
		Survival proportions	Beasmesderfer, 1995
		Annual mortality rate	Shuter, 1987
		Mortality rate estimates	Shrader, 1999

		YOY survival rates	Lawrence, 2015
		Natural mortality average	Beamesderfer, 1995
		Annual mortality by age	Paragamian, 1984
		Survival proportions	Loppnow, 2014
		Annual rates	Beamesderfer, 1995; Sterns,
		Annual rate, yoy	Dunlop, 2007; Knotek, 1998
		Annual rate, juvenile & adult	Shuter, 1987; Coble, 1971
		Annual rate, juvenile	Kilambi, 1977
		Annual rate, adult	Wrenn, 1980
		YOY temperature dependency	Kerr, 1966; Coutant, 1975; Shuter & Post, 1980
	Fecundity	*Egg deposition by age class*	Kilambi, 1977
		Density dependence	Rubenson, 2019
Stressors, Response	Temperature	*Tolerance, all*	Sorenson, 2012; Horning and Pearson, 1973
		Tolerance, embryo	Kerr, 1966
		Tolerance, yoy	Coutant and DeAngelis, 1983
		Tolerance, juvenile	Horning and Pearson, 1973
		Tolerance, adult	Wehrly, 2003
		Spawning	Shuter, 1980; Graham and Orth, 1986
		Optimal, all	Larimore and Duever, 1968
		Optimal, yoy	Wrenn, 1980; Barans and Tubb, 1973; Cherry, 1975
		Optimal, juvenile	Reynolds and Casterlin, 1976; Cherry, 1977
		Optimal, adult	Smale and Rebeni, 1995
		Crayfish consumption	Shuter, 1980; Hanke, 1981; Zweifel, 1999
		Gape limtations	Schake, 2014
	Bioenergetics	*Proportion*	Roell, 1993
		Metabolic cost	Lawrence, 2012
		Diet composition	Westhoff, 2016; Anderson, 1981
		Model constants	Westhoff, 2016
	Predation	*Nest failure*	Rubenson, 2019
		Nest abandonment	Baldridge & Lodge, 2012

1.11.3.2 Smallmouth bass initialization range

Data Records for Model Validation

Smallmouth Bass

Citation	Year	Type	Presence (#)	Absence (#)
(Wilson, 2007)	2010	Presence, Counts	4	N/A
(Wiley et. al., 2006)	2010	Presence, % Catch	3	45
(Wilson et. al., 2011)	2010	Presence, Counts	3	1
(DeHart et. al., 2012)	2010	Presence, Counts	2	1
(Lawrence et. al., 2012)	2010	Snorkel Survey	4	4
(Bare et. al., 2016)	2016	Presence, Counts	4	1
(Bare et. al., 2017)	2016	Presence, Counts	4	1
(Rubenson & Olden, 2019)	2020	Presence, Counts	27	7
(Rubenson & Olden, 2016)	2016	Snorkel Survey	2	2
(Franklin et. al., 2018)	2020	Snorkel Survey	2	1
(Bare et. al., 2019)	2020	Presence, Counts	4	0
(Henderson et. al., 2023)	2020	Presence, Counts	2	3

1.11.3.2 Rusty crayfish

Attribute		Parameter	Source
Life History	Site	*Initialization*	Larson & Olden, 2008
		Initialization	Olden, Adams, & Larson, 2009
		Initialization	Miller & Sytsma, 2014
	Population	*Frequency*	Corey, 1988
		Frequency	Hamr, 1997
		Age classes	Hein et al., 2006
		Age range	Holdich & Crandall, 2002
		Maturity	Lorman, 1980
		Maximum age	Prins, 1968
		*Age classes**	Sorenson, 2012
Movement & Range	Density Dependence	*Adult-juvenile effect*	Hansen et al., 2013
		Fecundity effect	Hein et al., 2006
		Survival	Momot, 1984
		Fecundity effect	Momot & Gowing, 1977
	Speed	*Range contraction*	Bobeldyk & Lamberti, 2008
		Distance maximum	Bryon & Wilson, 2001
		Habitat association	Bubb et al., 2004
		Range extension	Charlebois & Lamberti, 1996
		Lotic distribution	Dress & Swanson, 2013
		Annual rate	Hudina et al., 2009
		Annual rate	Jansen et al., 2009
		Interannual variation	Kuhlmann & Hazelton, 2007
		Sub-daily rate	Lorman, 1980
		Secondary spread, upstream	Momot, 1996
		Lentic distribution	Perry et al., 2001
		Annual rate	Puky & Schád, 2006
		Annual rate	Sorenson et al., 2012
		Annual rate	Wilson et al., 2004
	Spread Rate	*Predation pressure*	Bulter & Stein, 1985
		Dispersal, population growth	Gallien et al., 2010
		Predation pressure	Hill & Lodge, 1994
		Behavioral response	Kamran & Moore, 2015
		Environmental attributes	Kershner & Lodge, 1995
		Environmental attributes	Perry et al., 2001
	Velocity	*Behavioral response*	Bobeldyk & Lamberti, 2008
		Distance maximum	Bubb et al., 2004
		Behavioral response	Clark et al., 2008
		Density	Flinders & Magoulick, 2007
		Habitat association	Foster & Keller, 2011

	Depth	*Behavioral response*	Maude & Williams, 1983
		Sex	Maude & Williams, 1983
		Habitat association	Prins, 1968
		Sex	Sorenson, 2012
		Passive dispersal	Wubs et al., 2016
		Habitat association	Claramunt et al., 2005
		Habitat association	Hein et al., 2007
		Habitat association	Jansen et al., 2009
		Habitat association	Taylor & Redmer, 1996
	Temperature	*Minimum threshold*	Bubb et al., 2002
		Directional patterns	Buric et al., 2009
		Thermal optimum	Claussen, 1980
		Thermal optimum	Ellrott et al., 2007
		Minimum threshold	Hamr, 1997
		Daily patterns	Johnson et al., 2014
		Thermal optimum	Keller & Hazlett, 2010
		Behavioral response	Lozán, 2000
		Thermal optimum	Mundahl, 1986
		Thermal optimum	Mundahl, 1989
		Thermal optimum	Mundahl & Benton, 1990
		Minimum threshold	Prins, 1968
Demography	Reproductive Status	*Age 2-3*	Corey, 1988
		Age 1-3	Hamr, 1997
		Age 2-3	Kuhlmann, 2008
		Age 1-3	Langlois, 1935
		Age 1-3	Lorman, 1980
		Age 1-3	Prins, 1968
		Age 1-3	Sorenson, 2012
	Survival	*Non-native range*	Arcellea et al., 2014
		Age 3+	Corey, 1988
		Habitat association	Hansen et al., 2013a
		Age-based proportion	Hein et al., 2006
		Age 2	Hill & Lodge, 1999
		Habitat characteristics	Kershner & Lodge, 1995
		Seasonal patterns	Lorman, 1980
		Age 0	Momot, 1984
		Age 0	Momot & Gowing, 1977
		Size relationship	Prins, 1968
	Fecundity	*Age 1-3*	Vannote & Ball, 1963
		Recruitment class size	Arcella et al., 2014
		Inner population variation	Corey, 1987

Lotic production	Corey, 1988
Reproductive classification	Corey, 1990
Average production by age	Hein et al., 2006
Egg mortality/loss	Lorman, 1980
Egg mortality/loss	Mason, 1975
Clutch size variation	Momot and Gowing, 1977a
Egg production	Prins, 1968
Egg production, clutch size	Sorenson, 2012

Chapter 2. The impacts of an invasive crayfish (*Faxonius rusticus*) on the early life history of an anadromous salmonid (*Oncorhynchus mykiss*)

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2.1 Abstract

Invasive species directly threaten anadromous salmon and steelhead by competing for resources and consuming individuals in early stages of life. Understanding the ecological impacts of invaders in salmon-bearing streams is paramount to maintaining ecosystem functions and conserving biodiversity. Characterized by unique polytrophic feeding habits, crayfish are known to produce strong, negative effects that vary across levels of freshwater food webs. A greater understanding of how invasive crayfish alter ecosystem processes (i.e., primary productivity) and affect native biota (i.e., egg predation) will be critical as these events increase in frequency and scope. The aim of this study is to investigate direct effects of rusty crayfish (*Faxonius rusticus*) along the leading edge of invasion, where a density gradient spans prime spawning and rearing habitat of steelhead (*Oncorhynchus mykiss*). By examining trophic position, isotopic niche overlap, and macroinvertebrate community composition, we found highly variable resource use by rusty crayfish throughout the South Fork of the John Day River (Oregon, U.S.A.). Stable isotope analyses revealed that the average trophic position at upstream sites was significantly lower than downstream sites, potentially resulting from interspecific competition or opportunistic predation. We found considerable isotopic niche overlap between crayfish and smolts that suggest competitive effects may increase during the freshwater rearing phase. However, crayfish density does not appear to impact abundance of preferred prey for juvenile salmonids. While exploratory in nature, fatty acid analysis revealed relatively high levels of marine-derived nutrients in adult crayfish. This work demonstrates that invasive crayfish may have direct effects on juvenile steelhead, particularly during latter phases of development, through competition and predation.

2.2 Keywords

Freshwater ecology, stable isotopes, fatty acids, nonnative species, steelhead

2.3 Introduction

Invasive species remain a predominant threat to freshwater ecosystems worldwide, reducing biodiversity and disrupting ecological functions (Moorhouse & Macdonald, 2015; Reid et al., 2019; Ricciardi & Macisaac, 2010). Mounting evidence points to a diversity of ways in which invasive species can directly impact freshwater communities through mechanisms such as predation, competition, habitat alteration, and even disease transmission (Cucherousset & Olden, 2011; Gallardo et al., 2016; Olden et al., 2022). The increasing number of aquatic invasive species and the threats they pose emphasize the need for a greater understanding of the complex ways in which these species integrate into food webs (Vagnon et al., 2022). Such knowledge helps to anticipate the vulnerability of freshwater ecosystems to invasions and determine how best to prioritize prevention and control efforts in both space and time (Kumschick et al., 2012), including eradication programs that minimize undesirable consequences by accounting for multispecies interactions (Bode et al., 2015; Lurgi et al., 2018).

In the Pacific Northwest (PNW), many conservation challenges face anadromous salmon and steelhead that hold a significant role in ecological, economic, and cultural health (Ruckelshaus et al., 2002). Among the many threats related to environmental change and hatchery practices (Crozier et al., 2008; Macneale et al., 2010; McMillan et al., 2023), invasive species are an emerging and growing concern that impact the persistence of native salmonids (Sanderson et al., 2009). Considerable research has documented the impacts of direct predation of salmonid species by a host of species (Wells et al., 2025), including by native species (Beamesderfer et al., 1996; Carey et al., 2012) and nonnative sport fishes (Carey et al., 2011; Murphy et al., 2021; Sanderson et al., 2009). Much less recognized and understudied are

potential consequences of invasive crayfish that are becoming increasingly frequent and abundant in the region (Larson & Olden, 2011).

Invasive crayfish can impact freshwater ecosystems in consequential ways (Lodge et al., 2000). Successful crayfish invasions can be attributed to polytrophic feeding habits that are known to produce ecosystem-wide consequences, including effects on nutrient dynamics, submerged macrophytes, and benthic macroinvertebrates (Matsuzaki et al., 2009; Twardochleb et al., 2013). Direct effects of invasive crayfish (e.g., altered basal resource abundance, reduced benthic fish growth) have been shown to increase with density and remain detectable even at low densities (Galib et al., 2022). Despite the rising frequency and widespread distribution of crayfish introduction events, there is a limited number of studies that have explored the effects (e.g., competition, predation) of invasive crayfish on salmonid species (Olden et al., In press).

Impacts on salmon and steelhead may be considerable, as invasive crayfish commonly reach greater densities, exhibit aggressive behavior, and show comparatively high consumption rates of primary producers and consumers relative to native species (Faria et al., 2023; Twardochleb et al., 2013). Crayfish opportunistically feed on salmonid eggs and larvae (i.e., alevin), potentially lowering recruitment among salmonid populations (Claramunt et al., 2005). A variety of crayfish species have been documented searching for and consuming salmonid eggs (Olden et al., In press), and even demonstrating preference towards the nutrient-rich resource (Edmonds et al., 2011; Love & Savino, 1993). Research within this scope has exposed numerous biotic (e.g., predator abundance) and abiotic (e.g., burial depth, temperature) factors that determine interaction outcomes across the unique life histories of salmonids. These findings encourage additional research into the direct effects of invasive crayfish on populations of native salmon and steelhead in the PNW.

Field investigations offer opportunities to better understand the ecological consequences of invasive species. Biotracers, such as stable isotopes of carbon and nitrogen, are now commonly used to estimate resource use and the trophic niche of individuals (Newsome et al., 2007), particularly in response to changing environments (Alp & Cucherousset, 2022). Past studies have used stable isotope analysis (SIA) to explore the consequences of invasive species by evaluating changes in food web structure (Vander Zanden & Rasmussen, 1999), estimating diets of omnivorous invaders (Stenroth et al., 2006), and quantifying complex interactions between invaders across trophic levels (M. C. Jackson et al., 2012). SIA has proven particularly useful for revealing evidence of interspecific competition between native and invasive species by quantifying the degree of overlap between isotopic niches (Jardine et al., 2003; McCue et al., 2020; Walsworth et al., 2013). More recently, studies have leveraged the distinctively high nitrogen signatures found in anadromous fish to investigate predation by other species (Murphy et al., 2021; Rubenson et al., 2020). Resource competition and predatory impacts of crayfish can be more broadly assessed by combining these methodological approaches (Adey et al., 2018; Reynolds, 2011).

The John Day River is illustrative of present and future concerns regarding crayfish invasions in the PNW. First introduced over 20 years ago, nonnative rusty crayfish (*Faxonius rusticus*) has rapidly spread and reached extremely high densities in the John Day River and its tributaries. Rusty crayfish have been associated with declines in macrophytes, benthic invertebrates, and fish populations (Glon et al., 2016). In other invaded systems across North America, rusty crayfish have exhibited higher per-capita feeding rates than native crayfish species, depleting resources for other species. Rusty crayfish in the John Day River have expanded upstream toward the system's headwaters that provide spawning and rearing habitat for

two threatened, ESA-listed species: Chinook salmon (*Oncorhynchus tshawytscha*) and steelhead (*Oncorhynchus mykiss*). This includes widespread occurrence and high densities in the South Fork John Day River where they overlap with the range of summer-run steelhead. As steelhead spawning and rearing habitat becomes increasingly inundated by rusty crayfish, uncovering evidence of competitive interactions with and predatory behavior towards juvenile steelhead is pivotal for informing future management action.

This study seeks to understand whether, and if so how, rusty crayfish are impacting the early life history stages of steelhead through the mechanisms of competition and predation. The objectives were to: (1) examine the trophic ecology of rusty crayfish across an invasion density gradient in the South Fork of the John Day River, (2) quantify resource use of, and isotopic overlap between, rusty crayfish and young steelhead (i.e., fry, smolts), and (3) evaluate evidence for direct consumption of steelhead by rusty crayfish using stable isotopes and fatty acid analysis. By clarifying the trophic role of rusty crayfish, following recent introduction and expansion, we aimed to better understand potential impacts to steelhead and address knowledge gaps for future management of federally protected salmonid populations.

2.4 Methods

2.4.1 Study system

The South Fork of the John Day River (hereafter, SFJDR) is an 89-kilometer-long tributary to the John Day River and defines a 2,176 km^2 watershed. The SFJDR originates in the Ochoco and Aldrich Mountains and meets the mainstem near Dayville, Oregon. Boasting a diverse spread of shrubland, woodlands, and coniferous forests, the four major tributaries to the SFJDR are Deer Creek (DC), Wind Creek (WC), Black Canyon Creek (BC), and Murderers Creek (MC), with

Izee Falls representing a natural dispersal barrier (Figure 2.1). Adult steelhead spawn in the early spring and fry emerge from redds in early April to mid-June (Lindsay et al., 1989). Juvenile steelhead exhibit two life history strategies, either immediately out-migrating or rearing in freshwater for up to a year. Rusty crayfish, native to the Ohio River Basin, were first observed in the John Day River in 2005 but were likely released in 1999 after a small number of individuals were kept in a local classroom (Olden et al., 2009). Since the initial introduction, rusty crayfish population has spread rapidly downstream and upwards, encompassing over 700 kilometers of the John Day River, including the SFJDR up to Izee Falls (Messager & Olden, 2018).

2.4.2 Field surveys and sampling

Surveys across the SFJDR were conducted from June 19 to July, 2023, representing the end of steelhead spawning and the period of subsequent fry emergence (Carmichael et al., 2015). Field reconnaissance prior to the study informed the spatial distribution of survey efforts to ensure a range of crayfish densities that included un-invaded reaches. This resulted in a total of 20 primary sites (Table 2.1) that were evenly distributed throughout the South Fork (10 sites) and each of the major tributaries (7 sites in MC, 1 site in each of DC, WC, and BC). In addition, we surveyed (kick-seine, snorkeling) 10 secondary sites during the field study to further quantify spatial patterns in crayfish density and steelhead abundance.

For each primary and secondary site, we selected a downstream starting point based on proximity to the randomized coordinates and delineated a total reach length as the wetted channel width multiplied by 40, with a 150-m maximum length (CHaMP 2016). Snorkel surveys for steelhead were completed before the crayfish sampling. Starting at the downstream edge, two snorkelers surveyed opposite shorelines upstream while a third person recorded habitat unit type (e.g., riffle, run, pool, glide) and length with a range finder, along with fish counts and habitat

characteristics such as woody debris. After the snorkel survey, we delineated11 equally spaced transects spanning the entire reach. At each transect, riparian and geomorphic characteristics were measured at left, center, and right points (25-50-75% of wetted width), following National River and Streams Assessment protocol (EPA 2019). Habitat metrics were selected for their relevance to steelhead fry suitability, including canopy cover, depth, and substrate type. Table 2.1 summarizes characteristics by site. The order in which sites were surveyed was randomized.

At every other transect (6 out of 11 transects), we used area-standardized kick-seining to estimate the density of rusty crayfish. After delineating a 1-m^2 area with a chain, one person disturbed the substrate upstream of a seine net held by another team member to flush crayfish downstream, yielding a mean and standard deviation of crayfish density at each site. To ensure consistency in our measure of relative density, we exclusively sampled in runs (i.e. rather than pools or riffles) when possible, because runs provide the water velocity and depth needed for this sampling method to be most effective (Larson & Olden, 2016). Individual crayfish were identified by species, counted, and temporarily placed in a bucket for processing separately. We recorded the sex, carapace length (mm), weight (mg), and ovigerous status of each crayfish, using random selection when the total number of individuals exceeded 60, and placed whole specimens into sealable plastic bags that were frozen for stable isotope analysis. During the sampling period, we collected steelhead fry with permission from NOAA (Permit #1998-016-00). ODFW provided additional fish tissue samples, including caudal fin clips from out-migrating steelhead smolts (South Fork rotary screw trap), pre-spawn hens (Wallowa Fish Hatchery; Enterprise, OR), and whole-body alevin and fry (Irrigon Fish Hatchery; Irrigon, OR). Prior work suggests that fin clips, a non-lethal form of tissue collection, provide estimates of $^{13}C/^{12}C$ and $^{15}N/^{14}N$ that are similar to those from muscle tissue (Sanderson et al., 2009).

Sampling of benthic macroinvertebrates, periphyton, and filamentous algae was performed at each of the primary sites. First, we estimated community composition by collecting a single Surber sampler (area: 0.9 m^2; mesh size: 500 µm) at each of the 11 transects within a site. Each sample was gathered by manually disturbing the substrate up to 5 cm for a period of 1-minute and then stored collectively in ethanol. Second, we used a 500-µm D-net to collect representative samples of aquatic insects from various microhabitats that were then identified, sorted at a family-level, and stored for subsequent stable isotope analysis. Periphyton was removed from three rocks (cobble-size substrate) based on varying degrees of depth and sunlight exposure. We collected each sample by (1) delineating a 12 cm^2 area on the upper surface of each rock, (2) brushing with a stiff-bristled toothbrush for 30-seconds, (3) washing the dislodged periphyton with deionized water into a graduated cylinder (500 mL), and (4) if present, allowing fine sediment to settle. Samples were then concentrated from ~25 mL aliquots onto ashed 0.7-µm quartz fiber (QM-A) filters (Whatman, Maidstone, United Kingdom) using vacuum-pressurized filtration apparatus. We also collected filamentous algae samples (n = 3) at each site, following the selection protocol. All primary producer samples were placed in falcon tubes and stored away from sunlight until they could be frozen.

2.4.3 Laboratory processing

All samples were stored in a -20˚C freezer prior to returning from the field site but were then relocated to a -80˚C freezer for long-term storage. Benthic macroinvertebrate samples were identified and sorted under a stereomicroscope (Meiji EMZ-45957) in accordance with the U.S. Environmental Protection Agency Bioassessment Macroinvertebrate Protocols (Barbour, 1999). We classified all individuals within each sample to the lowest practical taxonomic level (typically order or family) and assigned functional feeding groups (e.g., scrapers, shredders,

predators) (Merritt & Cummins, 1997). A representative sample of crayfish (n = 12) was measured (carapace length, wet mass) and dissected to extract tail muscle and hepatopancreas tissue for stable isotope analysis and fatty acid analysis, respectively. Known assimilation rates for various species of freshwater crayfish informed our selection to process these two tissue types. Lipid extraction or acidification can be performed on samples that are isotopically light, but prior research suggests this additional step is unnecessary for those which exclude inorganic carbonate found in the exoskeleton (Pinnegar & Polunin, 1999). Additionally, lipid extraction does not significantly affect isotopic values within crayfish tail muscle tissue (Bodin et al., 2007). Following analysis, we reviewed carbon values from muscle tissue to confirm they were under the recommended threshold (Post et al., 2007).

All biological samples (n = 818) were prepared following the protocol developed in the Holtgrieve Ecosystem Ecology Lab (HEEL). We oven-dried the samples at 60˚C for a 48-hour period before homogenizing using a mortar and pestle. Due to their high lipid content, hepatopancreas samples were freeze-dried. Samples were weighed and packed into tins based on carbon and nitrogen content (%): macroinvertebrate tissue (1.25 µg ± 0.25), primary producers (3.50 µg ± 0.75), and steelhead tissue (1.00 µg ± 0.75). All samples were processed by the Cornell University Stable Isotope Laboratory (COIL) to determine carbon ($^{13}C/^{12}C$) and nitrogen ($^{15}N/^{14}N$) isotope ratios in reference to internal standards of COIL and international standards for carbon and nitrogen (Vienna Pee Dee Belemnite, atmospheric air). These isotope values are reported as delta values (δ) in per mil units (‰) and calculated as: $\delta^{13}C$ or $\delta^{15}N = [(R_{sample}/R_{standard}) - 1] \times 1000$, with R representing the ratio of heavy to light isotopes.

Fatty acids (FA), an additional ecological marker, have been paired with stable isotope ratios to elucidate trophic positions of benthic organisms (Nyssen et al., 2005). A random

selection of freeze-dried and homogenized hepatopancreas samples (n = 14; 1–1.5 mg) from rusty crayfish were processed following the protocol from HEEL, adapted from Brett et. al. (2009). Total lipids were extracted with a chloroform-MeOH (2:1) solution. Fatty acid methyl esters (FAME) were obtained by dissolving each sample in toluene, adding 1% H_2SO_4 in methanol, and incubating for 18 hours at 50˚C. FAMEs were analyzed using a HP6890 gas chromatograph with a DB-23 column (J&W Scientific) and flame ionization detector. The standards used to identify peaks were from Supelco 37 (Sigma Aldrich) and from instrument-specific data established by the Lake Lab in the University of Washington's Chemical and Environmental Engineering Department.

2.4.4 Stable isotope analysis

We examined the relative position of rusty crayfish and steelhead fry and smolts by analyzing $\delta^{13}C$ and $\delta^{15}N$ values in isotopic space (Layman et al., 2007). Additional insight into the structure of food webs can be revealed by quantifying shifts across spatial environmental gradients and isotopic niche space (Layman et al., 2012). In aquatic ecosystems, $\delta^{15}N$ values of primary consumers provide a baseline for inferring the trophic positions of other consumers (Vander Zanden & Rasmussen, 1999). Dispersion in isotopic space (i.e., niche width), paired with isotope values of potential food sources, were used to estimate both individual and population-level resource use (Newsome et al., 2007). All data processing and analyses were performed in R (Posit team, 2025).

Baseline corrections were calculated for $\delta^{13}C$ and $\delta^{15}N$ to account for variation in basal resource between sites (See Appendix). Following Olsson et al. (2009), we converted $\delta^{13}C$ values to corrected carbon isotope ratios ($\delta^{13}C_{corr}$) with the following equation:

$$\delta^{13}C_{corr} = (\delta^{13}C_c - \delta^{13}C_{meaninv}) / CR_{inv}$$

where $\delta^{13}C_{corr}$ is the corrected carbon isotope ratio of crayfish, $\delta^{13}C_c$ is the uncorrected isotope ratio of crayfish, $\delta^{13}C_{meaninv}$ is the mean invertebrate isotope ratio, and CR_{inv} ($\delta^{13}C_{max}$ - $\delta^{13}C_{min}$) is the invertebrate carbon range. We calculated the trophic position of crayfish using $\delta^{15}N$ values in the following equation:

$$TP_c = ((\delta^{15}N_c - \delta^{15}N_{base}) / 3.4) + 2$$

where TP_c is the trophic position of individual crayfish, $\delta^{15}N_c$ is the isotopic ratio of individual crayfish, and $\delta^{15}N_{base}$ is the isotopic ratio of primary consumers, 3.4 equals the trophic discrimination factor in $\delta^{15}N$, and 2 represents the trophic position of the baseline consumer. We used Western pearlshell (*Margaritifera falcata*) as the baseline ($\delta^{15}N_{base}$) given the longer lifespan and common occurrence of this species in the study region.

Potential prey of rusty crayfish were informed by previous studies that analyzed individual gut contents (Pacioglu et al., 2019; Wooster et al., 2024), and used to select a subset of macroinvertebrate samples to represent each functional feeding group (e.g., predators, collector-gatherers, collector-filterers, scrapers, shredders). The isotopic signature of crayfish was adjusted according to estimated fractionation rates, also known as trophic enrichment factors. While previous studies have reported isotopic turnover and fractionation rates for numerous crayfish species, these values vary with food source and experimental conditions (Glon et al., 2016; Jussila et al., 2015). Therefore, we applied fractionation rates that are most commonly used for aquatic ecosystems: $\delta^{13}C = 0.4 \pm -1.3‰$ and $\delta^{15}N = 3.4 \pm 1.0‰$ (Post, 2002). Because this analysis was limited to diet proportion estimation, we did not apply lipid correction to prey or consumers (Arostegui et al., 2019).

We investigated potential resource competition by comparing the isotopic niche widths of rusty crayfish, steelhead fry, and steelhead smolts using the SIBER package (version 2.1.9) in

SIAR (A. L. Jackson et al., 2011; Parnell et al., 2013). This Bayesian framework uses isotope values ($\delta^{13}C$ and $\delta^{15}N$) of individuals to define a standard ellipse area (SEA) that represents the core isotopic niche of a consumer group. We also examined variation in the niche width of rusty crayfish in relation to crayfish density. We applied the corrected version of standard ellipse area (SEA_c) to minimize potential biases arising from small sample sizes exhibited in 2 sites (< 12 samples) (A. L. Jackson et al., 2011). Resource use, as defined by the total area of SEA_c, was calculated for rusty crayfish, steelhead fry, and steelhead smolts, and proportional overlap between groups was calculated.

2.4.5 Macroinvertebrate community composition

To understand relative food availability for steelhead, we classified all identified macroinvertebrate taxa based on propensity to enter the drift of rivers (Rader, 1997). This system ranks benthic macroinvertebrates as having low (1), moderate (3), and high (2) drift propensity, thus providing insight into food availability for salmonids (Sullivan & White, 2017). We calculated a drift-weighted community mean relative density (D_s^{dw}) for each primary site using abundance estimates from community composition samples. More influence (i.e., greater weight) was assigned to taxa with more propensity to drift (low = 1, moderate = 2, high = 3). Taxon-specific relative abundance at each site was calculated as:

$$p_{i,s} = \frac{N_{i,s}}{\Sigma_i N_{i,s}}$$

where $N_{i,s}$ is the abundance of taxon i at site s and $\Sigma_i N_{i,s}$ is the total abundance across all taxa at site s. The value of D_s^{dw} for each site was then calculated as:

$$D_s^{dw} = \frac{\Sigma_i (p_{i,s} \times w_i)}{\Sigma_i w_i}$$

where $p_{i,s}$ is the relative density of taxon i at site s, and w_i is the drift propensity weight assigned to taxon i. This metric, D_s^{dw}, provides a weighted average of taxon-specific relative densities by giving greater weight to taxa more likely to drift.

2.4.6 Fatty acid analysis

To explore potential crayfish predation on steelhead following spawning events, we analyzed fatty acid profiles using a subset of individuals. We retained only dietary FAs (i.e., FAs acquired through consumption) for the following analysis (Budge et al., 2012; Iverson et al., 2004). Specifically, this analysis focused on essential fatty acids (EFAs) that are pronounced in marine organisms and critical for egg production (Fuiman et al., 2015); namely, docosahexaenoic acid (DHA, 22:6n3), eicosapentaenoic acid (EPA, 20:5n3) and arachidonic acid (ARA, 20:4n6).The proportional contribution of each FA, or mass divided by total mass, was used to produce consumer profiles. If the proportional contribution was <1%, we removed the FA from the individual dataset and re-normalized the profile.

2.5 Results

Isotopic signals of rusty crayfish, steelhead fry and smolts, and potential impacts of crayfish on drifting macroinvertebrates varies considerably across the South Fork of the John Day River (Figure 2.2). Community-wide carbon isotope values ranged from -39.9‰ to -17.2‰, and nitrogen values ranged from -3.6‰ to 12.1‰, with steelhead fry defining the upper limit. For rusty crayfish, $\delta^{13}C$ ranged from -24.2‰ to -29.1‰ and $\delta^{15}N$ ranged from 4.1‰ to 7.4‰ in alignment with expectations for a polytrophic consumer (Figure 2.3). Steelhead fry had an average $\delta^{13}C$ value of -21.9‰ (-21.2 – -23.0‰), and an average $\delta^{15}N$ value of 13.6‰ (12.5 – 14.9‰).

Across the sub-basin of the South Fork, rusty crayfish had an average TP_c of 2.4 (range = 1.9-2.9) and average C_{corr} value of 0.5 (range = 0.2-0.8). The TP_c of rusty crayfish averaged 2.3 in the South Fork (Figure 2.4A; range = 1.9-2.9), 2.4 in Murderers Creek (Figure 2.4B; range = 2.1-2.8), and 2.7 (2.6-2.8) in the smaller tributary sites (BC, DC, WC). The C_{corr} of rusty crayfish averaged 0.6 (range = 0.2-0.8), as compared with 0.5 (range = 0.3-0.6) in Murderers Creek and 0.6 (range = 0.6-0.7) across tributaries. Based on snorkel surveys, sites were divided by steelhead fry presence (n = 11; MC2, MC4-7, SF1, SF4, SF7-10) and absence (n = 5; MC3, SF2-3, SF5-6). Trophic position of rusty crayfish was significantly higher in sites occupied (mean = 2.41, SD = 0.2) versus un-occupied by steelhead fry (mean = 2.27, SD = 0.2) (one-tailed *t*-test, $t = 129.9$, $P < 0.0001$).

The isotopic niches of rusty crayfish and juvenile steelhead varied in both size and space (Figure 2.5, Table 2.2). Rusty crayfish had a SEA_c value of 2.08. Steelhead fry and smolts had SEA_c values of 4.79 and 5.15, respectively. Using mean and covariance from the maximum likelihood estimate, we calculated the overlap between crayfish and fry to be 1.32 (3.3% proportional overlap) as compared to 11.83 (37.6% proportional overlap) between crayfish and smolts.

Sites along the South Fork had average densities lower than those observed in Murderers Creek (Figure 2.7, Table 2.1). Drift-weighted community mean relative density showed a weak negative relationship with median density of rusty crayfish ($\beta = -0.0009 \pm$ SE, $t = -1.21$, $p = 0.24$), with substantial variability among sites and considerable uncertainty evidenced by wide confidence intervals (Figure 2.6). The linear model explained a small amount of variation in the response ($R^2 = 0.075$) to median density estimates across invaded sites. Smaller tributaries

representing un-invaded areas were included in these analyses but were not detected at any of the three sites.

FA profiles of rusty crayfish revealed relatively high proportions of FAs that could be attributed to marine energy sources (Figure 2.8; Table 2.3). Ratios (DHA:EPA) were highly variable among sampled individuals (20.6 ± 16.1). Based on our subset of samples processed for this analysis, we did not find a significant relationship between trophic position ($\delta^{15}N$) and proportional contribution of EFAs (Figure 2.9). Results from separate linear regressions showed weak, negative correlations for the three EFAs: ARA ($F_{1,8} = 2.7$, $p = 0.141$, $R^2 = 0.25$), DHA ($F_{1,8} = 4.1$, $p = 0.078$, $R^2 = 0.34$), and EPA ($F_{1,8} = 3.6$, $p = 0.094$, $R^2 = 0.31$).

2.6 Discussion

This study explored trophic interactions between an invasive omnivore and native anadromous fish in early stages of life, providing insight into varying effects along an invasion gradient. We assessed impacts to an imperiled steelhead population by using a wide range of methods: SIA (trophic metrics, niche analysis), community composition, and fatty acid analysis. Our approach supplied three major results. First, we found high variation in the trophic position of rusty crayfish and their carbon sources, with significantly higher values of both metrics detected in areas where steelhead fry were present. Next, there was considerable overlap between the isotopic niches of rusty crayfish and steelhead smolts, indicating potential competition for prey resources. Limited isotopic overlap between crayfish and steelhead fry was found, although this result was likely biased by maternal carry-over in isotopic composition. Finally, we identified a negative, yet weak relationship between rusty crayfish density and drift-weighted community mean relative density, offering some evidence that crayfish may reduce the availability of

macroinvertebrate prey for juvenile steelhead. Collectively, these results provide initial evidence that rusty crayfish engage in prey competition with juvenile steelhead, with instances of direct predation remaining uncertain.

The trophic range (1.9-2.9) occupied by rusty crayfish throughout our study system aligned with expectations for an omnivorous, opportunistic consumer. As a polytrophic organism, crayfish feed on diverse sources that include macrophytes, periphyton, detritus, smaller macroinvertebrates, and tissue from various animals. Their plasticity is a major factor in successful establishment and widespread invasions(Tobler & Morehouse, 2013). Spatial variation in isotopic metrics (TP and C_{corr}) show that rusty crayfish are interacting with the South Fork food web in diverse ways. Rather than occupying a fixed position, crayfish respond to site-specific resources throughout the invaded range. Individuals from MC and lower South Fork sites had higher $\delta^{15}N$ values compared to other sites, which constitutes consumption of prey from higher trophic levels, or assimilation of resources enriched in ^{15}N. The stream reaches where steelhead fry were observed, we found that rusty crayfish had significantly higher TP and C_{corr} values, implying crayfish feeding strategies change in response to steelhead presence or habitat preferred for spawning. These localized patterns may be the result of spatial differences in prey availability, reflective of abiotic stream characteristics. Further analysis with $\delta^{13}C$ was confounded by cross-site variability observed in primary producers. The differences in carbon signatures of periphyton, filamentous algae, and macroinvertebrates are indicative of a heterogeneous system and diverse resource pools (Sagouis et al., 2015). Thus, we interpreted $\delta^{13}C$ values from rusty crayfish as relative with one another rather than contributed resources. It is likely that this crayfish population uses an allochthonous source, terrestrial invertebrates or plant matter, that obscures additional quantitative results (Hollows et al., 2002). In spite of these

limitations, the spatial patterns we identified with isotope values provide evidence that support the highly context-dependent nature of crayfish behavior and role in novel food webs.

The isotopic niche of rusty crayfish was largely separate from that of steelhead fry. When visualized in isotopic space, we found marginal overlap (3.3%) between the ellipses of each species and distinct centroid positions. However, the isotope values from steelhead fry are likely not representative of actual consumption patterns, rather these values expectedly reflect marine-derived nutrients as a result of maternal carry-over—further evidenced by elevated $\delta^{15}N$ values (Murphy et al., 2021). This artificial enrichment, compared to resident species, hinders our ability to detect competition using this methodology and predation more broadly. However, samples collected from smolts accurately represent dietary sources because this population of steelhead spends 1-2 years rearing before out-migrating. In contrast to fry, we identified substantial isotopic niche overlap (37.6%) between rusty crayfish and steelhead smolts. These results suggest similar resource use throughout the South Fork and a higher likelihood for competitive interactions. Convergence of the two ellipses indicates potential similarities in prey selection and or habitat use. Overlap may also vary temporally as a result of ontogenetic shifts caused by seasonal variation in productivity (Litz et al., 2017) and development stages (Wood et al., 2017). Still, the relatively larger area occupied by smolts provides evidence of broad resource use that exceeds rusty crayfish, contrasting prior work that shows crayfish have a wider niche in invaded systems as compared to their native ranges (Olsson et al., 2009). Impacts could be more pronounced if the niche of rusty crayfish differs from that of the native signal crayfish (Pacioglu et al., 2019). In this system and others, rusty crayfish displace native crayfish species as behaviorally aggressive and morphologically superior competitors (Hill & Lodge, 1999).

Our study offers evidence that the invasion of rusty crayfish in the SFJDR has slightly reduced preferred prey availability for steelhead juveniles. Sites invaded by crayfish, relative to those left uninvaded, had a lower average relative density of macroinvertebrates with greater propensity to drift. We saw an inverse relationship among uninvaded sites, where density values decline in response to increasingly upstream survey locations. Past studies have found that abundance of taxa with moderate and high propensity to drift is lower when crayfish are present at low densities (Kreps et al., 2016; Nyström et al., 1999). While in support of these findings, our results reveal that crayfish abundance alone explains relatively little of the observed variation in macroinvertebrate density. The impacts of crayfish on benthic communities vary across the literature; reports describe little to no effect on macroinvertebrates despite notable reduction of macrophytes and snails (Lodge et al., 1994; Momot, 1995). Such variation supports the idea that crayfish effects are context dependent. In highly heterogenous ecosystems, abiotic factors (e.g., flow regime, substrate composition, water quality) may mediate the strength of impacts. Additionally, compensatory community responses can occur where individual taxa decline while others increase and produce relatively stable values. An overall lowered abundance of either moderate and high drift groups could potentially limit growth and survival of juvenile steelhead because of a heavy reliance on drift-feeding. More broadly, these results support concerns regarding the response of native fishes to reductions in prey abundance (Nilsson et al., 2012) and reiterate the importance of secondary production in benthic communities that support higher trophic levels (Vander Zanden & Vadeboncoeur, 2002). The magnitude of effects observed for multiple drift groups provides evidence that invasive crayfish modify prey availability at scales relevant to stream-rearing steelhead.

Our results confirm the presence of marine-derived lipids in rusty crayfish, with some individuals having notably higher levels of EFAs which are similarly abundant in anadromous fish species (Heintz et al., 2010). Compared to bulk carbon, EFAs may be transferred from sources to consumers more efficiently (Gladyshev et al., 2011), with similar patterns observed for both DHA and ARA (Carreón-Palau et al., 2013). Past studies have compared the size of individuals (e.g., fork length) and EFA concentrations (mg) to identify direct consumption (Fuiman et al., 2015). Preliminary analyses, which used carapace length and mass, did not yield evidence that support similar results for crayfish. However, numerous accounts of native and invasive populations of crayfish affirm the ability and preference to prey upon salmon and trout eggs (Ellrott et al., 2007; Fitzsimons et al., 2002; Jonas et al., 2005). Eggs of anadromous species serve as a notable nutritional resource for consumers and the ecosystem more broadly. In freshwater ecosystems, marine-derived nutrients supplied by anadromous fish can increase productivity (Heintz et al., 2010). Discrete spawning events provide energy-rich tissue (e.g., carcass flesh, eggs) to consumers, resulting in increased primary production (Johnston et al., 2004). The seasonal occurrence of steelhead spawning, and even the presence of juvenile Chinook, likely cause perturbations to the flow of energy through the John Day River food web (Rubenson et al., 2020). In respect to current and future management, we must also consider nutrient limitation within this ecosystem. The benefits of marine-derived nutrients are likely to support growth, survival, and other metrics of rusty crayfish—whether for the population or select individuals. Because these analyses were done in supplement to SIA, we acknowledge that this discussion is largely explorative in nature. However, these results provide additional rationale for quantifying EFA transfer among trophic levels (Landsman et al., 2018).

Further inference can be made by comparing our results to other studies that report tracers identified in captive crayfish and patterns found in potential prey (i.e., juvenile salmon). Specifically, Volk (2004) found that terrestrial sources contain low levels of polyunsaturated FA and insects generally lack 22:6n-3. The average proportion of 22:6n-3 among coho salmon fingerlings (7.4%) suggests DHA may help to distinguish sources of individual diets (Sheridan et al., 1985). Long-chain fatty acids (i.e., n-3, n-6) are metabolized more slowly by consumers as compared to short or medium-chain FAs, making them more powerful in determining a consumer's diet. The unique structure of Cetoleic acid (22:1n11) produces a similar effect but may match the accumulation of short or medium-chain FAs (Bremer & Norum, 1982). While not ubiquitous, FAs can be assimilated from more than a single energy source. A study on the biosynthesis of *Cherax destructor* revealed that invertebrate-fed individuals had higher proportions of DHA compared to detritus- or pellet-fed individuals. Greater proportions of EPA and ARA were consistent among individuals in the pellet treatment, where crayfish were given commercial aquaculture feed (McInerney et al., 2022). However, interpretations with EPA should be conservative because large quantities have been identified in tail muscle tissue and hepatopancreas of crayfish held in captivity (Ackefors et al., 1997). While we did not perform in-depth analysis with crayfish hepatopancreas samples, future work could leverage the differences in tissue turnover rates to design an efficient study across shorter time spans (Jussila et al., 2015). The diets of rusty crayfish, as reflected through FA profiles, remain illustrative of consumption habits and may also clarify mixing model results of underdetermined systems (Galloway et al., 2015).

Interpretation of stable isotope values requires a deep knowledge of ecological processes specific to the study system. Additional complexities arise when stable isotope methodology is

applied to omnivorous consumers, such as crayfish. The assimilation efficiency may differ across energy sources, so discrepancies can occur when individuals are – for example – simultaneously consuming black fly larvae and detritus from terrestrial plants (Phillips & Koch, 2002; Robbins et al., 2002). Further research may benefit from a multi-year structure, where similar analyses are performed to understand temporal variability (M. C. Jackson et al., 2017). Consumption patterns generate ecological effects that propagate throughout food webs but are highly dependent upon the functional diversity of invasive species (Strayer, 2010). Primary consumers employ various feeding strategies that support exponential population growth, forcing declines in the abundance and richness of primary producers (Stenroth & Nyström, 2003). Through omnivory or ontogeny, invaders can occupy multiple trophic levels to create additional linkages across benthic and littoral habitats (Moore et al., 2012; Tabor et al., 2007). The increasingly complex nature of invasion theory reflects a growing need to explore interactions throughout food webs, which could better reflect specific effects on species of conservation concern. This approach is particularly relevant for understudied species and systems, where the consequences of invasion may be more difficult to uncover (Simberloff et al., 2013).

For nearly three decades, Middle Columbia River steelhead have been recognized as 'threatened' under the ESA due to ongoing threats of habitat loss and climate change (Ford, 2022). The rapid growth of rusty crayfish, in both abundance and distribution, is an ever-growing concern for managing bodies due to overlap with salmon spawning and rearing habitat. Beyond the South Fork, invasive crayfish populations hold potential for widespread impacts to the persistence of salmon and steelhead. With the capacity to structurally alter dynamics in food webs, invasive species can affect aspects of ecosystems that are difficult to discern or quantify (Schindler & Scheuerell, 2002). In this context, identification of key factors like prey preferences

and feeding behavior are important to understanding impacts to the trophic ecology of invaded systems. Conservation of highly imperiled freshwater ecosystems, like the John Day River Basin, should addressed with consideration of how both human and ecological processes are negatively affected (Hand et al., 2018).

2.7 References

Ackefors, H., Castell, J., & ÖRde-Öström, I.-L. (1997). Preliminary Results on the Fatty Acid Composition of Freshwater Crayfish, Astacus astacus and Pacifastacus leniusculus, Held in Captivity. *Journal of the World Aquaculture Society*, *28*(1), 97–105. https://doi.org/10.1111/j.1749-7345.1997.tb00967.x

Adey, A. K., Kuehne, L. M., & Olden, J. D. (2018). Trophic Ecology of Olympic Mudminnow (Novumbra hubbsi) in Lake Ozette, Washington. *Northwest Science*, *92*(4), 267–277. https://doi.org/10.3955/046.092.0404

Alp, M., & Cucherousset, J. (2022). Food webs speak of human impact: Using stable isotope-based tools to measure ecological consequences of environmental change. *Food Webs*, *30*, e00218. https://doi.org/10.1016/j.fooweb.2021.e00218

Arostegui, M. C., Schindler, D. E., & Holtgrieve, G. W. (2019). Does lipid-correction introduce biases into isotopic mixing models? Implications for diet reconstruction studies. *Oecologia*, *191*(4), 745–755. https://doi.org/10.1007/s00442-019-04525-7

Barbour, M. T. (1999). Rapid bioassessment protocols for use in wadeable streams and rivers: Periphyton, benthic macroinvertebrates and fish. *US Environmental Protection Agency*.

Beamesderfer, R., Ward, D. L., & Nigro, A. A. (1996). Evaluation of the biological basis for a predator control program on northern squawfish (*Ptychocheilus oregonensis*) in the Columbia and Snake rivers. *Canadian Journal of Fisheries and Aquatic Sciences*, *53*(12), 2898–2908. https://doi.org/10.1139/f96-225

Bode, M., Baker, C. M., & Plein, M. (2015). Eradicating down the food chain: Optimal multispecies eradication schedules for a commonly encountered invaded island ecosystem. *Journal of Applied Ecology*, *52*(3), 571–579. https://doi.org/10.1111/1365-2664.12429

Bodin, N., Le Loc'h, F., & Hily, C. (2007). Effect of lipid removal on carbon and nitrogen stable isotope ratios in crustacean tissues. *Journal of Experimental Marine Biology and Ecology*, *341*(2), 168–175. https://doi.org/10.1016/j.jembe.2006.09.008

Bremer, J., & Norum, K. R. (1982). Metabolism of very long-chain monounsaturated fatty acids (22:1) and the adaptation to their presence in the diet. *Journal of Lipid Research*, *23*(2), 243–256. https://doi.org/10.1016/S0022-2275(20)38153-0

Brett, M. T., Kainz, M. J., Taipale, S. J., & Seshan, H. (2009). Phytoplankton, not allochthonous carbon, sustains herbivorous zooplankton production. *Proceedings of the National Academy of Sciences*, *106*(50), 21197–21201. https://doi.org/10.1073/pnas.0904129106

Budge, S. M., Penney, S. N., & Lall, S. P. (2012). Estimating diets of Atlantic salmon (*Salmo salar*) using fatty acid signature analyses; validation with controlled feeding studies. *Canadian Journal of Fisheries and Aquatic Sciences*, *69*(6), 1033–1046. https://doi.org/10.1139/f2012-039

Carey, M. P., Sanderson, B. L., Barnas, K. A., & Olden, J. D. (2012). Native invaders – challenges for science, management, policy, and society. *Frontiers in Ecology and the Environment*, *10*(7), 373–381. https://doi.org/10.1890/110060

Carey, M. P., Sanderson, B. L., Friesen, T. A., Barnas, K. A., & Olden, J. D. (2011). Smallmouth Bass in the Pacific Northwest: A Threat to Native Species; a Benefit for Anglers. *Reviews in Fisheries Science*, *19*(3), 305–315. https://doi.org/10.1080/10641262.2011.598584

Carmichael, R. W., Bliesner, K. L., Cameron, W., Clarke, L. R., Craft, N. M., Faber, D. M., Kurtz, D. G., Ruzychi, J. R., & Tinus, E. (2015). *A Compendium of Viable Salmonid*

Population Abundance and Productivity Field and Analysis Methods for Natural Origin Steelhead Populations in the Middle Columbia River DPS of Northeast Oregon from 1959 to 2015. ODFW, Northeast-Central Oregon Research and Monitoring. https://nrimp.dfw.state.or.us/DataClearinghouse/default.aspx?pn=ViewFile&att=ODFW%2fODFW_1100_2_Report_Compendium_VSP_Steelhead_Mid-C.pdf

Carreón-Palau, L., Parrish, C. C., del Angel-Rodríguez, J. A., Pérez-España, H., & Aguiñiga-García, S. (2013). Revealing organic carbon sources fueling a coral reef food web in the Gulf of Mexico using stable isotopes and fatty acids. *Limnology and Oceanography*, *58*(2), 593–612. https://doi.org/10.4319/lo.2013.58.2.0593

Claramunt, R. M., Jonas, J. L., Fitzsimons, J. D., & Marsden, J. E. (2005). Influences of Spawning Habitat Characteristics and Interstitial Predators on Lake Trout Egg Deposition and Mortality. *Transactions of the American Fisheries Society*, *134*(4), 1048–1057. https://doi.org/10.1577/T04-075.1

Crozier, L. G., Hendry, A. P., Lawson, P. W., Quinn, T. P., Mantua, N. J., Battin, J., Shaw, R. G., & Huey, R. B. (2008). Potential responses to climate change in organisms with complex life histories: Evolution and plasticity in Pacific salmon. *Evolutionary Applications*, *1*(2), 252–270. https://doi.org/10.1111/j.1752-4571.2008.00033.x

Cucherousset, J., & Olden, J. D. (2011). Ecological Impacts of Non-native Freshwater Fishes. *Fisheries*, *36*(5), 215–230. https://doi.org/10.1080/03632415.2011.574578

Edmonds, N. J., Riley, W. D., & Maxwell, D. L. (2011). Predation by *Pacifastacus leniusculus* on the intra-gravel embryos and emerging fry of *Salmo salar*. *Fisheries Management and Ecology*, *18*(6), 521–524. https://doi.org/10.1111/j.1365-2400.2011.00797.x

Ellrott, B. J., Marsden, J. E., Fitzsimons, J. D., Jonas, J. L., & Claramunt, R. M. (2007). Effects of Temperature and Density on Consumption of Trout Eggs by Orconectes propinquus and O. rusticus. *Journal of Great Lakes Research*, *33*(1), 7–14. https://doi.org/10.3394/0380-1330(2007)33%5B7:EOTADO%5D2.0.CO;2

Faria, L., Vitule, J. R. S., & Olden, J. D. (2023). Predation risk by largemouth bass modulates feeding functional responses of native and non-native crayfish. *NeoBiota*, *87*, 191–212. https://doi.org/10.3897/neobiota.87.108457

Fitzsimons, J. D., Perkins, D. L., & Krueger, C. C. (2002). Sculpins and Crayfish in Lake Trout Spawning Areas in Lake Ontario: Estimates of Abundance and Egg Predation on Lake Trout Eggs. *Journal of Great Lakes Research*, *28*(3), 421–436. https://doi.org/10.1016/S0380-1330(02)70595-9

Ford, M. J. (2022). Biological Viability Assessment Update for Pacific Salmon and Steelhead Listed Under the Endangered Species Act: Pacific Northwest. *NOAA Technical Memorandum NMFS-NWFSC-171*. https://doi.org/10.25923/KQ2N-KE70

Fuiman, L. A., Connelly, T. L., Lowerre-Barbieri, S. K., & McClelland, J. W. (2015). Egg boons: Central components of marine fatty acid food webs. *Ecology*, *96*(2), 362–372. https://doi.org/10.1890/14-0571.1

Galib, S. M., Sun, J., Gröcke, D. R., & Lucas, M. C. (2022). Ecosystem effects of invasive crayfish increase with crayfish density. *Freshwater Biology*, *67*(6), 1005–1019. https://doi.org/10.1111/fwb.13897

Gallardo, B., Clavero, M., Sánchez, M. I., & Vilà, M. (2016). Global ecological impacts of invasive species in aquatic ecosystems. *Global Change Biology*, *22*(1), 151–163. https://doi.org/10.1111/gcb.13004

Galloway, A. W. E., Brett, M. T., Holtgrieve, G. W., Ward, E. J., Ballantyne, A. P., Burns, C. W., Kainz, M. J., Müller-Navarra, D. C., Persson, J., Ravet, J. L., Strandberg, U., Taipale, S. J., & Alhgren, G. (2015). A Fatty Acid Based Bayesian Approach for Inferring Diet in Aquatic Consumers. *PLOS ONE*, *10*(6), e0129723. https://doi.org/10.1371/journal.pone.0129723

Gladyshev, M. I., Sushchik, N. N., Anishchenko, O. V., Makhutova, O. N., Kolmakov, V. I., Kalachova, G. S., Kolmakova, A. A., & Dubovskaya, O. P. (2011). Efficiency of transfer of essential polyunsaturated fatty acids versus organic carbon from producers to consumers in a eutrophic reservoir. *Oecologia*, *165*(2), 521–531.

Glon, M. G., Larson, E. R., & Pangle, K. L. (2016). Comparison of 13C and 15N discrimination factors and turnover rates between congeneric crayfish Orconectes rusticus and O. virilis (Decapoda, Cambaridae). *Hydrobiologia*, *768*(1), 51–61. https://doi.org/10.1007/s10750-015-2527-3

Hand, B. K., Flint, C. G., Frissell, C. A., Muhlfeld, C. C., Devlin, S. P., Kennedy, B. P., Crabtree, R. L., McKee, W. A., Luikart, G., & Stanford, J. A. (2018). A social–ecological perspective for riverscape management in the Columbia River Basin. *Frontiers in Ecology and the Environment*, *16*(S1), S23–S33. https://doi.org/10.1002/fee.1752

Heintz, R. A., Wipfli, M. S., & Hudson, J. P. (2010). Identification of Marine-Derived Lipids in Juvenile Coho Salmon and Aquatic Insects through Fatty Acid Analysis. *Transactions of the American Fisheries Society*, *139*(3), 840–854. https://doi.org/10.1577/T09-115.1

Hill, A. M., & Lodge, D. M. (1999). Replacement of Resident Crayfishes by an Exotic Crayfish: The Roles of Competition and Predation. *Ecological Applications*, *9*(2), 678–690. https://doi.org/10.1890/1051-0761(1999)009%5B0678:RORCBA%5D2.0.CO;2

Hollows, J., Townsend, C., & Collier, K. (2002). Diet of the crayfish Paranephrops zealandicus in bush and pasture streams: Insights from stable isotopes and stomach analysis. *New Zealand Journal of Marine and Freshwater Research - N Z J MAR FRESHWATER RES*, *36*, 129–142. https://doi.org/10.1080/00288330.2002.9517076

Iverson, S. J., Field, C., Don Bowen, W., & Blanchard, W. (2004). Quantitative Fatty Acid Signature Analysis: A New Method of Estimating Predator Diets. *Ecological Monographs*, *74*(2), 211–235. https://doi.org/10.1890/02-4105

Jackson, A. L., Inger, R., Parnell, A. C., & Bearhop, S. (2011). Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. *Journal of Animal Ecology*, *80*(3), 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Jackson, M. C., Donohue, I., Jackson, A. L., Britton, J. R., Harper, D. M., & Grey, J. (2012). Population-Level Metrics of Trophic Structure Based on Stable Isotopes and Their Application to Invasion Ecology. *PLoS ONE*, *7*(2), e31757. https://doi.org/10.1371/journal.pone.0031757

Jackson, M. C., Evangelista, C., Zhao, T., Lecerf, A., Britton, J. R., & Cucherousset, J. (2017). Between-lake variation in the trophic ecology of an invasive crayfish. *Freshwater Biology*, *62*(9), 1501–1510. https://doi.org/10.1111/fwb.12957

Jardine, T. D., McGeachy, S. A., Paton, C. M., Savoie, M., & Cunjak, R. A. (2003). Stable Isotopes in Aquatic Systems: Sample Preparation, Analysis, and Interpretation. *Fisheries and Oceans*, *2656*.

Johnston, N. T., MacIsaac, E. A., Tschaplinski, P. J., & Hall, K. J. (2004). Effects of the abundance of spawning sockeye salmon (*Oncorhynchus nerka*) on nutrients and algal

biomass in forested streams. *Canadian Journal of Fisheries and Aquatic Sciences*, *61*(3), 384–403. https://doi.org/10.1139/f03-172

Jonas, J. L., Claramunt, R. M., Fitzsimons, J. D., Marsden, J. E., & Ellrott, B. J. (2005). Estimates of egg deposition and effects of lake trout (*Salvelinus namaycush*) egg predators in three regions of the Great Lakes. *Canadian Journal of Fisheries and Aquatic Sciences*, *62*(10), 2254–2264. https://doi.org/10.1139/f05-141

Jussila, J., Ruokonen, T. J., Syväranta, J., Kokko, H., Vainikka, A., Makkonen, J., & Kortet, R. (2015). It takes time to see the menu from the body: An experiment on stable isotope composition in freshwater crayfishes. *Knowledge and Management of Aquatic Ecosystems*, (416), 25. https://doi.org/10.1051/kmae/2015021

Kreps, T. A., Larson, E. R., & Lodge, D. M. (2016). Do invasive rusty crayfish (*Orconectes rusticus*) decouple littoral and pelagic energy flows in lake food webs? *Freshwater Science*, *35*(1), 103–113. https://doi.org/10.1086/683358

Kumschick, S., Bacher, S., Dawson, W., Heikkilä, J., Sendek, A., Pluess, T., Robinson, T., & Kühn, I. (2012). A conceptual framework for prioritization of invasive alien species for management according to their impact. *NeoBiota*, *15*, 69–100. https://doi.org/10.3897/neobiota.15.3323

Landsman, S. J., Samways, K. M., Hayden, B., Knysh, K. M., & Heuvel, M. R. V. D. (2018). Assimilation of marine-derived nutrients from anadromous Rainbow Smelt in an eastern North American riverine food web: Evidence from stable-isotope and fatty acid analysis. *Freshwater Science*, *37*(4), 747–759. https://doi.org/10.1086/700598

Larson, E., & Olden, J. (2016). Field Sampling Techniques for Crayfish. In *Biology and Ecology of Crayfish* (pp. 287–324). CRC Press.

Larson, E. R., & Olden, J. D. (2011). The State of Crayfish in the Pacific Northwest. *Fisheries*, *36*(2), 60–73. https://doi.org/10.1577/03632415.2011.10389069

Layman, C. A., Araujo, M. S., Boucek, R., Hammerschlag-Peyer, C. M., Harrison, E., Jud, Z. R., Matich, P., Rosenblatt, A. E., Vaudo, J. J., Yeager, L. A., Post, D. M., & Bearhop, S. (2012). Applying stable isotopes to examine food-web structure: An overview of analytical tools. *Biological Reviews*, *87*(3), 545–562. https://doi.org/10.1111/j.1469-185X.2011.00208.x

Layman, C. A., Arrington, D. A., Montaña, C. G., & Post, D. M. (2007). Can Stable Isotope Ratios Provide for Community-Wide Measures of Trophic Structure? *Ecology*, *88*(1), 42–48. https://doi.org/10.1890/0012-9658(2007)88%5B42:CSIRPF%5D2.0.CO;2

Lindsay, R., Jonasson, B., Schroeder, R., & Cates, B. (1989). *Spring chinook salmon in the Deschutes River, Oregon* (Nos. 89–4; Wildlife Information). Oregon Department of Fish and Wildlife.

Litz, M. N. C., Miller, J. A., Copeman, L. A., Teel, D. J., Weitkamp, L. A., Daly, E. A., & Claiborne, A. M. (2017). Ontogenetic shifts in the diets of juvenile Chinook Salmon: New insight from stable isotopes and fatty acids. *Environmental Biology of Fishes*, *100*(4), 337–360. https://doi.org/10.1007/s10641-016-0542-5

Lodge, D. M., Kershner, M. W., Aloi, J. E., & Covich, A. P. (1994). Effects of an Omnivorous Crayfish (Orconectes Rusticus) on a Freshwater Littoral Food Web. *Ecology*, *75*(5), 1265–1281. https://doi.org/10.2307/1937452

Lodge, D. M., Taylor, C. A., Holdich, D. M., & Skurdal, J. (2000). Nonindigenous Crayfishes Threaten North American Freshwater Biodiversity: Lessons from Europe. *Fisheries*,

25(8), 7–20. https://doi.org/10.1577/1548-8446(2000)025%3C0007:NCTNAF%3E2.0.CO;2
Love, J., & Savino, J. F. (1993). Crayfish (Orconectes virilis) Predation on Zebra Mussels (Dreissena polymorphs). *Freshwater Ecology*, *8*(3), 253–259. https://doi.org/doi/10.1080/02705060.1993.9
Lurgi, M., Ritchie, E. G., & Fordham, D. A. (2018). Eradicating abundant invasive prey could cause unexpected and varied biodiversity outcomes: The importance of multispecies interactions. *Journal of Applied Ecology*, *55*(5), 2396–2407. https://doi.org/10.1111/1365-2664.13188
Macneale, K. H., Kiffney, P. M., & Scholz, N. L. (2010). Pesticides, aquatic food webs, and the conservation of Pacific salmon. *Frontiers in Ecology and the Environment*, *8*(9), 475–482. https://doi.org/10.1890/090142
Matsuzaki, S. S., Usio, N., Takamura, N., & Washitani, I. (2009). Contrasting impacts of invasive engineers on freshwater ecosystems: An experiment and meta-analysis. *Oecologia*, *158*(4), 673–686. https://doi.org/10.1007/s00442-008-1180-1
McCue, M. D., Javal, M., Clusella-Trullas, S., Le Roux, J. J., Jackson, M. C., Ellis, A. G., Richardson, D. M., Valentine, A. J., & Terblanche, J. S. (2020). Using stable isotope analysis to answer fundamental questions in invasion ecology: Progress and prospects. *Methods in Ecology and Evolution*, *11*(2), 196–214. https://doi.org/10.1111/2041-210X.13327
McInerney, P. J., Stoffels, R. J., Shackleton, M. E., Davey, C. D., Albert, J., & Rees, G. N. (2022). Dietary fatty acid profiles shape crayfish biosynthesis and performance: Implications for riverine food webs. *Freshwater Biology*, *67*(6), 978–990. https://doi.org/10.1111/fwb.13895
McMillan, J. R., Morrison, B., Chambers, N., Ruggerone, G., Bernatchez, L., Stanford, J., & Neville, H. (2023). A global synthesis of peer-reviewed research on the effects of hatchery salmonids on wild salmonids. *Fisheries Management and Ecology*, *30*(5), 446–463. https://doi.org/10.1111/fme.12643
Merritt, R., & Cummins, K. (1997). *An introduction to the aquatic insects of North America*. Kendall Hunt.
Messager, M. L., & Olden, J. D. (2018). Individual-based models forecast the spread and inform the management of an emerging riverine invader. *Diversity and Distributions*, *24*(12), 1816–1829. https://doi.org/10.1111/ddi.12829
Momot, W. (1995). Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science*, *3*(1), 33–63. https://doi.org/doi.org/10.1080/10641269509388566
Moore, J. W., Carlson, S. M., Twardochleb, L. A., Hwan, J. L., Fox, J. M., & Hayes, S. A. (2012). Trophic Tangles through Time? Opposing Direct and Indirect Effects of an Invasive Omnivore on Stream Ecosystem Processes. *PLoS ONE*, *7*(11), e50687. https://doi.org/10.1371/journal.pone.0050687
Moorhouse, T. P., & Macdonald, D. W. (2015). Are invasives worse in freshwater than terrestrial ecosystems? *WIREs Water*, *2*(1), 1–8. https://doi.org/10.1002/wat2.1059
Murphy, C. A., Romer, J. D., Stertz, K., Arismendi, I., Emig, R., Monzyk, F., & Johnson, S. L. (2021). Damming salmon fry: Evidence for predation by non-native warmwater fishes in reservoirs. *Ecosphere*, *12*(9), e03757. https://doi.org/10.1002/ecs2.3757

Newsome, S. D., Martinez del Rio, C., Bearhop, S., & Phillips, D. L. (2007). A niche for isotopic ecology. *Frontiers in Ecology and the Environment*, *5*(8), 429–436. https://doi.org/10.1890/060150.1

Nilsson, E., Solomon, C. T., Wilson, K. A., Willis, T. V., Larget, B., & Vander Zanden, M. J. (2012). Effects of an invasive crayfish on trophic relationships in north-temperate lake food webs. *Freshwater Biology*, *57*(1), 10–23. https://doi.org/10.1111/j.1365-2427.2011.02688.x

Nyssen, F., Brey, T., Dauby, P., & Graeve, M. (2005). Trophic position of Antarctic amphipodsenhanced analysis by a 2-dimensional biomarker assay. *Marine Ecology Progress Series*, *300*, 135–145. https://doi.org/10.3354/meps300135

Nyström, P., Brönmark, C., & Granéli, W. (1999). Influence of an Exotic and a Native Crayfish Species on a Littoral Benthic Community. *Oikos*, *85*(3), 545–553. https://doi.org/10.2307/3546704

Olden, J. D., Adams, J., & Larson, E. (2009). First record of Orconectes rusticus (Girard, 1852) (Decapoda, Cambaridae) west of the Great Continental Divide in North America. *Crustaceana*, *82*(10), 1347–1351. https://doi.org/10.1163/156854009X448934

Olden, J. D., Berghout, P., & Vaage, C. (In press). Crayfish threats to salmonids: A synthesis of current knowledge and research priorities. *Water Biology and Security*.

Olden, J. D., Chen, K., García-Berthou, E., King, A. J., South, J., & Vitule, J. R. S. (2022). *Invasive Species in Streams and Rivers* (Vol. 2). https://doi.org/10.1016/B978-0-12-819166-8.00083-9

Olsson, K., Stenroth, P., Nyström, P., & Granéli, W. (2009). Invasions and niche width: Does niche width of an introduced crayfish differ from a native crayfish? *Freshwater Biology*, *54*(8), 1731–1740. https://doi.org/10.1111/j.1365-2427.2009.02221.x

Pacioglu, O., Zubrod, J. P., Schulz, R., Jones, J. I., & Pârvulescu, L. (2019). Two is better than one: Combining gut content and stable isotope analyses to infer trophic interactions between native and invasive species. *Hydrobiologia*, *839*(1), 25–35. https://doi.org/10.1007/s10750-019-03990-8

Parnell, A. C., Phillips, D. L., Bearhop, S., Semmens, B. X., Ward, E. J., Moore, J. W., Jackson, A. L., Grey, J., Kelly, D. J., & Inger, R. (2013). Bayesian stable isotope mixing models. *Environmetrics*, *24*(6), 387–399. https://doi.org/10.1002/env.2221

Phillips, D. L., & Koch, P. L. (2002). Incorporating concentration dependence in stable isotope mixing models. *Oecologia*, *130*(1), 114–125. https://doi.org/10.1007/s004420100786

Pinnegar, J. K., & Polunin, N. V. C. (1999). Differential fractionation of δ13C and δ15N among fish tissues: Implications for the study of trophic interactions. *Functional Ecology*, *13*(2), 225–231. https://doi.org/10.1046/j.1365-2435.1999.00301.x

Posit team. (2025). *RStudio: Integrated Development Environment for R* [Computer software]. Posit Software, PBC. http://www.posit.co/

Post, D. M. (2002). Using Stable Isotopes to Estimate Trophic Position: Models, Methods, and Assumptions. *Ecology*, *83*(3), 703–718. https://doi.org/10.1890/0012-9658(2002)083%5B0703:USITET%5D2.0.CO;2

Post, D. M., Layman, C. A., Arrington, D. A., Takimoto, G., Quattrochi, J., & Montaña, C. G. (2007). Getting to the fat of the matter: Models, methods and assumptions for dealing with lipids in stable isotope analyses. *Oecologia*, *152*(1), 179–189. https://doi.org/10.1007/s00442-006-0630-x

Rader, R. B. (1997). A functional classification of the drift: Traits that influence invertebrate availability to salmonids. *Canadian Journal of Fisheries and Aquatic Sciences*, *54*(6), 1211–1234. https://doi.org/10.1139/f97-025

Reid, A. J., Carlson, A. K., Creed, I. F., Eliason, E. J., Gell, P. A., Johnson, P. T. J., Kidd, K. A., MacCormack, T. J., Olden, J. D., Ormerod, S. J., Smol, J. P., Taylor, W. W., Tockner, K., Vermaire, J. C., Dudgeon, D., & Cooke, S. J. (2019). Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biological Reviews*, *94*(3), 849–873. https://doi.org/10.1111/brv.12480

Reynolds, J. D. (2011). A review of ecological interactions between crayfish and fish, indigenous and introduced. *Knowledge and Management of Aquatic Ecosystems*, (401), 10. https://doi.org/10.1051/kmae/2011024

Ricciardi, A., & Macisaac, H. J. (2010). Impacts of Biological Invasions on Freshwater Ecosystems. In *Fifty Years of Invasion Ecology* (pp. 211–224). John Wiley & Sons, Ltd. https://doi.org/10.1002/9781444329988.ch16

Robbins, C. T., Hilderbrand, G. V., & Farley, S. D. (2002). Incorporating Concentration Dependence in Stable Isotope Mixing Models: A Response to Phillips and Koch (2002). *Oecologia*, *133*(1), 10–13.

Rubenson, E. S., Lawrence, D. J., & Olden, J. D. (2020). Threats to Rearing Juvenile Chinook Salmon from Nonnative Smallmouth Bass Inferred from Stable Isotope and Fatty Acid Biomarkers. *Transactions of the American Fisheries Society*, *149*(3), 350–363. https://doi.org/10.1002/tafs.10237

Ruckelshaus, M. H., Levin, P., Johnson, J. B., & Kareiva, P. M. (2002). The Pacific Salmon Wars: What Science Brings to the Challenge of Recovering Species. *Annual Review of Ecology and Systematics*, *33*, 665–706.

Sagouis, A., Cucherousset, J., Villéger, S., Santoul, F., & Boulêtreau, S. (2015). Non-native species modify the isotopic structure of freshwater fish communities across the globe. *Ecography*, *38*(10), 979–985. https://doi.org/10.1111/ecog.01348

Sanderson, B. L., Barnas, K. A., & Rub, A. M. W. (2009). Nonindigenous Species of the Pacific Northwest: An Overlooked Risk to Endangered Salmon? *BioScience*, *59*(3), 245–256. https://doi.org/10.1525/bio.2009.59.3.9

Sanderson, B. L., Tran, C. D., Coe, H. J., Pelekis, V., Steel, E. A., & Reichert, W. L. (2009). Nonlethal Sampling of Fish Caudal Fins Yields Valuable Stable Isotope Data for Threatened and Endangered Fishes. *Transactions of the American Fisheries Society*, *138*(5), 1166–1177. https://doi.org/10.1577/T08-086.1

Schindler, D. E., & Scheuerell, M. D. (2002). Habitat coupling in lake ecosystems. *Oikos*, *98*(2), 177–189. https://doi.org/10.1034/j.1600-0706.2002.980201.x

Sheridan, M., Allen, W., & Kerstetter, T. (1985). Changes in the fatty acid composition of steelhead trout, Salmo gairdnerii Richardson, associated with parr-smolt transformation. *Comparative Biochemistry and Physiology Part B: Comparative Biochemistry*, *80*(4), 671–676.

Simberloff, D., Martin, J.-L., Genovesi, P., Maris, V., Wardle, D. A., Aronson, J., Courchamp, F., Galil, B., García-Berthou, E., Pascal, M., Pyšek, P., Sousa, R., Tabacchi, E., & Vilà, M. (2013). Impacts of biological invasions: What's what and the way forward. *Trends in Ecology & Evolution*, *28*(1), 58–66. https://doi.org/10.1016/j.tree.2012.07.013

Stenroth, P., Holmqvist, N., Nyström, P., Berglund, O., Larsson, P., & Granéli, W. (2006). Stable isotopes as an indicator of diet in omnivorous crayfish (*Pacifastacus leniusculus*): The

influence of tissue, sample treatment, and season. *Canadian Journal of Fisheries and Aquatic Sciences*, *63*(4), 821–831. https://doi.org/10.1139/f05-265
Stenroth, P., & Nyström, P. (2003). Exotic crayfish in a brown water stream: Effects on juvenile trout, invertebrates and algae. *Freshwater Biology*, *48*(3), 466–475. https://doi.org/10.1046/j.1365-2427.2003.01020.x
Strayer, D. L. (2010). Alien species in fresh waters: Ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology*, *55*(s1), 152–174. https://doi.org/10.1111/j.1365-2427.2009.02380.x
Sullivan, S., & White, S. M. (2017). *Methods Supporting the Development of Food Web Metrics from Benthic Macroinvertebrate Data*. https://doi.org/10.13140/RG.2.2.25176.29446
Tabor, R. A., Warner, E. J., Fresh, K. L., Footen, B. A., & Chan, J. R. (2007). Ontogenetic Diet Shifts of Prickly Sculpin in the Lake Washington Basin, Washington. *Transactions of the American Fisheries Society*, *136*(6), 1801–1813. https://doi.org/10.1577/T06-031.1
Tobler, M., & Morehouse, R. L. (2013). Invasion of rusty crayfish, Orconectes rusticus, in the United States: Niche shifts and potential future distribution. *Journal of Crustacean Biology*, *33*(2), 293–300. https://doi.org/10.1163/1937240X-00002120
Twardochleb, L. A., Olden, J. D., & Larson, E. R. (2013). A global meta-analysis of the ecological impacts of nonnative crayfish. *Freshwater Science*, *32*(4), 1367–1382. https://doi.org/10.1899/12-203.1
Vagnon, C., Rohr, R. P., Bersier, L.-F., Cattanéo, F., Guillard, J., & Frossard, V. (2022). Combining food web theory and population dynamics to assess the impact of invasive species. *Frontiers in Ecology and Evolution*, *10*. https://doi.org/10.3389/fevo.2022.913954
Vander Zanden, M. J., & Rasmussen, J. B. (1999). Primary Consumer δ 13C and δ 15N and the Trophic Position of Aquatic Consumers. *Ecology*, *80*(4), 1395–1404.
Vander Zanden, M. J., & Vadeboncoeur, Y. (2002). Fishes as Integrators of Benthic and Pelagic Food Webs in Lakes. *Ecology*, *83*(8), 2152–2161. https://doi.org/10.1890/0012-9658(2002)083%5B2152:FAIOBA%5D2.0.CO;2
Volk, C. J. (2004). *Nutrient and biological responses to red alder (Alnus rubra) presence along headwater streams: Olympic Peninsula, Washington* [Ph.D., University of Washington]. https://www.proquest.com/docview/305106697/abstract/72B3D6BED04D4D48PQ/1
Walsworth, T. E., Budy, P., & Thiede, G. P. (2013). Longer food chains and crowded niche space: Effects of multiple invaders on desert stream food web structure. *Ecology of Freshwater Fish*, *22*(3), 439–452. https://doi.org/10.1111/eff.12038
Wells, B. K., Huff, D. D., Quinn, T. P., Santora, J. A., Gomes, D. G. E., Vasbinder, K., Barnas, K. A., Burke, B. J., Courtney, M. B., Crozier, L. G., Fiechter, J., Friedland, K. D., Hayes, S. A., Hunsicker, M. E., Juanes, F., Mantua, N. J., Michel, C. J., Phillips, E. M., Sanderson, B. L., … Trudel, M. (2025). When, where, and why salmon become vulnerable to predation. *ICES Journal of Marine Science*, *82*(9), fsaf162. https://doi.org/10.1093/icesjms/fsaf162
Wood, K. A., Hayes, R. B., England, J., & Grey, J. (2017). Invasive crayfish impacts on native fish diet and growth vary with fish life stage. *Aquatic Sciences*, *79*(1), 113–125. https://doi.org/10.1007/s00027-016-0483-2
Wooster, D., DeBano, S., Li, X., Skillman, V., & Frost, K. (2024). *not-yet-known not-yet-known not-yet-known unknown Variation in Invasion Syndromes: Behavior, Morphology, and*

Diet in Rusty Crayfish (Faxonius rusticus) Along an Invasion Front. Preprints. https://doi.org/10.22541/au.173168643.37194474/v1

2.8 Figures

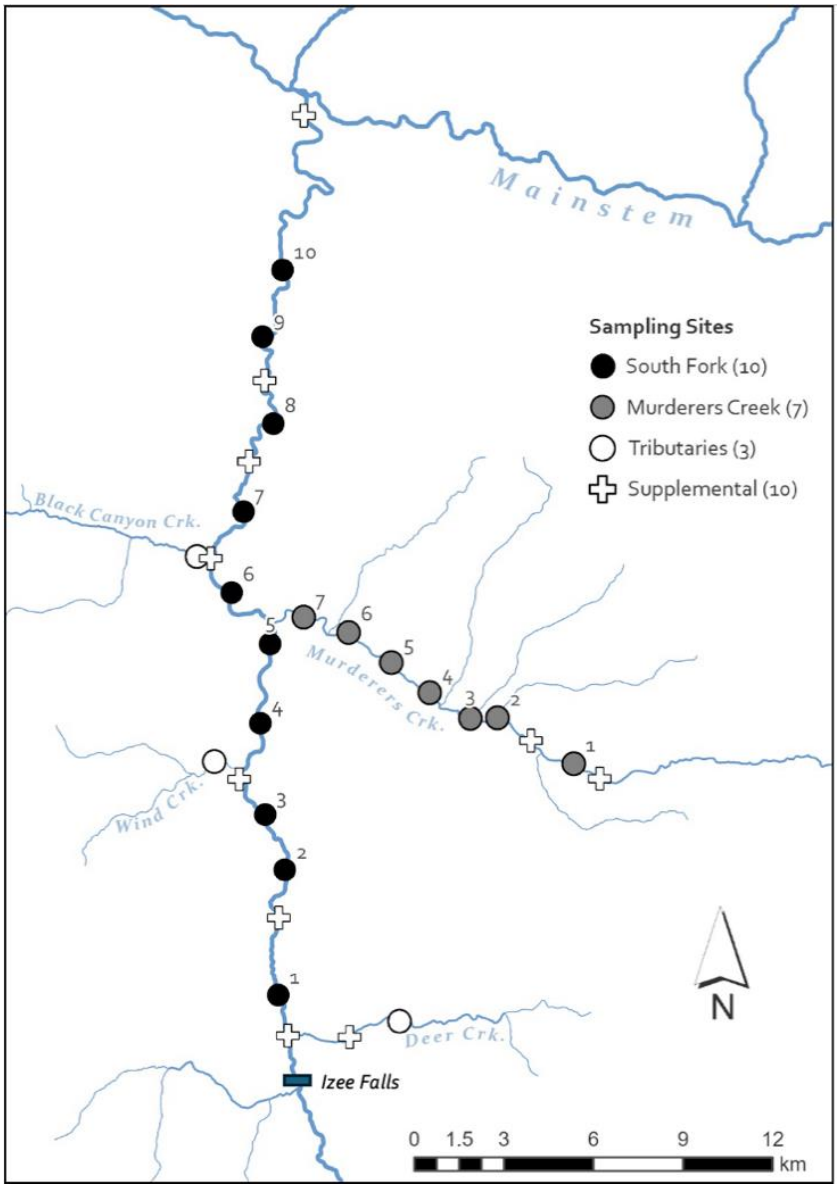

Figure 2.1. Survey sites across the South Fork of the John Day River, Oregon. Primary sites (circles) received all protocol detailed in 'Methods' section, while secondary sites (crosses) received a subset of such protocol. Primary sites are delineated by river sections and include South Fork (black), Murderers Creek (grey), and minor tributaries (white).

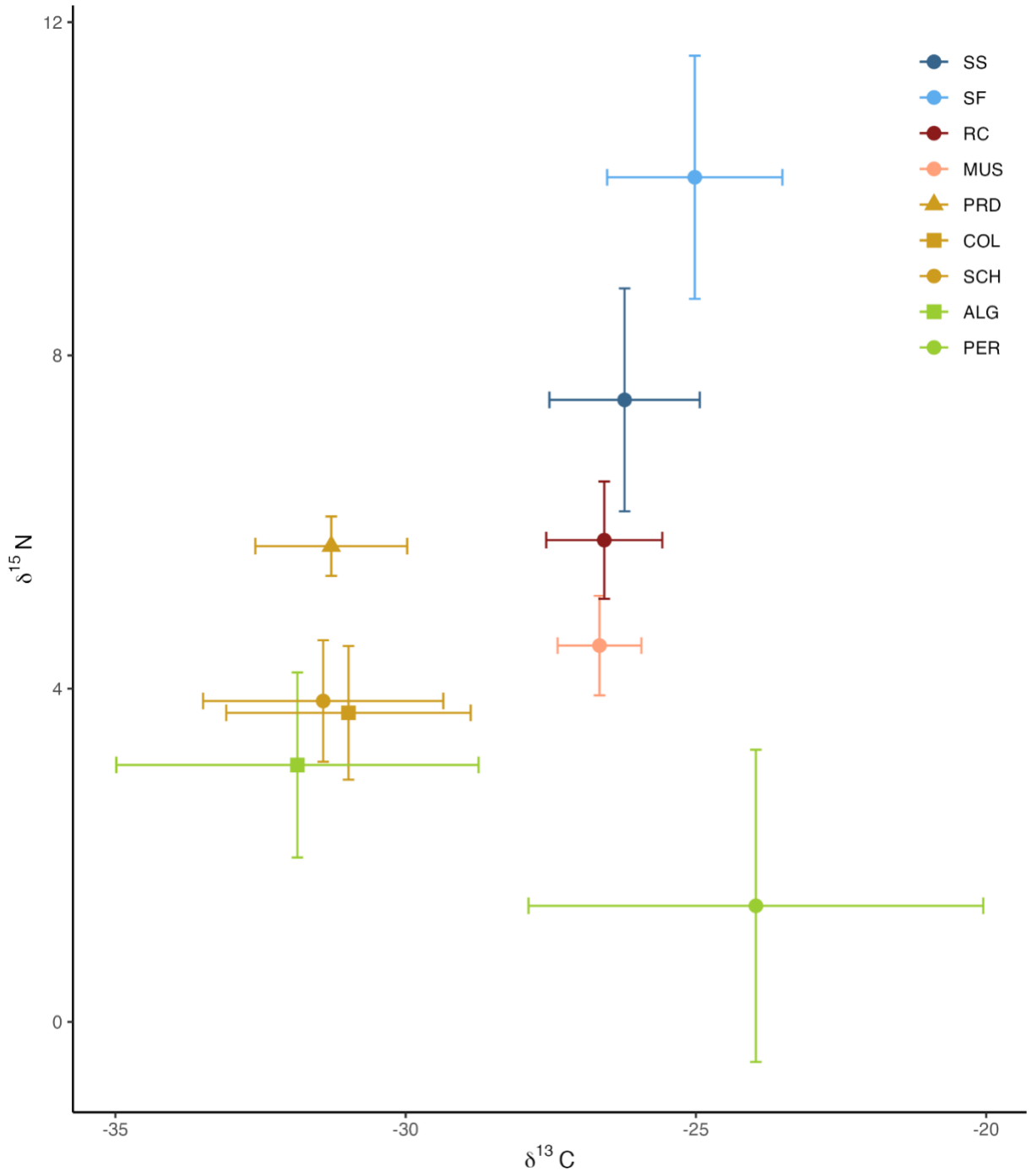

Figure 2.2. Relative position of collected species groups in isotopic space. Means (points) and standard deviation (lines) of the stable isotope signatures of each group. Sampled groups include aquatic insects, rusty crayfish (RC), steelhead fry (SF) and smolts (SS), mussels (MUS), periphyton (PER, square), filamentous algae (ALG, circle). Aquatic insects are grouped by 1-2 functional feeding groups: shredders & scrapers (SCH, circle), predators (PRD, triangle), collectors-gatherers & collectors-filterers (COL, square).

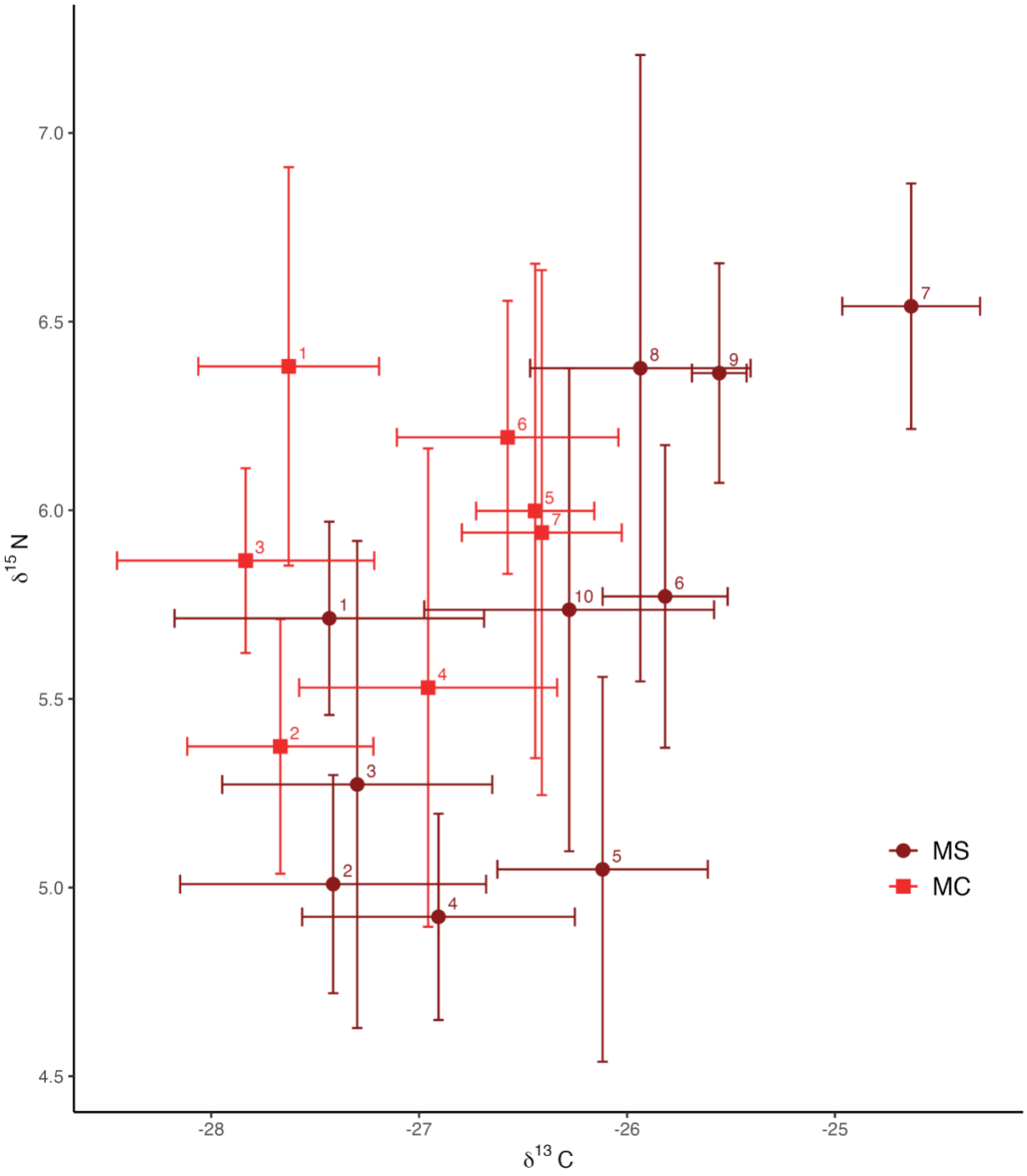

Figure 2.3. Relative position of rusty crayfish across core sampling sites in isotopic space. Means (points) and standard deviation (lines) of the stable isotope signatures of each site (MS = mainstem; MC = Murderers Creek).

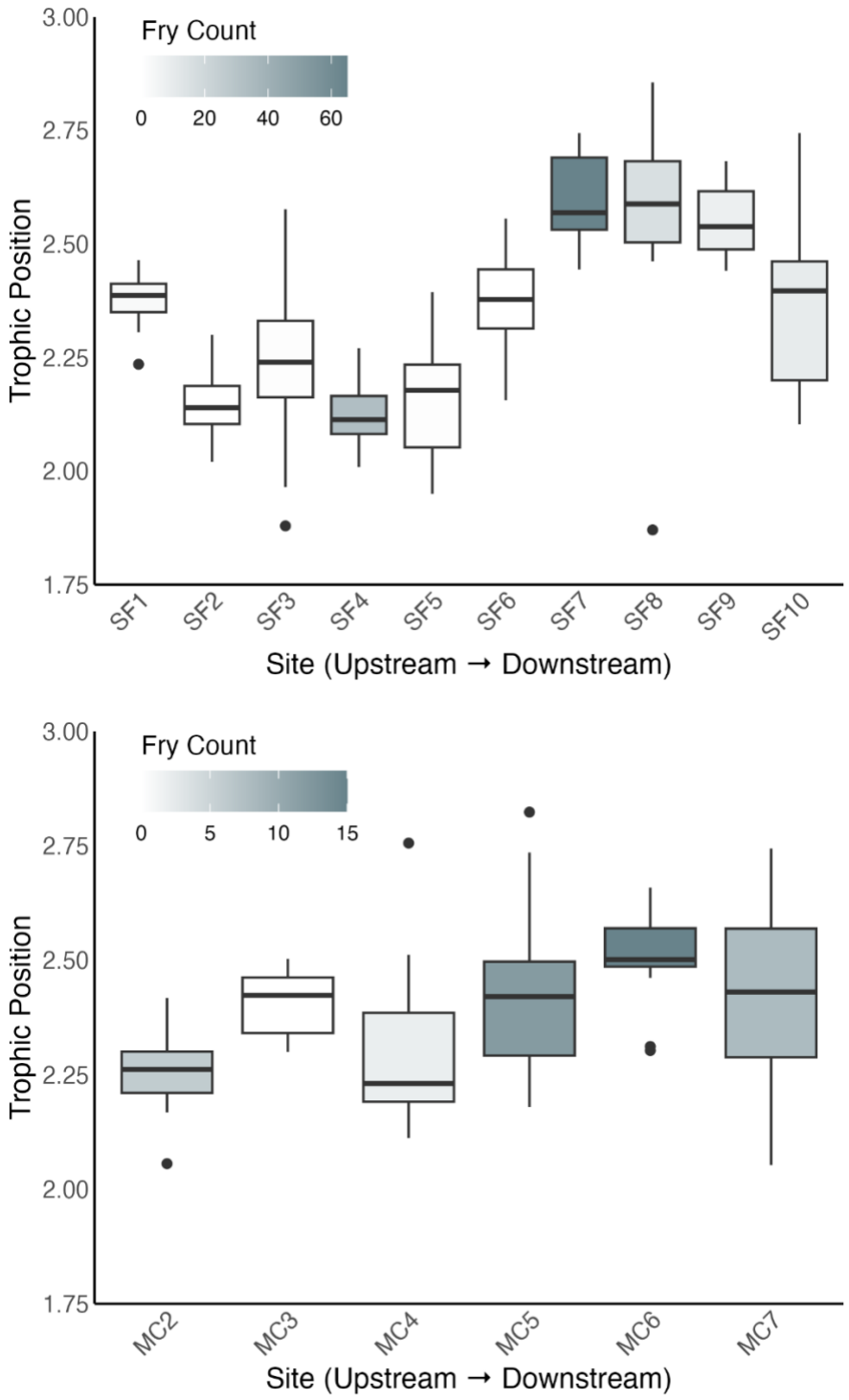

Figure 2.4. Average trophic position of rusty crayfish across **(A)** South Fork sites (n = 10) and **(B)** Murderers Creek sites (n = 7; rusty crayfish were absent at MC1). Error bars represent 1 standard deviation using site-specific averages. Color gradient of boxes represent raw counts of steelhead fry observed during snorkel surveys.

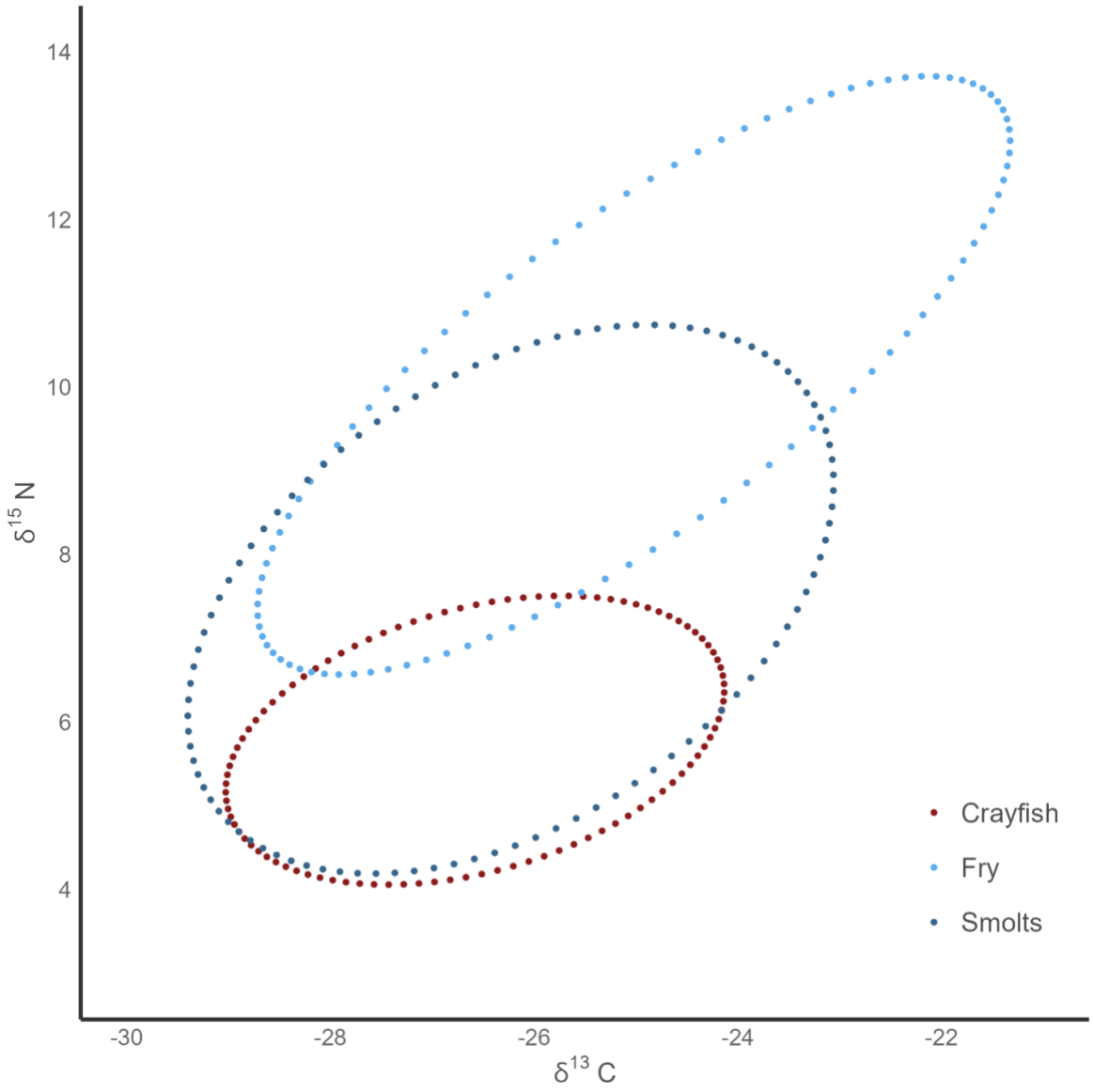

Figure 2.5. Maximum likelihood fitted standard ellipses of rusty crayfish, steelhead fry, and steelhead smolts estimated by the SIBER package (R). Ellipse locations represent the trophic position (central tendency), and size indicates variation in resource use. The dotted lines surround the standard ellipse area (SEAc) for each consumer.

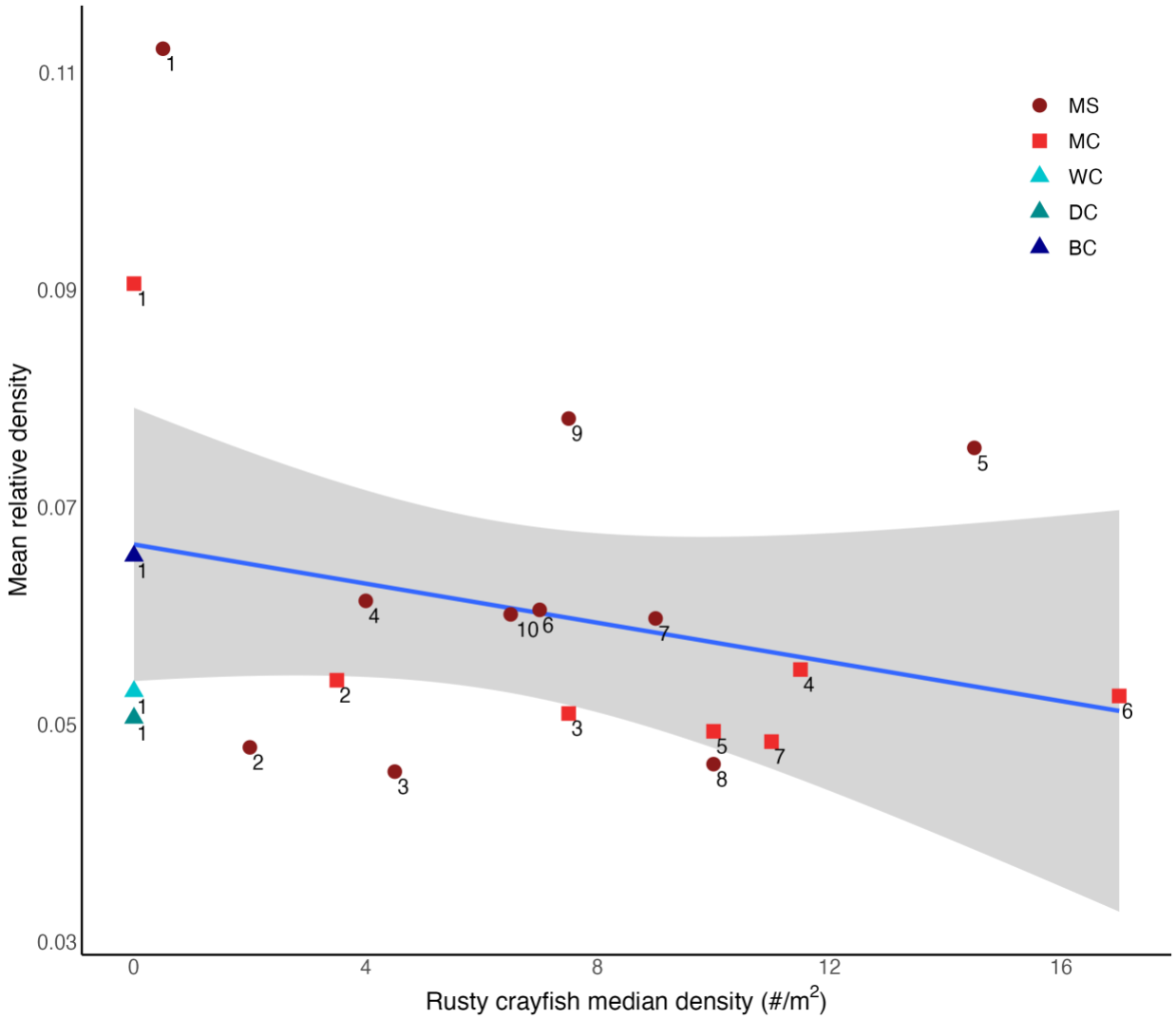

Figure 2.6. Relationship between median density (ind/m^2) of rusty crayfish and mean relative density of drift-weighted macroinvertebrate communities. Points represent site-specific values (MS = mainstem, MC = Murderers Cr., WC = Wind Cr., DC = Deer Cr., BC = Black Canyon Cr.)

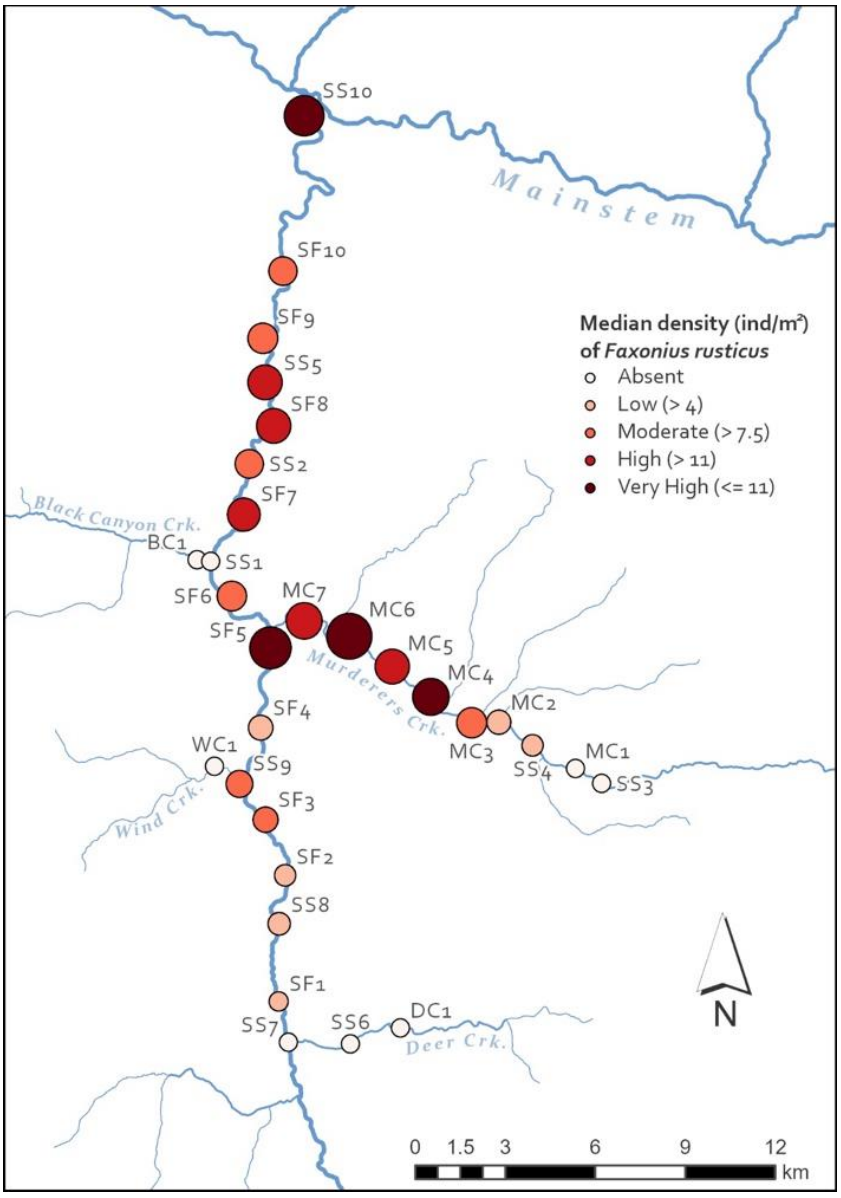

Figure 2.7. Adult crayfish density at survey sites across the South Fork of the John Day River, Oregon (n = 30). Estimates are the median of six samples, collected with kick-seines at six of twelve transects per site.

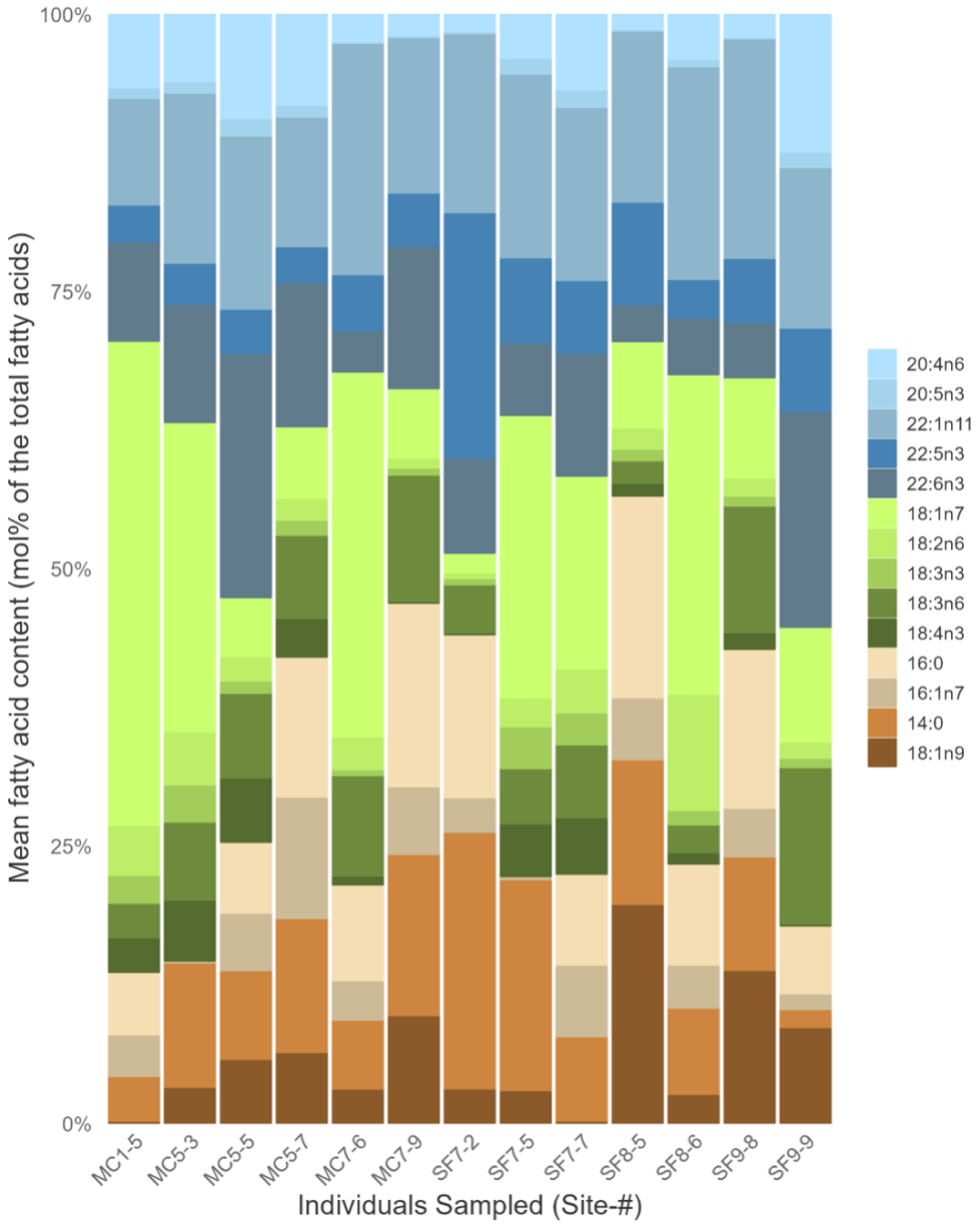

Figure 2.8. Rusty crayfish fatty acid profiles from South Fork (SF) and Murderers Creek (MC) sites. Each column shows the proportional contribution of dietary FAs: oleic acid (18:1n9), myristic acid (14:0), palmitoleic acid (16:1n9), palmitic acid (16:0), stearidonic acid (18:4n3), γ-linolenic acid (18:3n6), α-linolenic acid (18:3n3), linolenic acid (18:2n6), vaccenic acid (18:1n7), docosahexaenoic acid (22:6n3), docosapentaenoic acid (22:5n3), docosatricarboxylic acid (22:1n11), eicosapentaenoic acid(20:5n3), and arachidonic acid (20:4n6).

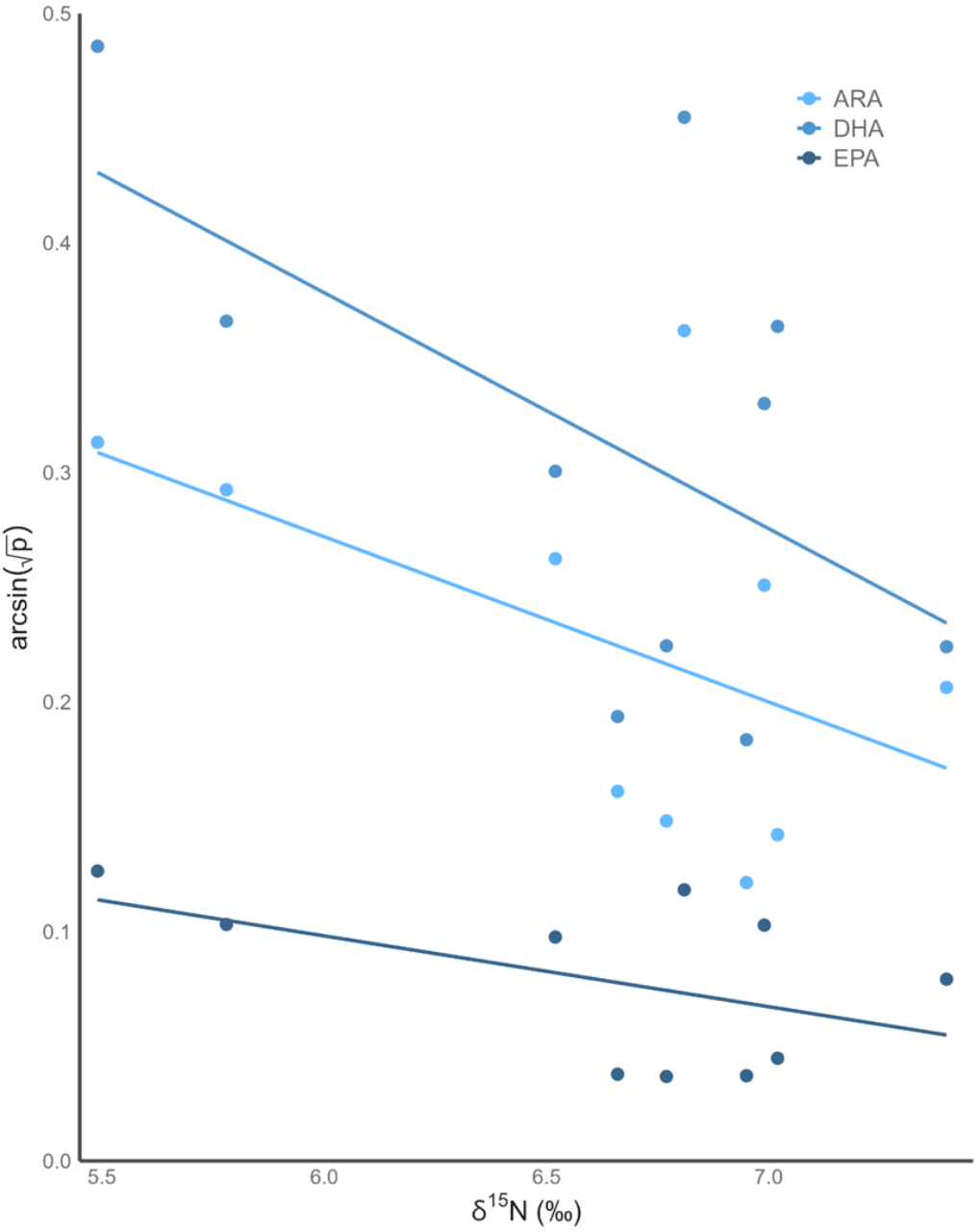

Figure 2.9. Relationships between the proportional contribution of essential fatty acids (EFAs) and $\delta^{15}N$ values of rusty crayfish across sites in the South Fork John Day River. Arcsine-square-root–transformed proportions (p) of ARA, DHA, and EPA are plotted against individual crayfish $\delta^{15}N$ values, with separate linear regressions shown for each EFA.

2.9 Tables

Table 2.1. Abiotic and biotic characteristics by survey site.

Site	Lat	Lon	Survey Length (m)	Max Pool Depth (cm)	Mean Depth (cm)	Wetted Width (m)	Fish Cover	Fry Count
SF1	44.204	-119.532	162	70	37.2	6.92	2	3
SF2	44.242	-119.531	163	115	41.5	7.13	1	0
SF3	44.258	-119.540	110	90	45.3	5.78	1	1
SF4	44.285	-119.543	176	105	41.6	8.19	2	33
SF5	44.309	-119.540	163	75	45.9	7.77	2	1
SF6	44.324	-119.557	212	152	57	10	2	0
SF7	44.348	-119.553	210	120	44.3	10.56	1	65
SF8	44.374	-119.542	195	100	58.1	11.38	1	16
SF9	44.400	-119.547	126	120	68	10.65	1	8
SF10	44.420	-119.540	294	105	61.8	11.76	1	10
MC1	44.276	-119.411	133	90	32.5	4.89	3	4
MC2	44.289	-119.444	137	71	29.5	5.06	1,2	6
MC3	44.288	-119.455	172	76	42.5	5.91	2	9
MC4	44.296	-119.472	139	90	37.5	4.78	2	2
MC5	44.304	-119.489	81	120	37	5.7	1,2	12
MC6	44.313	119.507	165	65	39.7	5.15	2	15
MC7	44.317	-119.526	130	58	45.5	4.86	2	8
BC1	44.334	-119.572	73	50	18.9	4.48	3	124
WC1	44.273	-119.562	103	73	28.4	4.84	3	90
DC1	44.197	-119.481	114	60	19.2	4.82	3	0

	Crayfish Density (ind./sq. m)			
Site	Mean	Min	Max	Median
SF1	0.3	0	1	0.5
SF2	3.2	0	20	2.0
SF3	2.9	1	14	4.5
SF4	3.0	1	12	4.0
SF5	9.4	11	28	14.5
SF6	8.7	2	57	7.0
SF7	6.6	3	25	9.0
SF8	7.7	6	33	10.0
SF9	4.7	4	17	7.5
SF10	4.0	5	13	6.5
MC1	-	-	-	-
MC2	2.5	0	10	3.5
MC3	3.8	2	11	7.5
MC4	7.1	5	29	11.5
MC5	6.2	5	20	10.0
MC6	9.5	9	27	17.0
MC7	5.9	5	15	11.0
BC1	–	–	–	-
WC1	–	–	–	-
DC1	–	–	–	-

Table 2.2. Isotopic metrics for analyzed populations of steelhead fry (Sfry), rusty crayfish (RC), and steelhead smolts (Ssmolts). Reported values include total area (TA), SEA (standard ellipse area), and SEAc (SEA corrected).

	Sfry	**RC**	**Ssmolts**
TA	10.97	7.43	18.04
SEA	2.07	4.31	4.92
SEAc	2.08	4.79	5.15
ML	Fitted Standard Ellipses		
Area	4.79	2.08	5.14
Overlap	0	-	0.29
ML	Predicted (95%) Standard Ellipses		
Area	28.66	12.47	30.82
Overlap	1.32	-	11.83
Overlap.p	0.03	-	0.38

Table 2.3. Mean contribution of FA types (%) to total dietary lipid profiles of crayfish (denoted as "RC-site-sample") in the mainstem South Fork (top) and Murderers Creek (bottom). Total proportions of saturated (SFA), monounsaturated (MUFA), and polyunsaturated (PUFA), and marine-derived nutrient ratios (DHA:EPA), are listed by individual crayfish (columns) and summarized (mean, standard deviation) across both tributaries.

	South Fork								
Variables	**RC-7-2**	**RC-7-5**	**RC-7-7**	**RC-8-5**	**RC-8-6**	**RC-9-8**	**RC-9-9**	**μ**	**± SD**
∑ SFA	38.2	19.2	16.1	31.6	17.2	24.9	8.1	22.2	10.2
∑ MUFA	24.1	45.3	39.5	48.5	54.3	46.9	34.8	41.9	10.1
∑ PUFA	37.8	35.5	44.3	20	28.4	28.2	57.1	35.9	12.2
14:0 (M)	23.3	19.2	7.8	13.2	7.9	10.4	1.8	11.9	7.3
16:0 (PA)	14.9	0	8.4	18.4	9.3	14.5	6.3	10.3	6.2
16:1n9 (POA)	3	0.3	6.3	5.4	3.7	4.2	1.3	3.5	2.1
18:1n7 (VA)	2	25.6	17.5	8	29	9.2	10.5	14.5	9.9
18:1n9 (OA)	3	2.9	0.1	19.6	2.5	13.7	8.5	7.2	7.1
18:2n6 (LIN)	0.5	2.6	4	1.9	10.5	1.6	1.5	3.2	3.4
18:3n6 (GLA)	4.5	5.1	6.7	2.2	2.7	11.6	14.3	6.7	4.6
18:3n3 (ALA)	0.4	3.6	2.7	0.9	1.1	0.7	0.7	1.4	1.2
18:4n3 (SDA)	0	4.6	4.9	1	0.9	1.4	0	1.8	2.1
20:4n6 (ARA)	1.7	4.1	7	1.5	4.2	2.2	12.5	4.7	3.9
20:5n3 (EPA)	0.2	1.4	1.5	0.1	0.6	0.1	1.4	0.8	0.7
22:1n11 (DCA)	16.1	16.6	15.6	15.4	19.2	19.8	14.5	16.7	2
22:5n3 (DPA)	22.2	7.7	6.6	9.1	3.5	5.6	7.5	8.9	6.1
22:6n3 (DHA)	8.4	6.4	10.9	3.3	4.9	5	19.3	8.3	5.5
DHA:EPA	42.5	4.5	7.1	24.1	7.9	36.6	13.9	19.5	15.2

	Murderers Creek							
Variables	**SC-1-5**	**RC-5-3**	**RC-5-5**	**RC-5-7**	**RC-7-6**	**RC-7-9**	**μ**	**± SD**
∑ SFA	10	11.5	14.7	25	15.2	31.4	18	8.4
∑ MUFA	57.1	46.6	31.8	35.4	60.3	36	44.5	12.1
∑ PUFA	32.9	41.9	53.5	39.5	24.5	32.6	37.5	9.9
14:0 (M)	4.2	11.4	8.2	12.2	6.4	14.7	9.5	3.9
16:0 (PA)	5.8	0.1	6.6	12.8	8.8	16.7	8.5	5.8
16:1n9 (POA)	3.6	0.1	5	10.8	3.4	6	4.8	3.5
18:1n7 (VA)	43.8	28.1	5.5	6.7	33.1	6.5	20.6	16.6
18:1n9 (OA)	0.1	3.1	5.7	6.3	3	9.6	4.6	3.3
18:2n6 (LIN)	4.5	4.8	2.2	1.9	3	0.9	2.9	1.5
18:3n6 (GLA)	3.3	7.2	7.8	7.7	9.2	11.5	7.8	2.7
18:3n3 (ALA)	2.4	3.2	0.9	1.2	0.4	0.5	1.4	1.1
18:4n3 (SDA)	3	5.3	5.6	3.3	0.6	0	3	2.3
20:4n6 (ARA)	6.7	6.2	9.5	8.3	2.6	2	5.9	3
20:5n3 (EPA)	0.9	1.1	1.6	1.1	0.1	0.2	0.8	0.6
22:1n11 (DCA)	9.6	15.3	15.6	11.7	20.9	14	14.5	3.9
22:5n3 (DPA)	3.4	3.7	4.1	3.3	4.9	4.8	4	0.7
22:6n3 (DHA)	8.8	10.5	21.8	12.8	3.7	12.7	11.7	6
DHA:EPA	9.2	10	13.7	12.1	25.9	63.2	22.4	20.9

2.10 Appendix

2.10.1 Baseline correction rationale

We examined $\delta^{13}C$ and $\delta^{15}N$ values of all potential energy sources (benthic macroinvertebrates, filamentous algae, and periphyton) for significant differences between sites prior to our analyses of these values for rusty crayfish. Significant differences in $\delta^{13}C$ were identified for multiple pairwise comparisons per source: benthic macroinvertebrates (n = 12; Kruskal-Wallis, $\chi^2 = 52.3$, $p < 0.0001$), filamentous algae (n = 3; Kruskal-Wallis, $\chi^2 = 49.5$, $p < 0.0005$), and periphyton (n = 2; Kruskal-Wallis, $\chi^2 = 33.5$, $p < 0.005$). Across all three sources, we found that $\delta^{13}C$ significantly varied between sites which limited our ability to perform further analysis (Figure 2.3A). In contrast, we found minimal evidence that supports any significant cross-site variation in $\delta^{15}N$ of all three sources. Significant differences in $\delta^{15}N$ were identified for a single site pairing per source: benthic macroinvertebrates (SF4 – SF10; Kruskal-Wallis, $\chi^2 = 22.1$, $p < 0.005$; Dunn-Holm, $Z = -3.4$, $p < 0.05$), filamentous algae (SF2 – SF10; Kruskal-Wallis, $\chi^2 = 47.7$, $p < 0.0005$; Dunn-Holm, $Z = -3.8$, $p > 0.05$), and periphyton (SF3 – SF9; one-way ANOVA, $F = 2.2$, $p < 0.05$; TukeyHSD, $p < 0.05$). Following these results, we assessed average $\delta^{15}N$ values of rusty crayfish. Sites lower in the South Fork (SF7-9) and sites in Murderers Creek (MC2-7) had higher $\delta^{15}N$ averages that were statistically similar, compared to a select number of SF sites (SF6, SF10) (Figure 2.3B; Kruskal-Wallis, $\chi^2 = 118.2$, $p = 3.5^{-16}$). See Table 2.A.1 for crosswise comparisons and coded significance.

Table 2.A.1. Output from Dunn-Holm test comparing $\delta^{15}N$ averages of rusty crayfish between sites.

Grp. 1	Grp. 2	n1	n2	*F*	*p*	*p* adjusted	Significance
MC1	SF2	7	12	-4.45	8.47e-06	1.04e-03	**
MC1	SF4	7	12	-4.69	2.68e-06	3.40e-04	***
MC1	SF5	7	12	-4.14	3.41e-05	4.10e-03	**
MC2	SF7	12	12	4.35	1.36e-05	1.65e-03	**
MC2	SF8	12	12	3.78	1.59e-04	1.83e-02	*
MC2	SF9	12	11	3.73	1.89e-04	2.13e-02	*
MC3	SF4	12	12	-3.56	3.75e-04	4.20e-02	*
MC4	SF7	12	12	3.82	1.35e-04	1.58e-02	*
MC5	SF2	12	12	-3.51	4.41e-04	4.90e-02	*
MC5	SF4	12	12	-3.79	1.48e-04	1.71e-02	*
MC6	SF2	12	12	-4.56	5.00e-06	6.25e-04	***
MC6	SF4	12	12	-4.85	1.26e-06	1.63e-04	***
MC6	SF5	12	12	-4.21	2.61e-05	3.16e-03	**
MC7	SF4	12	12	-3.77	1.66e-04	1.89e-02	*
SF2	SF7	12	12	5.69	1.27e-08	1.72e-06	****
SF2	SF8	12	12	5.12	3.13e-07	4.10e-05	****
SF2	SF9	12	11	5.04	4.59e-07	5.97e-05	****
SF3	SF7	12	12	4.47	7.84e-06	9.72e-04	***
SF3	SF8	12	12	3.90	9.80e-05	1.17e-02	*
SF3	SF9	12	11	3.85	1.18e-04	1.40e-02	*
SF4	SF7	12	12	5.97	2.37e-09	3.22e-07	****
SF4	SF8	12	12	5.40	6.81e-08	9.12e-06	****
SF4	SF9	12	11	5.32	1.05e-07	1.39e-05	****
SF5	SF7	12	12	5.33	9.84e-08	1.31e-05	****
SF5	SF8	12	12	4.76	1.98e-06	2.53e-04	***
SF5	SF9	12	11	4.69	2.72e-06	3.43e-04	***