Occupancy and Impacts of the Northern Crayfish (*Faxonius virilis*) in Tributaries of the North Saskatchewan River Basin

by

Victoria Adriana Van Mierlo

A thesis submitted in partial fulfillment of the requirements for the degree of

Master of Science

in

Conservation Biology

Department of Renewable Resources
University of Alberta

Abstract

Invasive species are the second greatest threat to biodiversity globally. Crayfish are especially robust invaders due to their omnivorous feeding behavior and ability to compete both directly and indirectly with native species. In the North Saskatchewan River (NSR) basin, the Northern Crayfish (*Faxonius virilis)* was introduced and has persisted since the early 1990s. Despite the North Saskatchewan River being an ecologically, economically, and culturally valuable watershed, the crayfish's impacts on Alberta's native fish communities have yet to be assessed.

I aimed to determine the in-stream environmental characteristics most associated with the crayfish's occupancy in the North Saskatchewan River and determined which currently unoccupied tributaries are most prone to future invasion. I deployed 24-hour baited crayfish traps and collected environmental measurements (water temperature (°C), turbidity (NTU), flow velocity (ms^{-1}), and physical complexity) at 37 tributary reaches along the Alberta portion of the North Saskatchewan River basin. Northern Crayfish were detected at 13 of 37 tributary reaches, with no occurrences detected west of the city of Edmonton, Alberta. Occupancy model selection and averaging revealed that water temperature alone drove occupancy of Northern Crayfish in tributaries of the North Saskatchewan River and that streams with mean summer water temperatures greater than 18.7 °C are 50% or more likely to be occupied by Northern Crayfish and thus, are at highest risk for subsequent invasion. Further, I found that streams with mean summer water temperatures below 15 °C were less than 25% likely to be or become occupied by Northern Crayfish. Cold water streams may have some natural protection against subsequent Northern Crayfish invasion.

I sampled ten North Saskatchewan River basin tributaries for *F. virilis* and six native common-generalist fish species and used stable isotope analysis to investigate if there is sharing of and/or exploitative competition for nutritional resources between *F. virilis* and native fish species. I also investigated if *F. virilis* sympatry was related to differences in fish isotopic characteristics and/or reduced body condition of native fishes. Moderate overlap (13.8-40.2%) of *F. virilis* and native fishes' fundamental isotopic niches indicated that *F. virilis* have the potential to consume the same dietary resources as secondary consumer native fishes. However, overall segregation of realized isotopic niches indicated a lack of resource sharing between *F. virilis* and native fishes in NSR tributaries. Similarity between the Bayesian estimated realized standard ellipse areas (SEA_B), carbon ranges, and body condition of *F. virilis* allopatric and sympatric native fish populations indicated that *F. virilis* sympatry did not have negative trophic effects on the native fish species in this study. My results suggest that *F. virilis* may be utilizing dietary plasticity to exploit a slightly different trophic niche than native fishes and in doing so, avoid exploitative competition for dietary resources. Dietary plasticity could facilitate the establishment and invasion of *F. virilis* populations in currently unoccupied tributaries of the North Saskatchewan River basin in the future.

The results from this thesis provide practical guidelines for watershed management of invasive Northern Crayfish populations in the North Saskatchewan River and Alberta. My findings also highlight the importance for watershed managers to continue to prevent further spread of *F. virilis* in the North Saskatchewan River basin to prevent potential negative impacts on native fish while further research is conducted.

Preface

This thesis is an original work by Victoria Adriana Van Mierlo. Ethics approval for this research project, for which this thesis is a part, was received from the University of Alberta Research Ethics Board, Animal Care and Use Committee “Assessing spread and impact of invasive species” AUP 00003578 and provincial Research License 20-3812 RL.

This thesis is dedicated to my nieces Elaine, Madeleine, and Charlotte.

Acknowledgements

This work was made possible with funding by Alberta Innovates and Alberta Environment and Parks. Thank you to Dr. Cristina Buendia-Fores and Dr. Faye Wyatt for their hard work in the Watershed Integrity Project's conception and funding securement. Thanks also goes to Dr. David Roberts for his analytical work in geospatial cluster determination. This work was also supported by scholarship funds provided by the University of Alberta and NSERC.

My thanks go to my supervisory committee which includes my primary supervisor Dr. Mark Poesch, co-supervisor Dr. Stephanie Green, and government professional committee member Dr. Craig Emmerton. To Mark, thank you for trusting me with this project as well as providing resources and invaluable mentorship throughout my degree. To Steph, thank you for demonstrating what a combination of strength, knowledge, and kindness look like and providing me with a template to model myself after as a woman in science. To Craig, thank you for your consistent helpfulness, leadership, and constructive input as the Watershed Integrity Project's leader. It has been a true privilege to work with all of you.

Next, I would like to thank all of my lab mates (Megan Edgar, Jacqueline Pallard, Chloe Christenson, Sebastian Theis, and Karling Roberts) and watershed management graduate student team members (Blake Stuparyk, Shelby Stenerson, and Jill Lightbown) for their friendship and encouragement. Despite the global pandemic's strains on social interaction, I felt truly close to each of you and will look back fondly on the happy memories of my friendships. For their hard work and comradery during field work data collection, I would like to thank Matthew Cunningham, Lauren Perras, Jesse Shirton, and Rebecca Huang.

To my parents Carol Van Mierlo and Adrian Van Mierlo, thank you for raising me to be strong, persevere, and believe in myself. There is no way I would have been able to make it through this degree without both of your unwavering support. I am extremely proud and thankful to be your daughter. Finally, I am grateful to my partner, Sebastian Theis, for being my love, my rock, and my best friend. Thank you for your love and support, always.

Table of Contents

List of Tables

Table A3.1 – Means and standard deviations for raw $\delta^{13}C$ and $\delta^{15}N$ muscle content as well as means and standard deviations for baseline corrected $\delta^{13}C$ ($\delta^{13}C_{corr}$) and baseline calculated trophic position (TP) for each species, within each reach sampled. Letter in parentheses beside unique reach code indicates *F. virilis* occupancy of that reach: p = present, a = absent. CPUE is the *F. virilis* catch per unit effort of each reach reported in mean number of individuals caught per single 24-hour overnight trapline survey. The number of individuals of each species within each reach (n) is provided. Species with $n < 3$ within each reach were excluded from realized niche width analyses with the exception of species of the genus *Catostomus*. If the sum of individuals of species of the genus *Catostomus* was $n \geq 3$, these individuals were combined into a group called *Catostomus spp.*. This *Catostomus spp.* group was included in realized niche width analyses. All individuals, including those with $n < 3$ within each reach were retained and included in fundamental niche width analysis.

List of Figures

that of *F. virilis*. Plotting of core isotopic niches and % overlap calculations were done using the *SIBER* R package.

Figure 3.3 – Corrected isotopic carbon (δ^{13}Ccorr) and trophic position (TP) biplots showing the core realized isotopic niche width of each species within each reach where northern crayfish were found to be present. Panel letters indicate the specific reach as follows: (**A**) BEA1, (**B**), BMD2, (**C**) BMD3, (**D**) VER4, and (**E**) WMD2. Isotopic niche widths are expressed in ‰^{2} and were calculated using small sample size corrected standard ellipse area (SEAc) which contains 1 SD around the mean or approximately 40% of the data for each species. Isotopic niches are labeled with the corresponding species' shorthand name, ellipse color, and marker type. Black open circles = *F. virilis*; red crossed circles = *L. lota*; orange open diamonds = *C. plumbeus*; blue open triangles = *R. cataractae*; and green open squares = *Catostomus spp.*. Percentages above species names indicate the percent overlap of fish species' isotopic niche with that of *F. virilis*. Lack of a percentage above a fish species name indicates a lack of trophic niche overlap with *F. virilis*. Plotting of core isotopic niches and % overlap calculations were done using the *SIBER* R package.

Figure 3.4 – Density plots of realized isotopic niche widths (SEA$_B$ ‰^{2}) of the three secondary consumer fish species ((**A**) *C. plumbeus* [*n*=35], (**B**) *R. cataractae* [*n*=65], and (**C**) *Catostomus spp*. [*n*=33]) compared where *F. virilis* are present vs. absent. Black dots represent the bootstrapped mean SEA$_B$ areas. Blue crosses represent the small sample size corrected standard ellipse area (SEAc). Boxes around means indicate the 95%, 75%, and 50% probability intervals of the SEA$_B$ area. Lower case letters indicate significant differences between mean SEA$_B$ values where different letters indicate significant differences with 95% confidence and like letters indicate statistically similar mean SEA$_B$ values. Unique reach codes appear below their respective bar.

Figure 3.5 – Range plot comparing reach specific δ^{13}Ccorr ranges (Δ‰) which are reflective of dietary source richness of the three secondary consumer fish species (*R. cataractae*, *C. plumbeus*, and *Catostomus spp.*) compared between where *F. virilis* are present (grey bars) vs. absent (white bars). Unique reach codes appear on the y-axis for each respective bar. The * symbol to the right of a carbon range bar indicates that that reach is located in the highly urbanized core of Edmonton, Alberta.

Figure 3.6 – Violin plots comparing mean body condition (as described by the relative weight condition metric [Wr] and reported in %) of *R. cataractae* (*n* = 43), *Catostomus spp*. (*n* = 9), and *C. plumbeus* (*n* = 13) over all reaches in which crayfish are present against all reaches where crayfish are absent. Significant difference between means is represented by an asterisk (*).

Figure A2.1 – Work flow of all statistical analyses conducted in this study. Individual boxes represent results section headings. Within each box, headings indicate the purpose of the analyses conducted while the italicized text in brackets list the statistical tools/analyses used to achieve the goal of each section. Boxes lined up horizontally make up the analysis work flow that resulted in the study's main findings. Boxes to either side contain analyses that were complimentary to the main results.

Figure A2.2 – Box and whisker plots comparing historic summer (June-August) water temperatures from 1954-2019 in the NSR basin (*n*=111) to the water temperatures sampled during this study in 2020 (*n* =37). No significant difference was found (p = n.s).

Figure A3.1 – Dissection images of two *L. lota* stomach contents which consisted of juvenile *F. virilis*. Both *L. lota* specimens were captured from reach WMD2. Arrows and numbers indicate the location and number of juvenile *F. virilis* in each stomach. Panel **A** shows that only one single *F virilis* juvenile was found in the stomach contents. Panel **B** shows six individual juvenile *F. virilis'* present in the *L. lota* stomach contents.

Chapter 1: General Introduction

Freshwater biodiversity provides human populations with invaluable ecosystem services, cultural significance, and economic resources (e.g. commercial and recreational fisheries) (Dudgeon et al., 2006). At the same time however, freshwater biodiversity is undergoing a steep decline. Freshwater vertebrate populations declined by 81% globally from 1970 to 2012 which is more than twice the declines seen in either terrestrial or marine vertebrates in the same time frame (McRae et al., 2017). Rapid decline of freshwater vertebrates can be attributed to human actives including habitat loss, overexploitation of stocks, and the introduction of invasive species (Dudgeon et al., 2006; Reid et al., 2019).

Invasive species are those that are established outside of their native range and cause harm (ecologically and/or economically) where they are introduced (Mack et al., 2000). Invasive species begin as non-native species that are transported outside of their native range either accidentally (e.g. fouled boat hulls or uncleaned muddy boots) or purposefully (e.g. stocking for recreational fishing) by humans. A non-native species must survive the initial transport, establish a self-sustaining population, spread to new locations, and cause negative ecological impacts in its extended range before it is classified as an invasive species (Lockwood et al., 2013; Mack et al., 2000). The majority of translocated non-native species are unable to move through the stages from introduction to spread leaving a small number of species that become invasive. These few invasive species can cause significant harm on native ecosystems in various ways including competition, predation, and ecosystem engineering (Lockwood et al., 2013). Aquatic invasive species (AIS) are an especially problematic invasive species group as they are difficult to detect, and once detected are difficult to control and contain (Ruppert et al., 2017). Crayfishes are a particularly worrying AIS group because in addition to their known generality, adaptability and omnivory, non-native crayfish species tend to become invasive species in a larger proportion than do other taxa (Longshaw & Stebbing, 2016).

The Northern Crayfish (*Faxonius virilis*; Hagen, 1870) is an inland crayfish species that is native to the central portions of Canada and the Northern United States (Williams, 2012). *F. virilis* resemble small lobsters and tend to be olive-brown in colour with yellow tuberculations on the chelae (Loughman, 2010). Although native to parts of the USA and Canada, *F. virilis* has established non-native populations of concern in the Southern United States, Eastern New

Brunswick, and Alberta (Donahou, 2020; McAlpine et al., 2007; Williams, 2012). In Alberta, *F. virilis* has been introduced to the Athabasca River, Battle River, Oldman River, Bow River, Milk River and North Saskatchewan River basins (Williams, 2012). Genetic microsatellite marker analysis of *F. virilis* populations in the North Saskatchewan River (NSR) basin revealed that their introduction was a result of both anthropogenic (capture and use as angler bait) and natural range expansion pathways (Williams, 2012). Currently, the province of Alberta has declared *F. virilis* a species of invasive concern and regulations to control the species' spread are in place. *F. virilis* can be legally captured without a license in any amount, all captured individuals must be killed prior to transport, and the use of *F. virilis* as anger bait is prohibited throughout the province (AEP, 2018).

Although present in multiple watersheds in Alberta, I concentrate on established *F. virilis* populations in the NSR basin. When last assessed, the NSR basin's ecosystem services were estimated to be worth $17.7 billion dollars which is equal to 18.3% of the total economic value (GDP) of the NSR basin and 6% of Alberta's provincial GDP in 2007 (North Saskatchewan Watershed Alliance [NSWA], 2010). Additionally, the basin supports multiple freshwater fish species at risk (Nelson & Paetz, 1992). Despite its ecological, economical, and social importance, the watershed is highly impacted by anthropogenic land-use stress and invasive species that have established themselves in this watershed, one of which is *F. virilis* (North Saskatchewan Watershed Alliance (NSWA), 2010).

To this point, few studies have evaluated the distribution of *F. virilis* populations in tributaries of the NSR with the most recent distribution data collected in 2011 (Williams, Proctor, et al., 2011). Other studies addressing NSR *F. virilis* populations are few and concentrate on their symbionts (e.g. Gale & Proctor, 2011; Williams et al., 2009; Williams, Williams, et al., 2011). Currently, no studies have investigated what local environmental characteristics are associated with *F. virilis* occupancy of NSR tributaries. Nor have any aimed to determine what trophic position *F. virilis* occupies in tributary food webs or evaluated the interactions and potential negative impacts that *F. virilis* may have on Alberta's native fish species.

I update the distribution of *F. virilis* populations in the NSR basin (Chapter 2). From there, I investigate the occupancy and impacts of *F. virilis* populations on native fishes of the North Saskatchewan River basin. Specifically, I surveyed 43 tributary reaches for *F. virilis* and developed an occupancy model to determine the local environmental characteristic(s) most associated with *F. virilis* occupancy. I averaged the best models to determine the parameter ranges most associated with *F. virilis* occupancy and advise on which currently unoccupied tributaries are at highest risk of future occupation (Chapter 2). I then conducted stable isotope analysis on specimens of *F. virilis* and six native fishes from a subset of ten tributary reaches to investigate what potentially detrimental trophic interaction(s) *F. virilis* has with native fishes (Chapter 3). The results of this thesis will provide watershed managers with practical advice on how to better prevent the spread of *F. virilis* within the NSR basin. My results will also add to the understanding of how non-native crayfish interact with and/or impact the trophic characteristics and body condition of native freshwater fish populations.

Chapter 2: Occupancy of invasive Northern Crayfish (*Faxonius virilis*) in the North Saskatchewan River basin is driven primarily by water temperature

Introduction

Non-native species are those that are present in an environment that is not part of their natural range. When non-native species become established in such an environment and their presence results in negative ecological, economic, and/or human-health impacts, they are described as "invasive species" (Mack et al., 2000). Invasive species have a marked adverse impact on global freshwater biodiversity, second only to habitat destruction, and more so than overexploitation, pollution and augmentation (Dudgeon et al., 2006; Mack et al., 2000). Aquatic invasive species (AIS) are an especially problematic invasive species group as they are challenging to detect. Once detected, they are difficult to control and contain (Ruppert et al., 2017).

Crayfish are a particularly problematic AIS group due to their generality in habitat use, adaptability, and omnivorous feeding behaviour. Their omnivorous feeding behaviour allows crayfish to compete with native fauna on multiple trophic levels simultaneously. Invasive crayfish have been documented as causing a host of ecological issues, including trophic cascades, truncation of food webs, and modification of habitat (Dorn & Wojdak, 2004; Geiger & Alcorlo, 2004; Jackson et al., 2014; Rodríguez et al., 2005). They have been known to compete with herbivores and modify habitat by decimating macrophytes that cause trophic cascades by reducing aquatic snail biomass, resulting in an increase in periphyton biomass (Jackson et al., 2014). Studies have also shown that crayfish directly affect the young-of-year of threatened fish species by consuming fish eggs and fry (Caroffino et al., 2010; Fitzsimons et al., 2002; Savino & Miller, 1991; Setzer et al., 2011). Finally, crayfish can compete indirectly for important microhabitats such as shelters that are essential for small and juvenile fish to evade predators (Longshaw & Stebbing, 2016).

Northern Crayfish (also known as the virile crayfish) (*Faxonius virilis,* Hagen, 1870), is an inland crayfish species that is native to large portions of Canada and the Northern United States. *F. virilis* live and reproduce in warm, well-oxygenated rivers with moderate flow velocity and turbidity (Brewer et al., 2009; Haddaway et al., 2015; Lehti-Koivunen & Kivivuori, 1994; Light, 2003; Olsson & Nyström, 2009; Smith et al., 1996). The thermal niche of *F. virilis* is challenged at low water temperatures (10-13 °C) where molting is slowed or halted completely (Momot, 1967; Rogowski et al., 2013). Additionally, crayfish reach sexual maturity later, embryonic development is slower, and juvenile mortality is higher at water temperatures below 16°C (Jin et al., 2019; Wetzel & Brown, 1993). Due to their inability to hold station under high flow velocity, *F. virilis* tend not to be found in fast-moving streams (Light, 2003; Maude & Williams, 1983).

Although native to Canada (from Quebec to Saskatchewan), established invasive populations of *F. virilis* are cause for concern in Eastern New Brunswick and Alberta (Donahou, 2020; McAlpine et al., 2007; Williams, 2012). In Alberta, *F. virilis* has been introduced to watersheds including the Athabasca River, Battle River, Oldman River, Bow River, Milk River and North Saskatchewan River (NSR) (Williams, 2012). The NSR runs through Edmonton, Canada's fifth-largest and North America's northern-most city with a population of over 1 million inhabitants. In 2007, NSR basin ecosystem services were estimated to be worth $17.7 billion dollars, equal to 18.3% of the total economic value (GDP) of the NSR basin and 6% of Alberta's GDP in the same year (North Saskatchewan Watershed Alliance [NSWA], 2010). Additionally, the basin supports aquatic species at risk (e.g. Bull Trout [*Salvelinus confluentus*] and Plains Sucker [*Catostomus platyrhynchus*]) as well as economically important sport fisheries (e.g. Northern Pike [*Esox* Lucius]) and culturally important species (e.g. Lake Sturgeon [*Acipenser fulvescens*]). Despite the watershed's ecological, economic, and cultural importance, the crayfish's impacts have yet to be assessed.

The goal of this study is to determine the environmental characteristics that are most associated with *F. virilis* occupancy in the NSR basin and which currently unoccupied tributary reaches are most susceptible to crayfish invasion. Specifically, I address the following two research objectives: (1) determine what suite of local (instream) habitat features best predict crayfish occupancy in tributaries of the NSR basin and what variables affect crayfish detection; and (2) identify which unoccupied tributary reaches ought to be prioritized for additional monitoring and prevention of subsequent invasion based on the suite of local habitat features identified in the first objective. I used occupancy modelling to meet these objectives. Occupancy models have been used to determine the distribution of crayfish as well as the ecological characteristics that best predict their occupancy while accounting for imperfect detection (e.g. Ficetola et al., 2011; Loughman et al., 2012; Magoulick et al., 2017; Smith et al., 2019; Yarra & Magoulick, 2018). Due to *F. virilis*' known affinity for warm, medium to low flow velocity, and productive, moderately turbid, physically complex environmental conditions, I hypothesize that the suite of environmental covariates that would best predict crayfish occupancy would consist of water temperature, flow velocity, turbidity, and in-stream physical complexity (Bowman, 2019; Longshaw & Stebbing, 2016; Maude & Williams, 1983; Momot, 1967; Olsson & Nyström, 2009; Rogowski et al., 2013; Rosewarne et al., 2014; Simon & Cooper, 2014; Wetzel & Brown, 1993).

Methods

Study area & tributary reach selection

The study area included tributaries of the Alberta portion of the North Saskatchewan River (NSR) basin. The basin is a glacier-fed watercourse that begins in the Rocky Mountains at the Saskatchewan Glacier. From the headwaters, the river's mainstem flows through Edmonton and continues to the Alberta-Saskatchewan border, just north of Lloydminster, Alberta. The basin includes multiple natural regions, including Alpine, Foothills, Parkland, and Prairie. Major tributaries of the basin from west to east include the Clearwater, Brazeau, Sturgeon, and Vermilion Rivers. Previous to *F. virilis* invasion, the basin possessed no endemic decapod species (Williams, 2012).

Field data were collected at 43 tributary reaches throughout the NSR basin from June through August 2020 (**Figure 2.1**). Each tributary reach (referred to hereinafter as "reach") consisted of a 200-300 m stretch of tributary and all reaches were located within a 4-6 Strahler stream order tributary of the NSR basin. No mainstem NSR reaches were sampled because mainstem environments were outside of the study's scope and because of low sampling feasibility due to high water velocity and depth. Reach selection was conducted based on a geospatial analysis which divided the basin into six clusters based on broad ecozones and accompanying geospatial data (Roberts, unpublished data). Alpine, Subalpine, and Montane natural regions were excluded from the analysis due to difficulty of access to these reaches and because these regions are relatively pristine (little anthropogenic footprint) compared to the rest of the basin. Forty-six variables describing various anthropogenic and natural characteristics of the basin were included in a Principal Component Analysis (PCA) and cluster analysis was used to assign each hydroshed unit (Lehner et al., 2008) to one of six clusters (Roberts, unpublished data). Reaches were selected based on these clusters to ensure even distribution of reaches throughout the basin and among natural basin features and anthropogenic land-uses. Further reach selection was conducted based on reconnaissance visits and were included or excluded based on accessibility and reach conditions.

Crayfish sampling

F. virilis were sampled using modified minnow traps with 2¼ inch diameter openings. Individual traps were tied to nylon rope 3 m apart in sets of six to form a trapline. Traplines were baited using Purina Friskies® salmon-based cat food-filled perforated film canisters in each individual trap and affixed to the river bank by a loop and rebar stake on the upstream end of the trapline. A total of four traplines were deployed at each reach for a total of 24 traps per reach. Traplines were deployed and numbered from first to fourth along the riverbank in an upstream direction and placed 50m apart to reduce spatial overlap between surveys and reduce spatial random effects (Acosta & Perry, 2000). A single trapline was the measurement unit considered as a single crayfish survey (**Figure 2.2**). Because crayfish are nocturnal and most active at night (Styrishave et al., 2007), baited traplines were left overnight to increase the chance of capture. The morning following deployment, traplines were retrieved from the water and presence/absence data as well as crayfish count data per trapline were recorded. All captured crayfish were humanely euthanized using a 15-minute ice bath and pithing in accordance with the University of Alberta, CCAC animal handling and ethics regulations (AUP No.: AUP00003578). Specimens were stored in ice-filled bags for transport to the University of Alberta for further analysis. No crayfish were returned to the water as they are a regulated invasive species in Alberta and all captured individuals must be euthanized on-site (AEP, 2021a). *F. virilis* presence/absence (p/a) data was compiled by trapline to create a detection history for the reach. For example, if presence is denoted as a 1 and absence as a 0, then at a reach were crayfish where detected in the first, third, and forth traplines, but not detected in the second trapline, the detection history including the four spatial sampling survey events at that reach is 1011 (**Figure 2.2**).

Covariate data collection

Local environmental characteristic measurements were collected after trapline retrieval so as not to disturb crayfish at the reach prior to sampling. Ten covariates (water temperature [C°], turbidity [NTU], flow velocity [ms^{-1}], p/a woody debris, p/a in-stream boulders, p/a overhanging banks [OHB], % rocky substrate cover, % macrophyte cover, % sand/silt cover, and water depth [cm]) were collected to model *F. virilis* occupancy and detection. Water temperature and turbidity were measured at each trapline using a Hydrolab DataSonde® 5X Multiprobe to the nearest tenth of a degree and NTU, respectively. Water depth was measured in five locations near each trapline that had been deployed using a Rickly USGS four-foot wading rod. Flow velocity was also measured in five random locations near each trapline that had been deployed at 40% depth (i.e. 60% distance off of the river bed) using a Marsh McBirney Flo-Mate^{TM} 2000. Presence or absence of woody debris, overhanging banks, and in-stream boulders was assessed visually within the vicinity of each trapline. Woody debris was defined as wood substrate (e.g. downed trees) that was totally or partially submerged in the water and was ≥ 15 cm in diameter (Zale et al., 2012). Overhanging banks were defined as river banks with eroded bottom portions that cause the top of the bank to hang over a portion of the river's surface (Zale et al., 2012). In-stream boulders were defined as rocky substrate that was ≥ 30cm in diameter and was fully or partially submerged in the water. Finally, the proportion of rocky cover, macrophyte cover, and sand/silt cover were visually assessed as the area covered by the substrate type, divided by the total area along the length of where the trapline was placed and within 50 dry cm and 50 wet cm of the water's edge. Rocky substrate included rock particles of cobble, gravel, and pebble size classes following the Wentworth Scale (Bunte & Abt, 2001).

Statistical analyses

Prior to analysis, reaches and covariates were inspected for missing values and data. This resulted in the exclusion of six reaches due to two or more missing covariate data values. The excluded reaches featured low physical complexity and near mean turbidity and flow velocity. Therefore, the exclusion of these six reaches likely did not have an effect on the study's results and was justified as it was necessary to retain all covariates in the data set. These six reaches were excluded, after which no missing values were found and all covariates were retained. A total of 37 reaches were included in the data set used for analysis. The existence of collinearity between covariates was inspected by conducting linear regression analysis between each unique pair of variables. Linear regression relationships between covariates with adjusted R^2 values exceeding 0.5 and significant p-values ($p < 0.05$) were considered colinear and redundant (Dormann et al., 2013; R Core Team, 2021).

Nonmetric multidimensional scaling

To reduce the total number of covariates and increase the parsimony of the models, the six physical complexity covariates (p/a woody debris, p/a in-stream boulders, p/a overhanging banks, % rocky substrate cover, % macrophyte cover, % sand/silt cover) were combined into a single gradient of in-stream physical complexity. I chose to use Nonmetric Multidimensional Scaling (NMDS) to achieve this over other multivariate statistical analyses (e.g. Principle Component Analysis [PCA]) because the physical complexity covariates were non-normally distributed and/or binary. Non-parametric analyses such as NMDS are much more flexible when working with non-normal and binary variables (Legendre & Legendre, 2012). First, I calculated the Bray-Curtis distance matrix (due to the binary nature of variables). It is important to note that % sand/silt cover was not included in the calculation as this variable is equivalent to the lack of % rocky substrate cover or % macrophyte cover and therefore, would have been redundant. I then performed NMDS ordination with two predefined dimensions of the final 37 reaches included in the model. The NMDS was calculated multiple times from random starts to achieve a stable solution using R *vegan* package (R function metaMDS, Oksanen et al., 2020). The first NMDS axis (NMDS1) was visually inspected in relation to covariate vectors and found to be a good representation of the physical complexity gradient. An inverse transformation (multiplication by -1) was applied to both NMDS axes so that positive values were associated with high physical complexity and negative values were associated with low physical complexity. I then replaced physical complexity covariate values with the complexity score values from NMDS axis 1 for each reach. These NMDS scores became the covariate representing in-stream physical complexity for the subsequent analysis: occupancy modelling.

Occupancy modelling and selection

The standard occupancy model assumes that the abundance of the target species is homogenous across all sample reaches (MacKenzie et al., 2017). However, during sampling it was apparent that *F. virilis* abundance was highly variable between reaches. For this reason, I elected to use the Royle & Nichols occupancy model in the R *unmarked* package (R function occuRN, Chandler et al., 2021) because it accounts for heterogeneity in target species abundance between reaches on the detection probability parameter (p) (Royle & Nichols, 2003). Detection probability may be larger in reaches with higher abundance because more individuals are present and thus, more likely to be detected. The Royle and Nichols model's occupancy parameter is denoted by lambda (λ) in lieu of psi (Ψ) which is used to denote the occupancy parameter in the standard occupancy model (MacKenzie et al., 2017). Occupancy was modelled using a global additive model with the following covariate structure for occupancy estimation and was based on hypotheses derived from the literature (**Table 2.1**): temperature + flow + turbidity + complexity. The detection estimation portion of the model included flow + depth covariate structure. Depth was included as a detection probability covariate because deeper reaches may prevent traplines from reaching the riverbed which would make the trapline inaccessible (physical survey barrier) to crayfish and therefore negatively impact detection (**Table 2.1**). Thus, the complete global model was λ (temp + comp + flow + turb), p(depth + flow). Goodness of fit of the global model to the data was assessed using Pearson's parametric bootstrapped chi-square test (MacKenzie & Bailey, 2004). If overdispersion was detected, standard error values were inflated by the square root of the overdispersion correction value (c-hat) to reduce the probability of a type I error (MacKenzie & Bailey, 2004). I then performed iteration of the global model and conducted model selection analysis with models comprised of all possible subsets of covariates to determine which covariates contributed positively to model parsimony. In particular, I calculated second-order bias-corrected Quasi-Akaike Information Criterion (QAICc) for each model, which is recommended for models with reasonable overdispersion (1> c-hat <4) and have n/K <40 where n is the number of observations and K is the total number of parameters in the model (Burnham & Anderson, 2004; MacKenzie et al., 2017). All strong (within ΔQAICc <2) and moderate (within ΔQAICc <3) models were considered to be plausible (MacKenzie et al., 2017). I interpreted covariates included in any of these models as having some potential to be incorporated into the most parsimonious explanation of the data. The directionality of covariate parameter estimates was used to interpret the relationship (negative or positive) of covariates with *F. virilis* occupancy. Covariate estimate standard errors were multiplied by 1.96 and added or subtracted from the estimate value to determine the upper and lower 95% confidence interval boundaries, respectively (Altman & Bland, 2005). The trapline and reach probabilities of detecting at least one crayfish were calculated using the detection intercept value from the best model (smallest QAICc) and principles of probability equations.

Water temperature standardized occupancy modelling and selection

To further investigate covariate-occupancy relationships, the effect of water temperature was controlled by repeating the above occupancy modelling procedures a second time on a subset of the data (n=19) including only reaches with water temperature measurements ≥ 18 C° (maximum = 22.2 C°) to achieve a standardized water temperature range spanning 4.2 C°. The aim of this analysis was to identify potential relationships between occupancy and turbidity, flow velocity, and physical complexity that may otherwise be masked by the effect of the strong water temperature gradient of the basin.

Model averaging

In order to make predictions about *F. virilis* occupancy based on covariate measurements among sampled streams, I used the R *AICcmodavg* package to perform model averaging of the temperature parameter estimate and calculate the model averaged-predictions and 95% confidence intervals (R functions modavg & modavgPred) based on the strongest (within ΔQAICc <2, Mazerolle, 2020) models (Buckland et al., 1997). Average occupancy probability was then plotted as a function of those predictors with 95% confidence intervals. The 50% probability of occupancy threshold was calculated based on the plot.

Generalized linear mixed model

To investigate potential random spatial effects that were unaccounted for in the occupancy model, I used the R *glmmTMB* package to create a generalized linear mixed model (GLMM) with the same covariate structure for occupancy estimation as the global occupancy model (λ [temp + comp + flow + turb]), with the addition of two spatial random variables: reach (n=37) and geospatial cluster (n=6) (R function glmmTMB, Magnusson et al., 2021). I chose a Poisson error distribution with a log link function for the GLMM because the response variable for this analysis was crayfish count per reach (Bolker et al., 2009). Random effect structure was assessed in four candidate global models, which differed in their random effect variable structures. Each global model possessed none (no random effects), reach, cluster, or reach + cluster (nested) as random effect variables. Model selection of these four candidate models was performed by calculating and comparing second-order bias-corrected Akaike Information Criterion (AICc) which is recommended for models with a small sample size but that are not overdispersed (Burnham & Anderson, 2004). The most parsimonious model with the smallest AICc value was the global model that included only reach as a random variable and was selected as the final global GLMM. I used the R *DHARMa* package to assess zero-inflation in the global model (R function testZeroInflation, Hartig & Lohse, 2021). Simulated residual plots were visually inspected and found to be normal, indicating proper model specification (Zuur et al., 2009). Iteration of the global model was then performed, followed by model selection analysis to determine which covariates contributed positively to model parsimony. Corrected Akaike Information Criterion (AICc) was then used to identify all strong (within ΔAICc <2) models which were considered to be plausible for identification and explanation of any deviation from the relationships found by the occupancy model that could result from random spatial effects. Covariate estimate standard errors were multiplied by 1.96 and added or subtracted from the estimate value to determine the upper and lower 95% confidence interval boundaries, respectively. Log-likelihood ratio test using a chi-square statistic was used to determine the significance of the random effect variable "reach" for all best models.

Model validation

To validate the reliability of the single season water temperature data and the accuracy of the occupancy model, I investigated two datasets (Government of Alberta Environment and Parks, 2021; Rosenberg et al., 2010).

The first data set was collected and provided by the Government of Alberta Department of Environment and Parks (AEP) and contains historic water temperature measurements from 1954-2019 at 726 individual tributary reach sites throughout the NSR basin. Water temperature data from water quality monitoring stations located on the NSR mainstream and its tributaries were included in the analysis. Only summer (June-August) water temperature observations were retained in the data set and minimum, maximum, and mean NSR basin summer water temperatures were calculated. Mean historic NSR basin water temperatures were then compared to mean water temperature measurements of the study's 37 sample reaches using a student's two-sided t-test and box and whisker plots (R Core Team, 2021).

The second dataset was provided by the Northwest Science and Information branch technical workshop report on invasive crayfish in the Lake Winnipeg River basin (Rosenberg et al., 2010). The data set includes both water temperature and *F. virilis* detection data at reaches (n=88) in tributaries of the Lake Winnipeg River basin. Lake Winnipeg River reach detection data was denoted as presence (1) and absence (0) and plotted as a function of reach water temperature. This was then overlaid on the model-averaged prediction of occupancy as a function of water temperature for comparison and model validation (**Figure 2.5**).

A work flow of all statistical analyses conducted during this study is available in the supplementary materials (**Figure A2.1**).

Results

A total of 239 *F. virilis* individuals were captured over the study period. *F. virilis* was positively detected in 14 of 43 reaches (mean=17 individuals per reach, range = 1-38) and no positive detections occurred west of the city of Edmonton, Alberta (**Figure 2.1**). Water temperature ranged from 9.4°C to 23.2°C with a mean water temperature of 17.4°C. The average flow velocity was 0.18 ms^{-1} with maximum and minimum flow velocities of 1.42 ms^{-1} and 0 ms^{-1}, respectively. Stream depth was an average of 42.6 cm deep (range = 3 - 107cm) and turbidity was 18.91 NTU on average (range = 0 - 174 NTU). There was a positive but insignificant correlation between temperature and turbidity ($R^2 = 0.290$, $p = 0.082$), and there was a negative but insignificant correlation between temperature and rocky stream cover ($R^2 = 0.093$, $p = 0.584$). No colinear or redundant relationships were identified.

Physical complexity gradient

A stable NMDS solution was reached after 20 random starts and had a stress value of 0.074 indicating low stress and a good fit to the data with two predefined dimensions. A gradient that described high (dominated by rocky substrate and boulders), medium (dominated by woody debris and macrophyte cover), and low (dominated by sand/silt cover) complexity was represented by axis 1 of the NMDS ordination (**Figure 2.3**). Axis 2 was not chosen to represent the physical complexity gradient as five out six covariate vectors possessed larger x-axis components than y-axis components and because % Rocky and Boulder vectors had very small y-axis components and would have not been well represented in the complexity gradient, had axis 2 been chosen. Positive loadings from the gradient (axis 1) were associated with high physical complexity, while negative loadings were associated with low physical complexity. The loadings from NMDS axis 1 were synthesized into the dataset as a single covariate for in-stream physical complexity hereafter referred to as physical complexity. Linear regressions of physical complexity with other covariates (water temperature, turbidity, flow velocity, and depth) resulted in weak and insignificant relationships ($R^2 < 0.5$, $p > 0.05$), indicating that the physical complexity covariate was independent from other covariates.

Covariate modelling

The Goodness of Fit test indicated a good fit of the global model (λ [temp + comp + flow + turb], p[depth + flow]) to the data (p = 0.20) and slight overdispersion (c-hat = 1.56). To account for the effect of overdispersion the square of the overdispersion correction factor was added to standard errors in each of the best models prior to 95% confidence interval calculation.

Iteration of the global model resulted in two models that were strongly supported by the data and four models that were moderately supported by the data (**Table 2.2**). The detection intercept value for the best model was found to be 0.67. Using the principles of probability, the probability of detecting at least one crayfish is equal to one minus the probability of not detecting a single crayfish in each of the four traplines at a reach. If the failure to detect a crayfish in trapline one, two, three, and four are represented by A', B', C', and D', respectively, then the detection probability at each reach is P(reach) = 1 - [p(A') x p(B') x p(C') x p(D')]. The probability of failing to detect a crayfish in a trapline at a reach where crayfish are present is equal to 1 – p = 1 – 0.67 = 0.33. Subbing the detection failure value into the reach detection probability equation: P(reach) = $1 - (0.33)^4 = 0.988$ = 98.8%.

The three best models lacked any detection covariates. Flow velocity and water depth appear as detection covariates in the fourth and sixth models, respectively. However, these estimates are not significant ($0 \subset$ 95% CI).

Water temperature was present as an occupancy covariate in all six best models (**Table 2.2**). The parameter estimates for water temperature in all six best models and the global model was positively (λmax = 0.418, λmin = 0.345) and significantly related to occupancy (95% CI: [0.130, 0.621]) (**Table 2.2**). Physical complexity was present as a covariate only in the second-best model (ΔQAICc = 1.35); however, the parameter estimate was insignificantly related to occupancy (λ = -2.666, 95% CI: [1.206, -6.538]). Turbidity and flow velocity were present in the third and fourth-best models, respectively (**Table 2.2**). Neither covariate estimate was significant in either of the models ($0 \subset$ 95% CI).

Temperature standardized covariate modelling

The Goodness of Fit test of the data subset of reaches with ≥ 18 C° and the same global model structure indicated poor fit ($p < 0.05$) and moderate overdispersion (c-hat = 2.25). To account for overdispersion, the square of the overdispersion correction factor (c-hat) was again added to standard errors in each of the best models prior to 95% confidence interval calculation.

Iteration of the global model resulted in two models that were strongly supported by the data (**Table A2.1**). The best model (QAICc = 33.95) did not include the water temperature covariate, indicating successful temperature standardization. Both best models lacked any detection covariates. Water temperature appeared as a positive ($\lambda = 0.472$) and significant occupancy covariate in the second model (95% CI: [0.0.56, 0.888]). No other covariate relationships were present, indicating that the strong effect of water temperature in the basin was not masking any other occupancy-covariate relationships (**Table A2.1**).

Temperature prediction with model averaging

Model averaging was performed on the full occupancy model only and not on the temperature standardized occupancy model. Model averaging of the water temperature occupancy parameter from the two best occupancy models (ΔQAICc <2) indicated that the probability of *F. virilis* occupancy of a stream reach is greater than 50% when mean summer water temperature is ≥ 18.7 °C (**Figure 2.4**).

Generalized linear mixed model

Iteration of the global model resulted in two best models that were strongly supported by the data (**Table 2.3**). Both water temperature and physical complexity were included as covariates in both best models. The water temperature parameter estimate was positive and significantly related to *F. virilis* abundance while the physical complexity gradient was negative and significantly related to *F. virilis* abundance (temp 95% CI: [0.274, 1.178]; comp 95% CI: [-1.360, -16.223]) (**Table 2.3**). Flow velocity was absent from the best model but present and positive in the second-best model; however, it was insignificantly related to *F. virilis* abundance ($0 \subset$ 95% CI). Variance and standard deviation of the reach random effect variable were 6.016 and 2.453 in the best model and 4.044 and 2.011 in the second-best model, respectively. The log-likelihood ratio test indicated that the random effect of reach was significant ($p<<0.05$) for both top GLMM models.

Model validation

Mean historic NSR basin summer (June-August) water temperature was 16.2 °C (max = 22.3 °C, min = 5.7 °C) between the years of 1954 and 2019. The student's two-sided t-test of difference of means between historic NSR basin water temperatures and water temperatures of the 37 reaches sampled in this study revealed that there was no significant difference between means ($p = 0.89$). Visual inspection of the box and whisker plot also indicated no significant difference in medians, maximums, or minimums (**Figure A2.2**).

Water temperature among the 88 reaches sampled in the Lake Winnipeg River basin crayfish-water temperature dataset had a mean of 22.2 °C and a maximum and minimum water temperature of 18.2 C° and 24.4 C°, respectively. *F. virilis* was positively detected in 92% (81 of 88) of reaches whereas only 8% (7 of 88) of reaches were absent of *F. virilis* (**Figure 2.5**).

Discussion

Detection probability of *F. virilis* was high across sample reaches, and there were no significant relationships between probability and detection covariates present in any of the best models. In particular, the detection intercept value of the best model ($p = 0.67$) indicates that there is a 67% probability of detecting a crayfish in each trapline given that crayfish are present at the sampling reach (**Table 2.2**). Further, the probability of detecting at least one crayfish at a reach, given that crayfish are present, was found to be 98.8%. In addition to this high reach detection probability, the absence of significant relationships between detection covariates (flow velocity and depth) indicates that in this study, crayfish detection is only affected by random and/or unmeasured variables. Consequently, imperfect crayfish detection was accounted for and determined to be negligible.

My analyses revealed that *F. virilis* occupancy is positively and significantly related to water temperature (**Table 2.2**). In contrast to expectations, neither flow velocity nor turbidity were significant drivers of *F. virilis* occupancy in the North Saskatchewan basin (**Table 2.2**). While both of these covariates have been shown in previous studies to affect the survival, growth, and/or reproduction of crayfish in the lab and in the field (Light, 2003; Longshaw & Stebbing, 2016; Maude & Williams, 1983; Rosewarne et al., 2014), the ranges in which these effects occur were not present in the study reaches. Maude & Williams demonstrated in their slip speed laboratory experiments that *F. virilis* individuals showed a mean slip speed of 0.3 ms^{-1} on Plexiglas surface

(1983). I initially hypothesized that flow velocity would be negatively correlated with crayfish occupancy; however, the mean flow velocity among study reaches was determined to be 0.18 ms^{-1}, which is nearly half that of the average slip speed for *F. virilis* found by Maude & Williams (1983). Therefore, the average flow velocities in the North Saskatchewan River basin were simply outside of the range in which they would preclude *F. virilis* from occupying and establishing a population at a given reach. This may explain the lack of a significant negative relationship of *F. virilis* occupancy and flow velocity.

In the case of turbidity, I hypothesized that turbidity would be negatively correlated with occupancy as crayfish gills are susceptible to fouling by suspended solids as well as bacteria and parasites in the water column (Holdich, 2003; Bauer, 1998). Further, gill function of the white-clawed crayfish and signal crayfish was found to be impaired at high suspended solids concentrations, specifically 133 mg l^{-1}, which is equivalent to 399 NTU (Rosewarne et al., 2014). In comparison, mean and maximum turbidity within study reaches were 18.91 and 174 NTU, respectively. Thus, mean and maximum NSR turbidity are approximately 21 and 2.3 times lower than the concentration of suspended solids in which crayfish begin to experience the adverse effects of suspended solids gill fouling, which is likely why turbidity was not found to be a major driver of crayfish occupancy in this study.

Water temperature alone was the most important driver of *F. virilis* occupancy at tributary reaches along the North Saskatchewan River basin. In all six best models, water temperature was found to be positively and significantly related to occupancy. The basin has a strong water temperature gradient from west to east, with high altitude cold-water streams originating in the Rocky Mountains and low altitude warm-water streams originating in the prairies. As poikilotherms, crayfish cannot regulate their own body temperature and rely on the external water temperature to meet their physiological and biochemical requirements. In general, crayfish prefer and require warmer water temperatures (Bowman, 2019; Longshaw & Stebbing, 2016; Momot, 1967; Rogowski et al., 2013; Wetzel & Brown, 1993). *F. virilis* have demonstrated a preference for 22°C water temperature when exposed to a temperature gradient tank ranging between 18 and 22°C (Bowman, 2019). Further, in a laboratory study conducted by Wetzel & Brown, 3rd instar *F. virilis* were exposed to water temperatures of 10, 15, 20, 25, and 30°C for 21 days during which their survival and growth were consistently monitored (1993). They found that the juvenile crayfish group subject to 10°C demonstrated the lowest survival at 53% and experienced the most stunted growth in both body length and weight compared to warmer water exposed groups (Wetzel & Brown, 1993). Low instar survival at cold water temperatures may be what is driving the strong positive relationship of occupancy with water temperature, in that juvenile crayfish may suffer high mortality in westerly cold-water streams, preventing *F. virilis* population survival and growth (**Table 2.2**). In addition to the cold-water treatments, Wetzel & Brown found that 3rd instar survival and growth were maximized at 25 degrees °C (1993). This finding is also reflected in the model averaging results that indicate that the probability of *F. virilis* reach occupancy reaches 50% at water temperature 18.7°C and approaches 100% around 21°C (**Figure 2.4**). This strong relationship of occupancy to water temperature may explain the insignificant relationship of occupancy with physical complexity.

Counter to my original hypothesis, physical complexity was also not significantly related to occupancy in all models (**Table 2.2**). This result is in contrast to previous studies that demonstrated the importance of physical complexity, specifically the positive effect of rocky substrate and in-stream boulders on crayfish growth, survival and reproduction (e.g. Olsson & Nyström, 2009; Simon & Cooper, 2014). I contend that this result is likely due to the inverse relationship between the geological and temperature gradients within the basin. As mentioned previously, the NSR is a glacier-fed basin that flows from the Rocky Mountains, through the foothills, and into the prairies of eastern Alberta. As such, the geological structure of headwater streams is predominantly composed of boulders, rocky substrate, and downed tree woody debris. Therefore, reaches located in headwater streams obtained a high physical complexity gradient in the NMDS ordination (**Figure 2.3**). However, *F. virilis* cannot occupy these highly complex reaches due to the cold-water temperatures that exceed their thermal niche and temperature tolerance. In contrast, the geological structure of reaches located in downstream prairie tributaries is dominated by overhanging banks, some aquatic macrophyte cover and a much smaller proportion of rocky substrate, resulting in lower overall physical complexity value. However, these reaches also possess optimal water temperatures for *F. virilis* survival and growth (Bugnot & López Greco, 2009; Jin et al., 2019). I therefore infer that rather than physical complexity being ecologically insignificant for *F. virilis* occupancy (which would contradict existing literature), it may be that the water temperature gradient of the basin is driving occupancy. Further, water temperature may be such a strong driver of *F. virilis* occupancy in the basin, that it effectively masks the effect of instream physical complexity.

The generalized linear model (GLMM) indicated that crayfish abundance had a similarly significant positive relationship with water temperature in both best models (**Table 2.3**). As discussed above, this is likely due to warmer water temperatures maximizing growth and reproduction, which allows *F. virilis* populations to occupy the reach and increase the number of individuals in the population. This is supported by studies which found that crayfish sperm production and embryonic development is optimized in water temperatures in the mid-twenty degrees Celsius range (Bugnot & López Greco, 2009; Jin et al., 2019) and that warmer water temperatures promote crayfish growth and decrease age of sexual maturity, thereby increasing egg production and abundance (Wetzel & Brown, 1993; Whitmore & Huryn, 1999). Also similar to the occupancy model results, flow velocity is included as a covariate in the second-best model, but is insignificantly related to crayfish in-reach abundance (**Table 2.3**). In contrast to the occupancy model results, physical complexity was significantly and negatively related to crayfish abundance (**Table 2.3**). This is likely due to the same effect of the inverse temperature and physical complexity gradients described above, in that it is *not* that physical complexity has a detrimental effect on abundance. Rather, highly complex cold-water reaches that crayfish are unable to occupy may be driving the significant negative relationship between physical complexity and abundance that is seen in the model outputs, but is actually a product of the positive relationship between water temperature and crayfish abundance. Regarding random effects of the GLMM, significant results of the chi-square log-likelihood ratio test indicate that there is/are in-reach characteristics that explain a significant amount of the variance in crayfish abundance among reaches on top of the variance that is already explained by the fixed variables. Since I only collected measurements of the covariates relevant to the research objectives (local in-stream environmental characteristics), it is not surprising that other factors (environmental or anthropogenic) could impact *F. virilis* occupancy across the basin. Examples of covariates driving this inter-reach variance in abundance could include the reach water toxin concentration and/or proximity to high human population density centers. Heavy metals and other contaminants related to mining have been shown to bioaccumulate and cause mass crayfish die-off events (Svobodová et al., 2017), while reaches in close proximity to highly populated human cities may be highly degraded and as a result, populated with fewer predatory fish species allowing populations to expand quickly and without correction.

This phenomenon of invasive species being more successful in already anthropogenically degraded reaches is well documented in the literature (e.g. Kennard et al., 2005; Meador et al., 2003).

Understanding the local instream environmental characteristics that drive crayfish occupancy is essential for managing and preventing current and future *F. virilis* invasive populations. My results indicate that *F. virilis* can tolerate a wide range of instream physical complexity and that both turbidity and flow velocity ranges within the basin are within the tolerance limits of *F. virilis*. However, *F. virilis*' water temperature requirements dictate that they may only occupy easterly warm-water streams as the cold-water streams of the headwaters exceed their thermal niche. Thus, rather than a suite of drivers, temperature appears to be the single local environmental variable driving crayfish occupancy in the basin.

Cold-water streams may therefore have some natural protection against subsequent invasion (Kurylyk et al., 2015). A management implication of this study and the answer to the second research objective is that currently unoccupied NSR streams with mean summer water temperatures ≥18°C ought to be prioritized for *F. virilis* monitoring and introduction prevention. Occupied streams with the same mean summer water temperatures should instead be prioritized for targeted *F. virilis* invasive species management such as mass culls, trapping derbies, etc. The NSR basin is projected to be affected by climate change resulting in thermal regime shifts as well as changes in streamflow, discharge volume, snowpack and more (Carr et al., 2019; Kienzle et al., 2012). As climate change progresses, westerly located cold water streams in the basin could potentially warm to the point where crayfish can occupy them. Watershed managers should consider the effects of climate change and real-time water temperature measurements when selecting tributaries of priority for *F. virilis* management.

These findings could also be extended to basins with similar temperature and geologic regimes as the NSR; For example, the model validation analysis indicated that 92% of reaches with water temperatures ≥18°C in the Lake Winnipeg River basin were occupied by *F. virilis* (**Figure 2.5**). In addition, similar occupancy susceptible water temperature thresholds (≥18°C) were found in other Alberta watersheds where crayfish were detected previously in 2011 (Williams, Proctor, et al., 2011) including the Oldman and Bow River watersheds (D. Watkinson, personal communication).

A limitation of this study is that it was conducted during a single season and used spatial rather than temporal replicate crayfish surveys. Although analysis of historic basin water temperatures demonstrated that water temperatures collected during this study were a good representation of average summer basin temperatures (**Figure A2.1**), the data collected in this study provided a “snapshot” of *F. virilis* occupancy and environmental variables during the summer of 2020. Further studies should utilize temporal replicate surveys, which would result in both intra-season and inter-year temporal information that could provide insight into how *F. virilis* distributions change over time and in response to environmental differences over time (e.g. flood events, dry years, etc.).

Conclusion

This study demonstrated that water temperature is the primary local environmental characteristic that strongly affected *F. virilis* occupancy and abundance in the NSR basin. Other local environmental characteristics (flow velocity, turbidity, and physical complexity) do not have strong effects on *F. virilis* occupancy or abundance. However, some variance in crayfish abundance was explained by differences between reaches that were not investigated in this study. Model averaging indicated streams possessing water temperature 18.7 C° or warmer have a +50% probability of being or becoming occupied by invasive *F. virilis*. This threshold could also be extended to other basins in Alberta that are similar to the NSR basin. Detection of *F. virilis* was highly accurate over the sampling season, giving us confidence in these results. This study provides practical guidelines for the management of invasive *F. virilis* populations in the NSR basin and Alberta.

Tables & Figures

Table 2.1 – Hypothesized relationships of covariates included in the global occupancy model with crayfish detection and/or occupancy and literary support for hypothesis. Symbols + and – indicate expected positive and negative relationships, respectively.

Covariates	Model aspect	Hypothesis	Literary support
flow velocity (ms^{-1})	detection	-	Maude & Williams, 1983; Light, 2003
depth (cm)	detection	-	no citation – physical survey barrier
flow velocity (ms^{-1})	occupancy	-	Maude & Williams, 1983; Light, 2003
turbidity (NTU)	occupancy	-	Rosewarne et al., 2014
water temperature (C°)	occupancy	+	Momot, 1967; Rogowski et al., 2013; Wetzel & Brown, 1993; Bowman, 2019
physical complexity (NMDS1)	occupancy	+	Longshaw & Stebbing, 2016; Simon & Cooper, 2014; Olsson & Nystrom, 2009

Table 2.2 – Global and best occupancy models ranked by QAICc weights within ΔQAICc <3 and corresponding covariate relationship estimates. Flow velocity (flow), turbidity (turb), physical complexity (comp), and water temperature (temp) were predictor variables for the occupancy parameter (λ). Water depth (dep) and flow velocity (flow) were predictor variables for the detection probability parameter (p). Significant relationships appear in bold.

	Detection estimates			Occupancy estimates				Ranking statistics		
Model	Intercept	depth (cm)	flow (ms^{-1})	flow (ms^{-1})	turbidity (NTU)	physical complexity	water temperature (C°)	QAICc	ΔQAICc	weight
λ(temp),p(.)	0.67	-	-	-	-	-	**0.376**	64.51	0	0.298
λ (temp + comp),p(.)	0.62	-	-	-	-	-2.666	**0.345**	65.87	1.35	0.213
λ (temp + turb),p(.)	0.65	-	-	-	-0.008	-	**0.418**	66.63	2.12	0.146
λ (temp),p(flow)	1.03	-	-0.011	-	-	-	**0.375**	67.07	2.55	0.117
λ (temp + flow),p(.)	0.66	-	-	0.9184	-	-	**0.377**	67.09	2.57	0.116
λ (temp),p(dep)	0.72	-0.001	-	-	-	-	**0.376**	67.20	2.68	0.110
global: λ (temp + comp + flow + turb), p(depth + flow)	1.30	-0.002	-0.021	2.990	-0.010	-3.168	**0.408**	77.06	12.55	-

Table 2.3 – GLMM models ranked by AICc weights within ΔAICc < 2 and corresponding covariates relationshsip estimates. Flow velocity (flow), turbidity (turb), physical complexity (comp), and water temperature (temp) were fixed predictor variables (F), while reach code (reach) was the random (r) variable in the model. Significant relationships determined by confidence intervals appear in bold.

	Fixed abundance estimates				Random effects		Ranking statistics		
Model	flow (ms-1)	turbidity (NTU)	physical complexity	water temperature (C°)	variance	standard deviation	AICc	ΔAICc	weight
F(temp+comp), r(site)	-	-	**-8.27**	**0.73**	6.016	2.453	141.41	0	0.598
F(temp+comp+flow), r(site)	6.21	-	**-8.79**	**0.72**	4.044	2.011	142.21	0.80	0.402

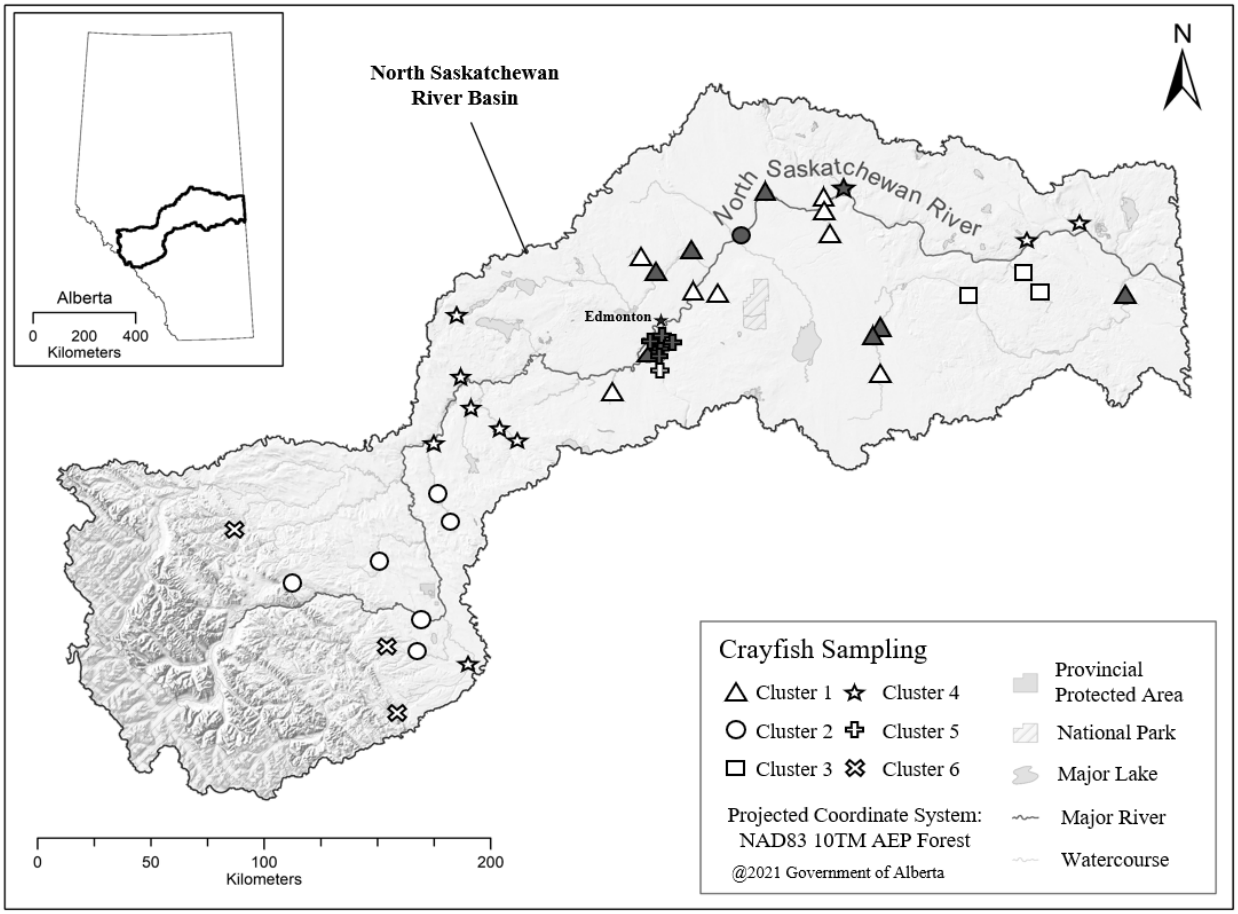


Figure 2.1- Tributary reaches where crayfish traplines were deployed throughout the North Saskatchewan River basin during June-August 2020. *F. virilis* presence and absence are represented by filled and open symbols, respectively. Type of symbol represents the reach's cluster with triangle = cluster 1, circle = cluster 2, square = cluster 3, star = cluster 4, cross = cluster 5, and tilted cross = cluster 6.

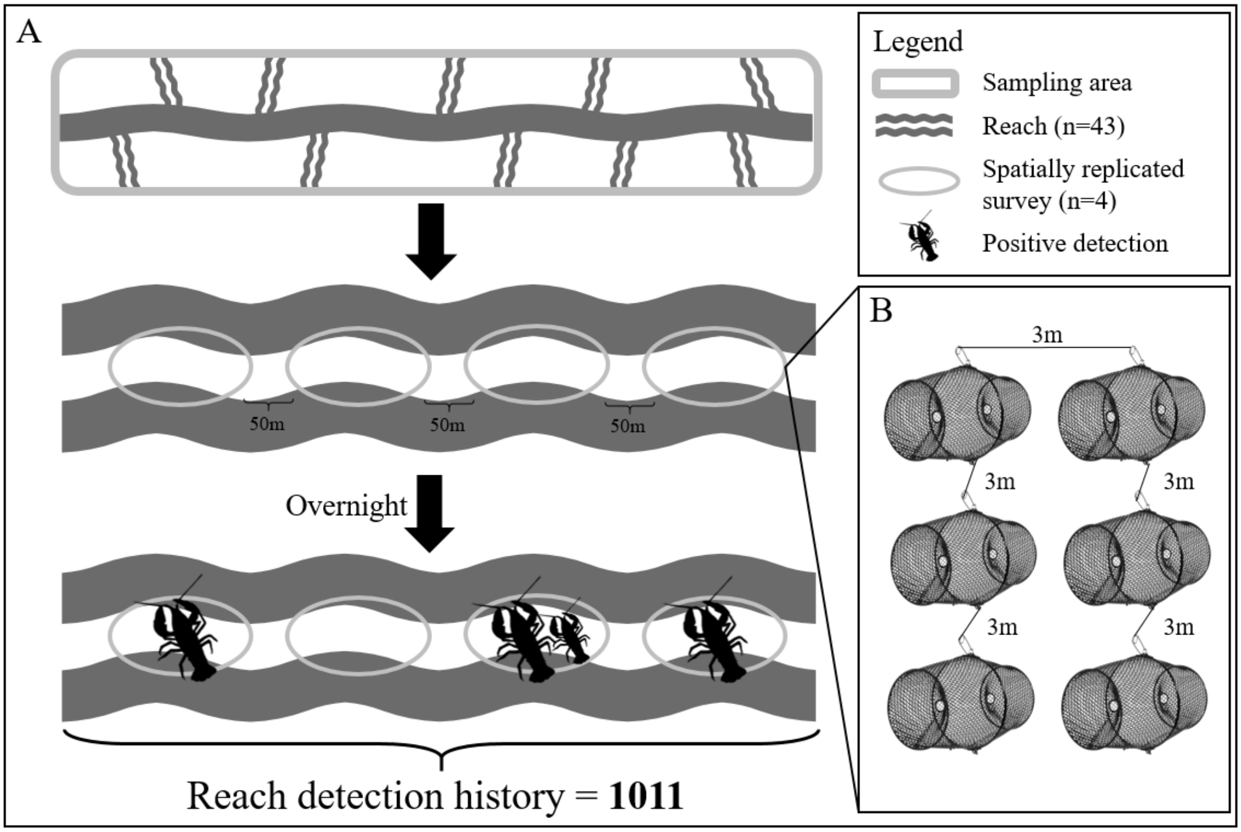


Figure 2.2 – Field sampling methods for *F. virilis* reach occupancy modeling. (**A**) 200-300m tributary reaches are selected as sampling reaches within the study area (North Saskatchewan River basin). Four spatially replicated crayfish surveys (baited traplines – depicted in panel **B**) are conducted within each reach. Traplines are set 50m apart along the river's edge, secured with a rebar stake, and left overnight. The following day, *F. virilis* presence/absence in each trapline is recorded as the reach detection history.

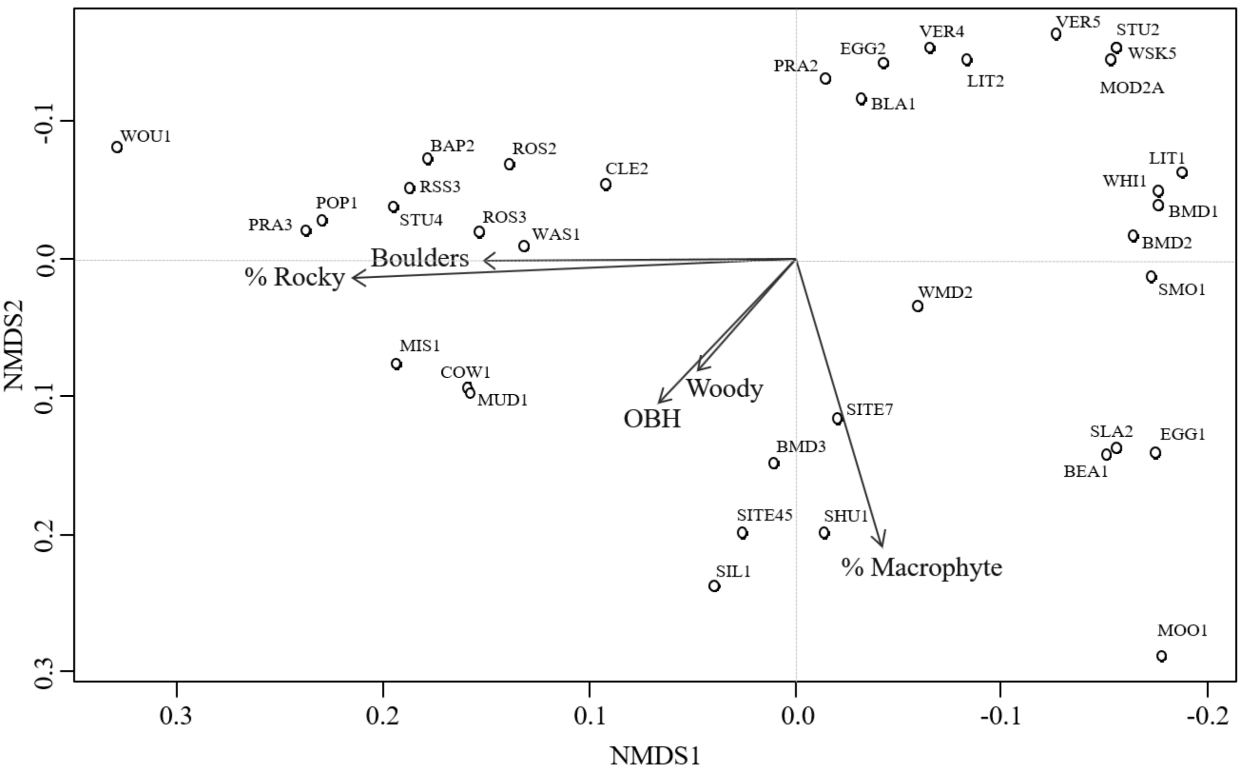


Figure 2.3 – Meta NMDS ordination of sample reaches based on five physical complexity variables. NMDS axis 1 represents the instream physical complexity gradient from reaches with high complexity (positive values) to those with low complexity (negative values). Reach identity and location within the ordination space are represented by unique reach codes and open circles, respectively. Vectors are defined as % Rocky = % rocky cover, Boulders = p/a in-stream boulders, OHB = p/a overhanging banks, Woody = p/a woody debris, % Macrophyte = % macrophyte cover. Dashed lines pass through the origin (0,0).

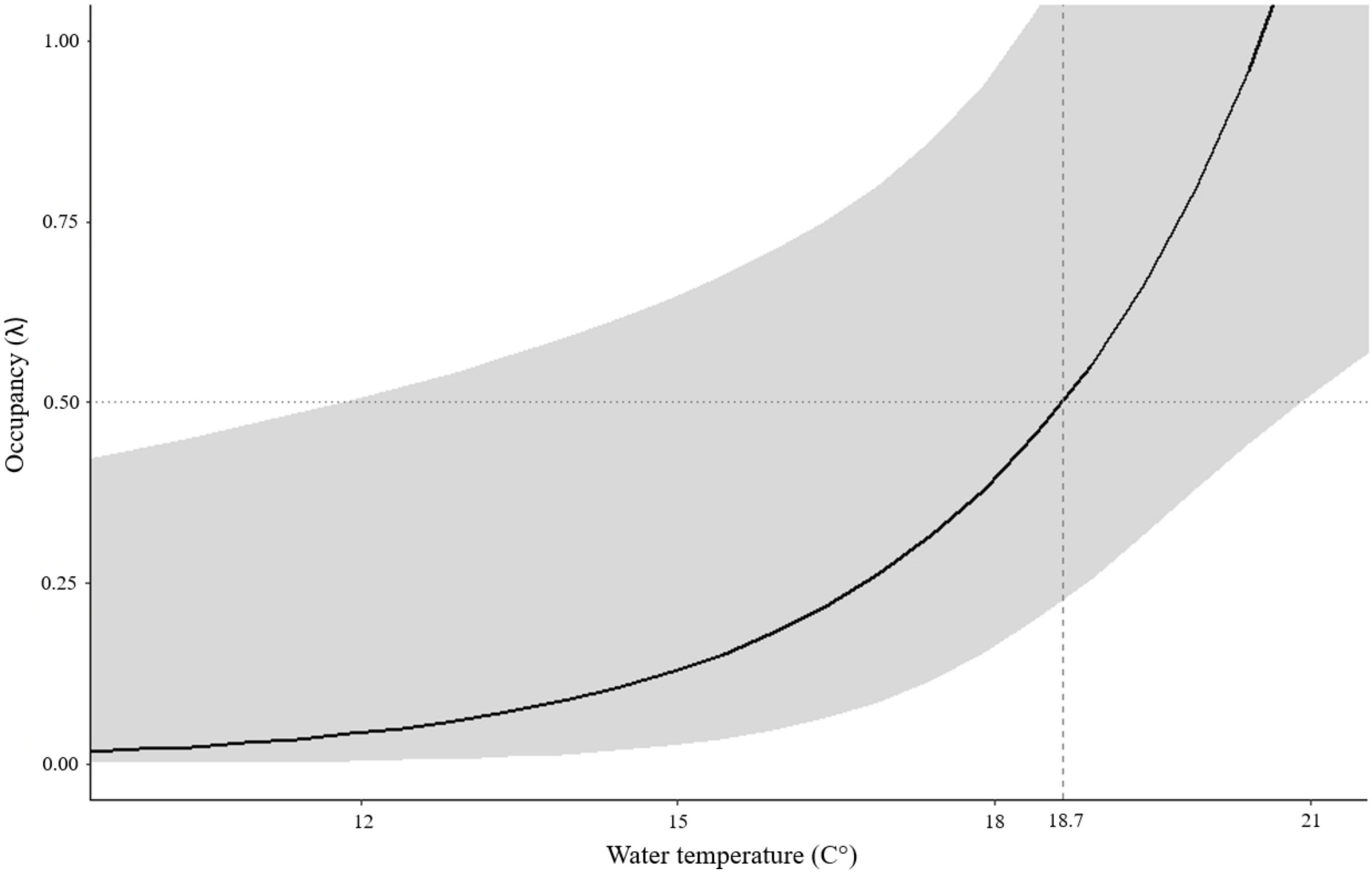


Figure 2.4 – Model averaged prediction of occupancy as a function of water temperature (C°) with 95% confidence intervals about occupancy (gray bands) and reference lines indicating 50% probability of occupancy estimate (dotted) and 18.7°C (dashed).

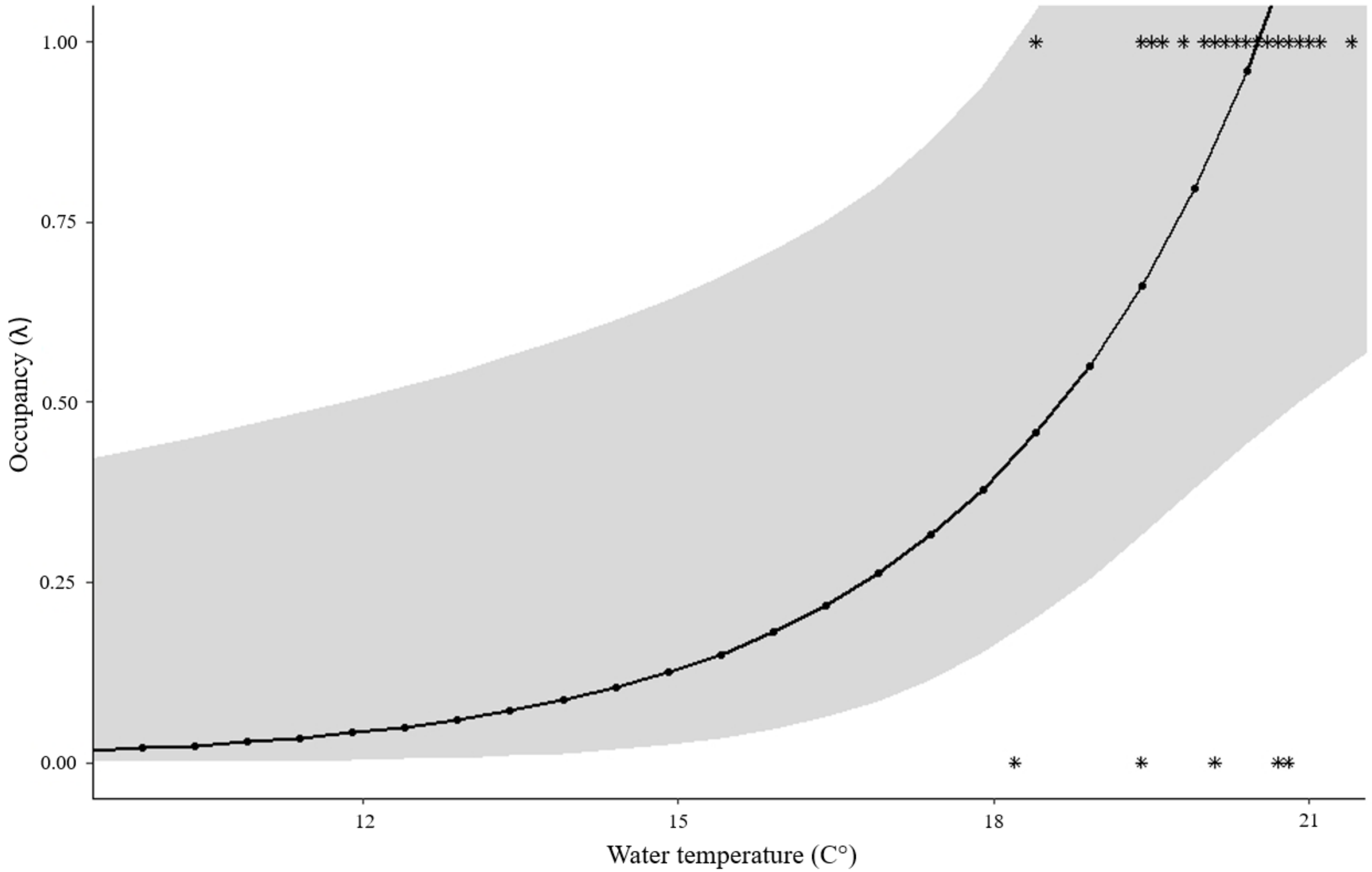


Figure 2.5 – Presence (1.00) and absence (0.00) of *F. virilis* as a function of temperature (C°) in the Lake Winnipeg River Basin overlaid on model averaged prediction of occupancy as a function of water temperature for model validation. Lake Winnipeg River basin presence and absence data are represented by * (n=88).

Chapter 3: Stable isotope analysis indicates differential trophic niche occupation between invasive Northern crayfish (*Faxonius virilis*) and native fishes in the North Saskatchewan River Basin

Introduction

Nutritional resource use is an important aspect of a species' ecological niche, which was described by Hutchinson (1957) as the multidimensional space within which a species can maintain a viable population based on environmental conditions and resource availability. The trophic niche is the space within the ecological niche that is explicitly based on the nutritional resources that are available and consumed by a particular species (Bearhop et al., 2004). In the absence of interspecific interactions such as predation and competition, the trophic niche is called the fundamental trophic niche and represents the potential extent of nutritional resource use by the species. Conversely, the realized trophic niche represents the actual nutritional resource use of species in the presence of interspecific interactions (Hutchinson, 1957). When the trophic niches of two species overlap significantly, competition for nutritional resources can result in either resource partitioning that causes a shift in trophic niche space and allows the species to co-exist or extirpation of the less competitive species (Tilman, 2020).

Invasive species are those that are established in an area outside of their native range and whose presence results in negative ecological or economic impacts, including those on native species (Mack et al., 2000). One way invasive species can negatively affect native species is by exploiting the same limited nutritional resources that native species rely upon (David et al., 2017). When the nutritional resource being shared is a limited one, the indirect effect of one species exploiting that resource to the detriment of the other is a form of indirect competition referred to as exploitative competition (Holomuzki et al., 2010; Tilman, 2020).

Exploitative competition between native and invasive species can be assessed by evaluating their trophic niches, which can be estimated by determining and analyzing the stable isotope concentrations of carbon (^{13}C) and nitrogen (^{15}N) in animal tissues as these concentrations have a predictable relationship with diet (Bearhop et al., 2004; Fry, 2006). Many studies have used stable isotopes as a tool to estimate the dietary habits of species and to estimate the position and size of core fundamental and realized trophic niches as well as the degree of overlap between species' trophic niches (e.g. Baltensperger et al., 2015; Jackson & Britton, 2014; Olsson et al., 2009; Ponce et al., 2021). Stable isotopes have been useful in determining species' trophic habits and interactions under natural conditions and those exposed to stressors such as climatic change and invasion events (Baltensperger et al., 2015; Botta et al., 2018; Zambrano et al., 2010).

The negative impacts of invasive crayfish have been documented worldwide (Longshaw & Stebbing, 2016; Reynolds, 2011). A particularly robust invader species of crayfish is the Northern Crayfish (*Faxonius virilis* [Hagen, 1870]), which is capable of decimating macrophytes, changing invert assemblages, causing trophic cascades, and competing with fish outside of its native range (Fitzsimons et al., 2002; Hanson et al., 1990; Nyström et al., 1999; Rodríguez et al., 2005; Savino & Miller, 1991). This invader was introduced to Alberta, Canada's North Saskatchewan River (NSR) basin in the early 1990s and has since established populations in the central and eastern portions of the basin (Williams, 2012). Currently, the province of Alberta has declared *F. virilis* a species of invasive concern and regulations to control the species' spread are in place (AEP, 2018). Throughout Alberta, *F. virilis* can be harvested legally without a license in any amount and all captured individuals must be killed prior to transport. Additionally, it is illegal for anglers to use crayfish as bait in the Province of Alberta as this is a significant vector of the species' movement (AEP, 2018; Williams, 2012). Despite the concern regarding *F. virilis*, their potential detrimental effects on the native fishes of the NSR basin have yet to be assessed.

Here stable isotope analyses were used to determine whether: (1) *F. virilis* is sharing and/or potentially competing exploitatively for the same nutritional resources consumed by native fishes in tributaries of the NSR, and (2) generating detrimental effects on the isotopic characteristics (proxies for resource use) and/or body condition of native species due to sympatry with *F. virilis*. To do this, I collected samples of *F. virilis* and six native fish species from ten tributary reaches within the NSR basin, five occupied by *F. virilis* and five not. I then used three metrics of stable isotope analysis to investigate the potential negative effects of *F. virilis* sympatry on native fishes. Standard ellipse area (SEA) is the isotopic niche width of a fish species and represents the core trophic niche space occupied by that species, while % ellipse overlap of SEA for two species indicates to what degree they are consuming the same nutritional resources (Baltensperger et al., 2015; Jackson et al., 2011). Carbon range reflects the richness of nutritional resources consumed by a species with narrower carbon ranges indicating lower richness in nutritional resources (Fry, 2006; Layman et al., 2007). I use these three isotopic metrics (SEA, % ellipse overlap, and carbon range) as well as fish body condition (Relative Weight) between *F. virilis* sympatric and allopatric native fish populations to address the two research objectives (Jackson et al., 2011; Layman et al., 2007; Ogle, 2018; Wege & Anderson, 1978).

I hypothesized that omnivorous *F. virilis* consume significantly similar nutritional resources as native secondary consumer fish and that this would be demonstrated by significant (≥60%) overlap of the core fundamental and core realized SEA of *F. virilis* with native secondary consumer fish species. Crayfish have been shown to have wide isotopic trophic niches due to their omnivory which can increase pressure on grazing species that consume the same resources (Linzmaier et al., 2020). Pressure on native species can take the form of reduced access to resources, leading to lowered body condition (e.g. Light, 2005). Therefore, I also hypothesized that the realized trophic niche space (estimated by SEA), carbon ranges, and body condition of native secondary consumer fish species to be reduced when sympatric with *F. virilis* as a result of pressure from sharing and/or exploitative competition for nutritional resources.

Methods

Study area, reach selection & crayfish sampling

I used *F. virilis* specimens obtained during collection of the dataset used previously to examine occupancy of *F. virilis* in the NSR basin. Methods pertaining to the study area, reach selection, and crayfish sampling can be found in chapter two (pg. 6). *F. virilis* relative abundance was estimated using catch per unit effort (CPUE) and calculated for each reach as the mean number of individuals captured in a single 24-hour overnight baited trapline survey and was reported in units of individuals per trapline (Zale et al., 2012)(**Table A3.1**).

Fish & benthic macroinvertebrate sampling

Fish were extensively sampled at ten reaches where *F. virilis* were also sampled during June-August 2020 (**Figure 3.1**). Three reaches (WMD2, BMD2, and BMD3) are located in the highly developed and populated urban core of Edmonton, Alberta, while the other seven reaches (COW1, BAP2, ROS2, POP1, BEA1, SMO1, and VER4) are located in less developed and lower populated rural and/or natural areas. Each 300m reach was subdivided into six 50m transects within which fish were sampled via backpack electrofishing in a sweeping systematic pattern. All fish captured were identified to species, measured, enumerated, and released back into the river from which they were caught, with the exception of individuals of target fish species. Target native fish species included invertivore/herbivore secondary consumers; Longnose Dace (*Rhinichthys cataractae* [Valenciennes, 1842]), Lake Chub (*Couesius plumbeus* [Agassiz, 1850]) and Trout Perch (*Percopsis omiscomaycus* [Walbaum, 1792]); native detritivorous consumers Longnose Sucker (*Catostomus Catostomus* [Forster, 1773]) and White Sucker (*Catostomus commersonii* [Lacepede, 1803)]); and one native piscivorous species: Burbot (*Lota lota* [Linnaeus, 1758]). Individuals of target fish species were humanely euthanized via single-blow blunt force trauma followed by pithing to ensure death (Research License 20-3812 RL). Mechanical euthanasia was conducted instead of a chemical euthanasia overdose of MS-222 because MS-222 is a carcinogenic agent capable of harming the natural environment in which sampling was conducted and because tissues exposed to chemical agents of euthanasia have been shown to alter $\delta^{13}C$ and $\delta^{15}N$ stable isotope values (Nahon et al., 2017). As stable isotope analysis is the primary method of investigation for this study, mechanical euthanasia was used to prevent isotope alteration. Whole specimens were promptly transported to the University of Alberta and frozen at -20 °C prior to sample processing and analysis. All fish sampling and euthanasia was conducted in accordance with the University of Alberta, CCAC animal handling and ethics regulations (AUP No.: AUP00003578), and under a valid Research License issued by the Government of Alberta (RL# 20-3812).

Benthic macroinvertebrates were collected for isotopic baseline calculation. At all ten reaches, benthic macroinvertebrate communities were sampled using a single zig-zag pattern kick net. Each kick net was conducted in a sweeping fashion over erosional zones (riffles and runs) and standardized to three-minute sampling effort. All collected material was placed into enamel pans where invertebrates were visually separated from stream bed substrate using forceps and wash water bottles on site. All samples were preserved in 70% ethanol filled 500ml Nalgene bottles (Hobson et al., 1997) and transported back to the University of Alberta. Benthic invertebrate samples were identified and enumerated to the family or genus when further identification was possible and necessary to resolve functional trait discrepancies. All identification of aquatic insect taxa and their functional feeding traits were determined following the work of Merritt et al. (2019). Microscopy was performed using an Olympus SZ61 Dissecting Microscopes and a Zeiss Primo Star 1 Optical Microscope. Sorted samples were separated by family and preserved in 70% ethanol prior to stable isotope analysis (Hobson et al., 1997).

Laboratory specimen & sample processing

Dissections were conducted for each fish specimen during which the weight (g), fork length (mm), total length (mm), sex (m/f), gonad weight (g), and liver weight (g) were measured. Subsequently, samples of caudal fin, pectoral fin, gonad, liver, and dorsal muscle tissues (two replicates per tissue type) were collected from each specimen. Ventral and fillet tissue samples were also collected from all specimens of sufficient size. Finally, the stomach contents of *L. lota* specimens were inspected for evidence of *F. virilis* consumption. Complete dissections were also conducted on a total of 69 crayfish specimens. The weight (g), carapace length (mm), total length (mm), and sex (m/f) of each individual was measured and recorded. Two replicated samples of gill, hepatopancreas, claw, and abdominal (tail) muscle tissue were collected. All tissue samples were preserved at -20 °C prior to stable isotope analysis.

Fish dorsal muscle tissue samples and crayfish abdominal muscle tissue samples were freeze dried at -55°C and 0.015 Barr for 24 hours in a LABCONO® FreeZone 1 Liter Benchtop Freeze Dry System (Labconco, 2021) to constant weight. Benthic invertebrate samples (separated by taxonomic family) were dried whole to constant weight at 60°C for 24 hours in a Precision® Compact Gravity Convection Oven (Thermo Scientific, 2009). Once dried, each sample was ground into a fine, homogenous powder and weighed into a six by eight mm tin capsule to 0.4000-0.4999 mg using the UMX2 Ultra-microbalance (Mettler Toledo, 2004). Tins were then sealed for carbon and nitrogen stable isotopic analysis. Samples were analyzed by continuous flow isotope ratio mass spectrometry using the Thermo Delta V Advantage Isotope Ratio Mass Spectrometer (IRMS). Within the instrument, each sample was flash combusted and converted to CO^2 and N^2 gases. These gases were separated using chromatography and the intensities of heavier isotopes (^{13}C & ^{15}N) were quantified and compared against international reference scales of vPDB and Air, respectively. Final isotopic values were reported in standard delta ($\delta^{13}C$ vPDB and $\delta^{15}N$ VAir) notation for analysis.

Data cleaning & inspection

Prior to analysis, raw isotope data were inspected for carbonate contamination using multiple linear regression analysis to compare $\delta^{13}C$ and percent carbon of samples of each reach (Hayden, 2021; Jardine et al., 2003). Slope values indicating a positive trend of $\geq 0.5\ \delta^{13}C$‰ (five times that of the instrument's precision for $\delta^{13}C$ content detection [Kracht, 2011]) would indicate carbonate contamination. No carbonate contamination was detected. Next, the presence of carbon depletion caused by lipid richness was investigated by calculating the ratio of $\delta^{13}C:\delta^{15}N$ of all samples by dividing the total carbon (TC) of each sample by the total nitrogen (TN) of each sample ($\delta^{13}C:\delta^{15}N = TC/TN$). Samples with $\delta^{13}C:\delta^{15}N$ ratios > 4 were considered lipid rich and in need of correction (Hayden, 2021). No lipid richness was detected and no corrections were conducted. Finally, *C. commersonii* and *C. catostomus* samples where combined into a single group called *Catostomus spp.* because the sample size of *C. catostomus* (*n*=6) was insufficient for subsequent SIA analysis. This combination was based on the two species sharing a very close taxonomic lineage (same genus) and similar life history, morphology, and diet (Scott & Crossman, 1973).

Baseline selection and calculation

To account for spatial isotopic variability among reaches and to compare stable isotope metrics between reaches, primary consumer benthic macroinvertebrate samples from each reach were used to calculate isotopic baselines. Primary consumer benthic invertebrates were used for baseline source estimates rather than long-lived primary consumers such as clams and snails because these long-lived organisms were unavailable. However, benthic invertebrates have been demonstrated as good indicators of baseline $\delta^{13}C$ and $\delta^{15}N$ values in previous studies (Anderson & Cabana, 2007; Busst & Britton, 2017; Jackson & Britton, 2014; Svanbäck, 2015). Baseline $\delta^{15}N$ values were calculated following the suggestions of Anderson & Cabana (2007) to convert raw $\delta^{15}N$ and $\delta^{13}C$ values into trophic position (TP) and corrected $\delta^{13}C$ ($\delta^{13}C_{Corr}$), respectively. Pairwise t-tests were conducted on macroinvertebrate functional feeding group's mean $\delta^{15}N$ values to determine which functional feeding group was the most nitrogen depleted over all ten reaches. There were no significant differences between the mean $\delta^{15}N$ values of functional feeding groups but collectors were the most depleted with the lowest mean $\delta^{15}N$. Similar pairwise t-tests were then conducted to compare collector taxonomic families' mean $\delta^{15}N$ values. The family Elmidae was the most nitrogen depleted and present in 60% of sampled reaches. Therefore, Elmidae mean $\delta^{15}N$ value was used to calculate TP and $\delta^{13}C_{Corr}$ in reaches where it occurred. In reaches where Elmidae did not occur, the collector taxonomic family with the lowest $\delta^{15}N$ value present in that reach was corrected to that of Elmidae. Correction was made by calculating the difference in mean $\delta^{15}N$ values between Elmidae and the reach's most nitrogen depleted family and subtracting this difference from the replacement family's mean $\delta^{15}N$ value (Anderson & Cabana, 2007). For example, in reach WMD2, Elmidae were not present and the most nitrogen depleted family found at that reach was Simuliidae. The difference in mean $\delta^{15}N$ between Elmidae and Simuliidae was 4.5‰. Therefore, 4.5‰ was subtracted from the mean $\delta^{15}N$ value of Simuliidae and then used as the baseline value for reach WMD2.

Trophic position and corrected $\delta^{13}C$ calculation

Trophic position was calculated for each consumer's muscle tissue sample using the previously calculated reach-specific baseline $\delta^{15}N$ values, and then these baseline values were substituted into the single source trophic position model described by Post (2002):

$$TP_{con} = \lambda + (\delta^{15}N_{con} - \delta^{15}N_{base})/\Delta_n$$

Where TP_{con} is the trophic position of the consumer, λ is the trophic position of baseline organisms ($\lambda = 2$ for herbivorous benthic invertebrates), $\delta^{15}N_{con}$ is the raw isotopic nitrogen value of the consumer, $\delta^{15}N_{base}$ is the calculated baseline $\delta^{15}N$ value, and Δ_n is the trophic enrichment factor equal to 3.4‰ (SD = 1‰) which is accepted as the widely applicable fractionation value determined by Post and has been accepted and used widely as the fixed nitrogen trophic enrichment factor (Dionne et al., 2016; McCutchan et al., 2003; Post, 2002; Zanden & Rasmussen, 2001).

I corrected $\delta^{13}C$ consumer values in each reach based on the same benthic invertebrate baseline sources as was used for calculation of trophic position using the equation described by Olsson et al. (2009).

$$\delta^{13}C_{corr} = (\delta^{13}C_{con} - \delta^{13}C\mu_{baseline})/CR_{baseline}$$

Where $\delta^{13}C_{corr}$ is the basal isotopic corrected $\delta^{13}C$ value of the consumer, $\delta^{13}C_{con}$ is the raw $\delta^{13}C$ value of the consumer, $\delta^{13}C\mu_{baseline}$ is the reach specific mean $\delta^{13}C$ value of the reach's baseline source, and $CR_{baseline}$ is the range of the reach's baseline source $\delta^{13}C$ values. Trophic position and corrected $\delta^{13}C$ values were used in all subsequent statistical analyses. Summary statistics of trophic position and δ^{13}Ccorr are available by species (**Table 3.1)** and by reach (**Table A3.1**).

Fundamental and realized niche interactions

To determine the size and position of each species' trophic niche, maximum likelihood fitted small sample size corrected standard ellipse area (SEA_c) was calculated and used as a measure of each species core trophic niche width using the R *SIBER* package (Jackson & Parnell, 2020). SEA_c represents approximately 40% of the spread of the data and is ideal for calculating the core trophic niche of a species when working with small sample sizes (n < 30) (Jackson et al., 2011). To determine the fundamental core trophic niche of each species over the basin, samples were pooled by species across all reaches and the niche width of each species was calculated and plotted. By pooling individuals of the same species over all study reaches, differential resource availability and interspecific interactions such as competition and predation of the multiple reaches are not accounted for (Baltensperger et al., 2015). Therefore, the standard ellipse area of conspecific individuals that are pooled over all study reaches is representative of that species' fundamental niche width. (**Figure 3.2**).

In contrast, when the species' standard ellipse areas of individual reaches are plotted separately they represent the core realized niche of the species present in that reach. This is because all individuals in a reach are subjected to the same kinds and magnitude of interspecific interactions and resource availability. Therefore, I plotted the standard ellipse areas within each reach to inspect the core realized niche widths of each species (Baltensperger et al., 2015)(**Figure 3.3**). Niche widths were reported in units of $‰^2$ area (**Table 3.2**).

To detect if nutritional resources were being shared by native fish and *F. virilis*, the fundamental and realized niche widths were inspected for presence and degree of overlap between *F. virilis* and native fishes. The degree of overlap was calculated as a proportion using the R *SIBER* package (Jackson & Parnell, 2020). Proportional overlap values were then calculated as a proportion of the non-overlapping area of the two ellipses using the following equation:

$$P_{\text{overlap}} = [V_{\text{overlap}} / (V_{\text{ellipse2}} + V_{\text{ellipse1}} - V_{\text{overlap}})]/100$$

Where P_{overlap} is the unitless proportion overlap of the two trophic niches' being compared; V_{overlap} is the $‰^2$ area value of overlap of the two species' trophic niches being compared; and V_{ellipse2} and V_{ellipse1} are the calculated trophic niche area of species 1 and species 2, respectively. The final proportional overlap was reported as a percentage between 0% and 100% with overlap of 0% indicating the ellipses are completely unique and overlap of 100% indicating perfect overlap. Any overlap of two isotopic niches that was >60% was taken as the potential to (fundamental niche overlap) or indication of actual (realized niche overlap) significantly similar nutritional resource consumption. The significant overlap threshold of 60% was first given by Schoener (1968) when comparing the diets of island lizard species and has since been applied as the threshold for significant isotopic niche overlap by multiple studies (e.g. Guzzo et al., 2013; Rosinski et al., 2020; Vaudo & Heithaus, 2011).

Impacts of *F. virilis* sympatry on native secondary consumer fish species

The following analyses were conducted on three native secondary consumer fish species (NSCFS) (*R. cataractae, C. plumbeus,* and *Catostomus spp.*) only. *P. omiscomaycus* and *L. lota* were excluded from the following analyses due to insufficient total sample size ($n_{P.\ omiscomaycus} = 3$, $n_{L.\ lota} = 11$).

To detect potential trophic impacts of *F. virilis* sympatry on NSCFS, I calculated the Bayesian estimate of realized standard ellipse area (SEA_B) using the R *SIBER* Package (Jackson & Parnell, 2020). A null prior distribution was used to estimate the SEA_B niche widths with 95% probability intervals for each NSCFS population over 20,000 iterative runs of the Bayesian bivariate distribution model. I then compared the SEA_B niche widths of populations that were sympatric with *F. virilis* and those that were not. SEA_B niche widths were considered significantly different from each other when the 95% probability intervals around the means being compared did not overlap (Jackson et al., 2011; Pettitt-Wade et al., 2015) (**Figure 3.4 & Table 3.3**).

In addition to differences in SEA_B niche widths, the $\delta^{13}C_{Corr}$ carbon range of NSCFS were calculated and compared between *F. virilis* sympatric populations and allopatric populations to determine if the richness of consumed nutritional resources was reduced when sympatric with *F. virilis*. Carbon range was calculated as the difference between the greatest individual $\delta^{13}C_{Corr}$ value and smallest $\delta^{13}C_{Corr}$ value and was expressed in units of Δ‰ (**Figure 3.5**).

In order to determine if *F. virilis* sympatry and/or trophic niche overlap may have caused detrimental effects on the body condition of NSCFS, the relative weight (Wr) fish condition metric was calculated as described by Wege & Anderson (1978). Wr was calculated using the R *FSA* Package (Ogle, 2019), using equations derived by Bister et al. (2000) and Giannetto et al. (2011 & 2012). The standard weight intercept and slope values for riffle daces (genus: *Rhinichthys*) and brook chub were used to calculate the relative weights of *R. cataractae* and *C. plumbeus*, respectively. The relative weight intercept and slope values of brook chub were used because the relative weight equation for *C. plumbeus* does not yet exist and brook chub and lake chub share similar life history, taxonomy, and morphology (Giannetto et al., 2012). The relative weight intercept and slope values of *C. commersonii* were used to calculate relative weights of *Catostomus spp.*. Relative weight is expressed as a percentage of the previously determined standard weight for individual of that species and fork length (Ogle, 2018). For example, a relative weight of 100 indicates that the individual is the exact expected weight for a typical individual of its size and species from a reference population. Relative weights of <100 or >100 indicate that the individual is under or overweight for an individual of its size and species, respectively (Ogle, 2018; Wege & Anderson, 1978). Mean relative weight and standard deviation of each NSCFS in *F. virilis* occupied and unoccupied reaches were calculated and the two-sided t-test was used to determine if there were significant differences between the mean relative weight of each species dependent on the natural treatment of *F. virilis* occupancy (i.e *F. virilis* occupied reaches vs. unoccupied reaches) (R Core Team, 2021). The two-sided t-test has been shown to be sufficiently accurate when used to determine significant difference in means with sample sizes $n \geq 5$ (de Winter, 2013). Relative weight distributions of each species for each natural treatment were plotted using violin plots (**Figure 3.6**).

Results

A total of 69 crayfish were captured from five of ten reaches (**Figure 3.1**). Among the five reaches where *F. virilis* were detected, maximum and minimum relative abundances were 9.50 indv/trapline at reach WMD2 and 3.25 indv/trapline at reach BEA1, respectively (**Table A3.1**). A total of 35, 38, 11, 3, and 67 individuals of *Catostomus spp.*, *C. plumbeus*, *L. lota*, *P. omiscomaycus*, and *R. cataractae* were sampled across the ten sample reaches, respectively (**Table 3.1**). Dissection and stomach content analysis of *L. lota* revealed that two out of four individuals found in sympatry with *F. virilis* had evidence of *F. virilis* consumption, with one individual's stomach containing a single *F. virilis* juvenile and the other containing six (**Figure A3.1**).

Across all reaches, mean fish stable isotope values ranged from -31.58 to -27.78‰ for $\delta^{13}C$ and 8.41 to 11.39‰ for $\delta^{15}N$ while *F. virilis* had means of -28.01‰ for $\delta^{13}C$ and 10.40‰ for $\delta^{15}N$ (**Table 3.1**). After baseline correction, mean $\delta^{13}C_{corr}$ values ranged from -0.21 to 0.14‰ in fish and was <0.01‰ in *F. virilis*. Trophic position ranged from 3.84 to 4.44 in fish with *P. omiscomaycus* having the lowest mean trophic position and *C. plumbeus* having the highest mean trophic position of fish species (**Table 3.1**). *F. virilis* had the lowest mean trophic position overall, lower than the trophic positions of all fish species sampled (mean TP = 3.60) (**Table 3.1**).

Fundamental niche interactions

L. lota possessed the smallest core fundamental niche (SEA_c) width of all fish species with an area of 0.132‰^{2} (**Table 3.2**). The largest core fundamental niche width of all fish species belonged to *R. cataractae* with an area of 0.665‰^{2} (**Table 3.2**). The core fundamental niche width of *F. virilis* was found to be 0.417‰^{2}, falling between *R. cataractae* and *Catostomus spp*. with the second largest core fundamental niche width over all species (**Table 3.2**). *F. virilis*' core fundamental niche occupied the lowest trophic position but had among the largest $\delta^{13}C_{corr}$ components of all species which was reflective of the species omnivory (**Figure 3.2**). The core fundamental niche of *F. virilis* was found to be overlapping with three out of five native fish species: *P. omiscomaycus*, *Catostomus spp.*, and *R. cataractae* (**Figure 3.2**). Two of these were moderate overlaps with the core fundamental niche of *P. omiscomaycus* overlapping 13.8% with that of *F. virilis* and the core fundamental niche of *Catostomus spp.* overlapping 23.3% with that of *F. virilis* (**Figure 3.2 & Table 3.2**). The core fundamental niche of *R. cataractae* overlapped more substantially with that of *F. virilis* with an overlap value of 40.2% (**Figure 3.2 & Table 3.2**). In contrast with my predictions, all core fundamental niche overlapping events were <60%.

Realized niche interactions

Core realized niches (SEA_C) were plotted for each species in the five reaches where crayfish were present. Unexpectedly, core realized niches were mostly segregated in isotopic space (**Figure 3.3 & Table 3.2**). Out of a total of seven potential overlap events with native fish species, the core realized niches of *F. virilis* overlapped with those of native fish only twice (**Figure 3.3C & E**). These overlaps occurred in reach BMD2 with a minor overlap of <0.1% with *Catostomus spp.* and in reach WMD2 with a minor overlap of 11.3% with *L. lota* (**Table 3.2**). Additionally, core realized niche overlap appears to be independent of *F. virilis* abundance as overlap with native fishes occurred in reaches BMD3 and WMD2 which had the first and third highest relative *F. virilis* abundances, but did not occur in VER4 which had the second highest relative abundance (**Table A3.1**).

Impacts of *F. virilis* sympatry on native secondary consumer fish species

Against expectations, the mean Bayesian estimated core realized niche width area (SEA_B) of *R. cataractae* and *Catostomus spp.* in *F. virilis* sympatric reaches were found to be statistically similar (95% probability intervals overlapping) to those of their conspecifics in *F. virilis* allopatric reaches (**Figure 3.4A & C; Table 3.3**). Additionally, the core realized niche of *C. plumbeus* that were in sympatry with *F. virilis* (reach WMD2) had a significantly larger core realized niche width than conspecifics found in *F. virilis* absent reaches BAP2, ROS2, and SMO1 (**Figure 3.4B & Table**

3.3). This implied that *F. virilis* sympatry had a positive effect on the core realized niche of *C. plumbeus*; a result that is in opposition with the original hypothesis.

Where I expected the carbon ranges of NSCFS sympatric populations to be narrowed compared to allopatric populations, the results were unexpected for one species and expected for two others. The carbon ranges of *Catostomus spp.* were similar in both *F. virilis* occupied and absent reaches. In contrast, carbon ranges of both *R. cataractae* and *C. plumbeus* were between -0.3 and -0.51Δ‰ and -0.37Δ‰ smaller when sympatric with *F. virilis*, respectively (**Figure 3.5**). It is also important to note that the fish populations collected from the reaches located in the highly urbanized downtown core of Edmonton, Alberta consistently had the narrowest carbon ranges within each species comparison (**Figure 3.5**).

Finally, the mean relative weighs of *C. plumbeus*, *R. cataractae*, and *Catostomus spp.* were 79.56% (SD= 5.11%), 79.23% (SD= 9.66%), and 80.59% (SD= 7.35%), respectively. Surprisingly, there was also no significant difference in mean relative weight between NSCFS sympatric and allopatric of *C. plumbeus* (t-test p-value = 0.1772), *R. cataractae* (t-test p-value = 0.8038), or *Catostomus spp.* (t-test p-value = 0.7582) (**Figure 3.6**).

Discussion

Fundamental and realized niche interactions

The first research objective was to evaluate species' core fundamental and core realized niche widths over ten reaches in the North Saskatchewan River basin to determine if there is evidence of nutritional resource sharing and/or potential exploitative competition between *F. virilis* and native fishes. Moderate core fundamental niche overlaps between *F. virilis* and three native fishes indicated that *F. virilis* have the potential to consume the same nutritional resources as native fish species (**Figure 3.2; Table 3.2**). I expected *F. virilis* to consume the same nutritional resources as native secondary consumer fish species and this was true for three of the four species evaluated. In particular, niche overlap at the basin scale seen between *F. virilis* and *Catostomus spp.*, *P. omiscomayus*, and *R.cataractea* is consistent with diet studies which reveal that these three fish species, like crayfish, are known benthic feeders who readily consume benthic macroinvertebrates, macrophytes, and/or benthic detritus (Brazo et al., 1978; Scott & Crossman, 1973). In contrast, the lack of core fundamental niche overlap between *F. virilis* and *C. plumbeus* was unexpected, but may be explained by the latter's foraging in the water column while *F. virilis* are benthic feeders (Longshaw & Stebbing, 2016; Scott & Crossman, 1973).

In contrast with core fundamental niche analyses and the original hypothesis, within individual reaches the core realized niche of *F. virilis* was largely segregated from those of native fishes (**Figure 3.3; Table 3.2**). Lack of significant overlap of core realized niches indicated that while crayfish do consume the same resources as native fish at the basin scale, *F. virilis* and native fishes are not sharing a significant amount of nutritional resources when in sympatry. The lack of consumption of the same nutritional resources as shown in the realized niche analysis therefore suggests that *F. virilis* is not competing exploitatively for nutritional resources with this study's native fish species. A possible explanation for this unexpected lack of realized trophic overlap, may be that *F. virilis* utilize plasticity in food resource selection to avoid exploitative competition for nutritional resources with sympatric native fishes *R. cataractea* and *Catostomus spp.*.

Omnivory and the ability of crayfish to exercise dietary plasticity is well documented in the literature (Dorn & Wojdak, 2004; Longshaw & Stebbing, 2016; Momot, 1995; Veselý et al., 2020). While *F. virilis* show a preference for consuming animal tissues and eggs, they are able to successfully subsist on less preferred nutritional resources by readily consuming macrophytes, benthic macroinvertebrates, and detritus (Chambers et al., 1991; Dorn & Wojdak, 2004; Hanson et al., 1990; Love & Savino, 1993; Momot, 1995). The ability of *Faxonius* genus crayfish to be highly plastic in their diet has been seen in response to environmental stimuli such as seasonal changes in nutritional resource availability (Tran & Manning, 2019; Veselý et al., 2020). Tran & Manning's study demonstrated that during times of high animal tissue abundance (e.g. invertebrate emergence in the late spring), crayfish preferentially consumed animal tissue. However, during times of low animal tissue abundance, crayfish increased their consumption of diatoms and detritus significantly (Tran & Manning, 2019). Veselý et al. found similar patterns of dietary plasticity through time and among populations with different nutritional resources (Veselý et al., 2020). The utilization of dietary plasticity to avoid trophic overlap with native species has also been documented in studies specifically investigating crayfish invasion. In mesocosm experiments, the dietary plasticity of macroinvertebrate consumption was implicated as the mechanism that facilitated niche differentiation between native and non-native crayfishes (Jackson et al., 2014). In a study of invasive crayfish and fishes, crayfish seem to employ dietary plasticity to occupy a different trophic niche than sympatric fish species (Jackson & Britton, 2014). Similarly to my results, it was found that the core fundamental isotopic niches of invasive crayfish overlapped moderately with those of two sympatric cyprinid species while the core realized niches of crayfish and fish were largely segregated (Jackson & Britton, 2014). It was concluded that crayfish were utilizing dietary plasticity as a mechanism to select food resources and avoid trophic niche overlap with fish (Jackson & Britton, 2014).

Overall, the results of the first objective do not support the original hypothesis. Rather, I contend that similarly to previous literature, the dietary plasticity of *F. virilis* may be allowing for the selection of nutritional resources to avoid exploitative competition with native fishes and occupy a slightly different trophic niche.

Impacts of *F. virilis* sympatry on native secondary consumer fish species

The second objective was to evaluate the potential impact of *F. virilis* sympatry on both the isotopic characteristics and body condition of the three native secondary consumer fish species (NSCFS). I expected the realized SEA_B trophic niches and body condition of sympatric NSCFS populations to be significantly reduced due to interspecific exploitative competition for limited nutritional resources. My results indicate the exact opposite trend, but are consistent with the segregation of *F. virilis'* realized trophic niche from those of native fishes. In all but one case, the niche widths (SEA_B) and body condition of sympatric NSCFS populations were statistically similar to conspecific allopatric populations (**Figures 3.4 & 3.6; Table 3.3**). I expected that sympatry with *F. virilis* would cause native fishes to be evicted from parts of their trophic niche by exploitative competition with *F. virilis* which would be represented by sympatric NSCFS populations having smaller trophic niches than conspecific allopatric populations. I further hypothesized that if the lost portion of the trophic niche included higher quality nutritional resources, it could result in reduced fish body condition in sympatric populations. However, the similarity of realized SEA_B niche widths and body condition of *F. virilis* sympatric and allopatric native fish populations indicates that *F. virilis* have exerted no significant detrimental trophic effects on these fishes. This finding is consistent with the results of the first objective that indicated *F. virilis* and native fish are consuming different nutritional resources and not participating in exploitative competition when in sympatry.

In line with my hypotheses, the carbon ranges of *F. virilis* sympatric *R. cataractea* and *C. plumbeus* populations were narrower than those of populations allopatric of *F. virilis* (**Figure 3.5)**.

This indicates that the richness of nutritional sources consumed by these species is reduced for sympatric populations. However, given the rest of the balance of evidence for this study suggests a lack of exploitive competition between *F. virilis* and NSCRS, this effect may be the result of reach-specific differences in the number of nutritional resources available, rather than *F. virilis* sympatry. In particular, the reaches in which carbon ranges were narrowed were WMD2 and BMD3 which are both located in the downtown core of Edmonton, Alberta. It is well documented that riparian areas located in high levels of urbanization, in general, suffer reduced biodiversity compared to those located in less developed rural and natural areas (e.g. McKinney, 2002; Pennington et al., 2010). As such, I contend that rather than a product of *F. virilis* sympatry, the reduced biodiversity in primary producers in these highly urbanized reaches is the cause of the reduced carbon ranges seen in these populations.

In summary, this study suggests that *F. virilis* do not negatively affect the isotopic characteristics or body condition of native fishes in this region. Lack of apparent impacts on fishes coupled with little evidence of nutritional resource sharing further supports the notion that *F. virilis* may be utilizing dietary plasticity to exploit a slightly different trophic niche than those occupied by native fishes and in doing so, avoid exploitative competition for nutritional resources.

Implications

The ability of *F. virilis* to utilize dietary plasticity to occupy a trophic niche that is unoccupied by native species could facilitate the species' establishment in currently unoccupied areas of the basin. This study indicates that *F. virilis* are not competing with the native fishes *R. cataractea*, *C. plumbeus*, or *Catostomus spp.*. However, these species are generalist, common, and generally robust (Scott & Crossman, 1973). Currently unoccupied reaches/streams with slightly different species assemblages containing rare, specialist, and/or sensitive fish species may be vulnerable to *F. virilis* in different ways. If introduced, *F. virilis* could exert a host of negative effects on sensitive species by way of indirect and/or direct competition, predation, habitat modification etc. Further, of all the provinces, excluding the Maritimes and Territories, Alberta is the most fish species poor (Scott & Crossman, 1973). This is due to dispersion barriers for routes from glacial refugia after the last (Late Wisconsian) glaciation (Nelson & Paetz, 1992). As biotic resistance to invasion is positively correlated with biodiversity (Elton, 2020), species poor NSR tributaries may be especially vulnerable to new invasions by *F. virilis*. However, the relationship between biodiversity and biotic resistance is highly nuanced depending on the invasive species, native community, and abiotic factors and cannot be assumed as strictly positive in all cases (Levine & D'Antonio, 1999; Lockwood et al., 2013). To prevent further movement and potential impacts of *F. virilis* in native fish, watershed managers should continue to implement and practice measures preventing further expansion of *F. virilis* within the NSR basin and other Alberta watersheds.

Future directions

This study evaluated the trophic effects of *F. virilis* on three common and generally robust native species (Scott & Crossman, 1973). While little evidence was found that *F. virilis* is competing exploitatively with these native fishes for nutritional resources, this study does not exclude the possibility that *F. virilis* may be competing exploitatively for resources with other NSR basin fish species. Further investigation should be made into the trophic effects of *F. virilis* on native rare and sensitive fish species as they could be more vulnerable to *F. virilis* presence than the species studied here. For example, crayfish have been shown to compete exploitatively with benthic carnivorous fish species for spatial resources (Longshaw & Stebbing, 2016; Reynolds, 2011). Furthermore, crayfish have been shown to force juvenile *L. lota* to leave preferred shelter habitats which can make juveniles more vulnerable to predators (Hirsch & Fischer, 2008), while sculpins are displaced form shelters and spend increased time fleeing in the presence of crayfish which resulted in reduced growth rates and lowered body condition (Light, 2005).

My results also do not rule out the possibility that *F. virilis* exert direct negative effects on native fish by way of predation. For example, instream experiments have shown that crayfish actively prey upon adult benthic darter species (Thomas & Taylor, 2013) as well as the eggs and fry of threatened fish species (e.g. Fitzsimons et al., 2002; Savino & Miller, 1991). Considering predation in the other direction, stomach content analysis revealed that *F. virilis* are being preyed upon and consumed by at least one piscivorous fish species in the NSR basin, *L. lota* (**Figure A3.1**). *L. lota* have been documented to prey upon crayfish as a natural prey item in their native range (Jacobs et al., 2010). However, crayfish have been shown to compound issues of bioaccumulation in piscivorous species (Prestie et al., 2019).

Lastly, the impacts of *F. virilis* in the NSR basin may not be limited to fishes. *F. virilis* have been known to change the species assemblages of benthic macroinvertebrates drastically, decimate native snail and clam biomass, and cause trophic cascades (e.g. Hanson et al., 1990; Nyström et al., 1999; Rodríguez et al., 2005). Future studies would do well to investigate *F. virilis*' behavioral interactions with juvenile piscivorous fish; if NSR basin sculpin species are being preyed upon by *F. virilis*; the mercury concentrations of *F. virilis* and their contribution to bioaccumulation in benthic predatory fish such as *L. lota*; and/or the potential effects of *F. virilis* on benthic invertebrate communities using stable isotope mixing models and diversity indices.

Conclusion

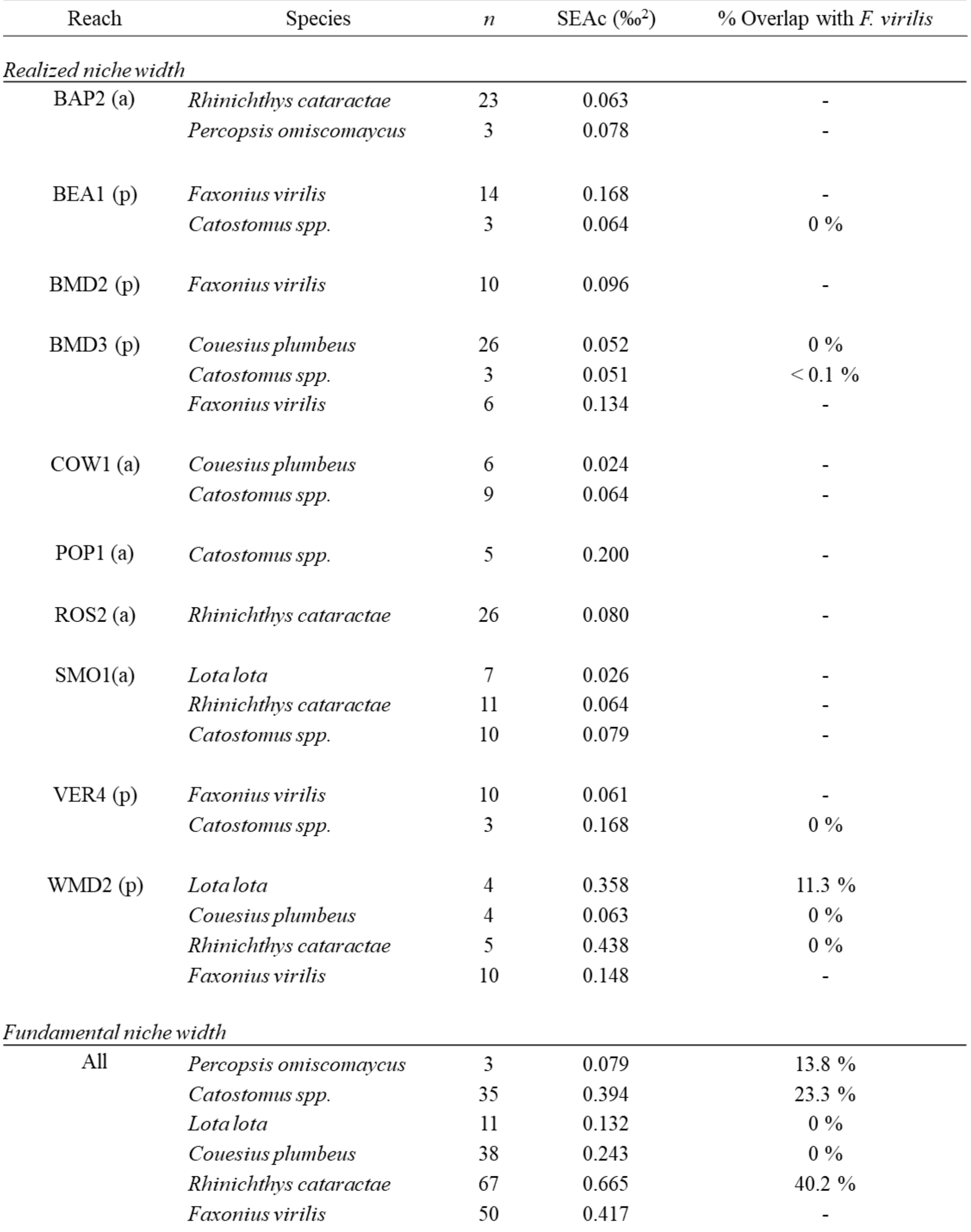
Moderate overlap of *F. virilis*' core fundamental niche with those of native fishes indicated that *F. virilis* have the potential to consume the same resources as and/or compete with native fishes. However, segregation of core realized niches showed a lack of resource sharing or exploitative competition within communities of the NSR basin. My results indicate that rather than participate in exploitative competition, *F. virilis* may be utilizing dietary plasticity to exploit a slightly different trophic niche than those occupied by native fishes and in doing so, avoid exploitative competition for nutritional resources. While *F. virilis* were not found to negatively affect the common, generalist fish species in this study, dietary plasticity may facilitate the invasion of *F. virilis* in currently unoccupied tributaries. Watershed managers should therefore continue to prevent *F. virilis* introductions into currently unoccupied tributaries to prevent potential negative effects on sensitive native fish species.

Tables & Figures

Table 3.1 – Means and standard deviations of raw $\delta^{13}C$ and $\delta^{15}N$ as well as means and standard deviations for baseline corrected $\delta^{13}C$ ($\delta^{13}C_{corr}$) and baseline calculated trophic position (TP) for each species, taken over all reaches. The number of individuals of each species over all reaches (*n*) is provided.

		Muscle $\delta^{13}C$ (‰)		Muscle $\delta^{15}N$ (‰)		Muscle $\delta^{13}C_{corr}$ (‰)		Trophic Position (TP)	
Species	*n*	Mean	St. Dev	Mean	St. Dev	Mean	St. Dev	Mean	St. Dev
Faxonius virilis	50	-28.01	0.78	10.40	0.56	< -0.01	0.29	3.60	0.44
Catostomus spp.	35	-31.54	1.78	10.38	1.45	-0.21	0.29	3.98	0.44
Couesius plumbeus	38	-27.78	0.77	11.39	1.60	0.11	0.22	4.44	0.51
Lota lota	11	-28.62	1.21	10.93	0.79	0.11	0.24	4.30	0.23
Percopsis omiscomaycus	3	-31.18	0.63	8.41	0.27	0.14	0.16	3.84	0.08
Rhinichthys cataractae	67	-30.33	1.76	9.18	3.39	0.02	0.26	4.04	0.86

Table 3.2 – Core isotopic niche widths (SEA_c) of each species within in each reach (realized niche width), among reaches (fundamental niche width), and the % overlap of each fish species with *F. virilis* (if applicable). Letter in parentheses beside unique reach code indicates *F. virilis* occupancy of that reach: p = present, a = absent. Realized niche widths and fundamental niche widths (SEAc) correspond with the plotted niche width spaces in Figures 1 & 2, respectively. Percent niche width overlap with *F. virilis* was calculated as the area of niche overlap as a proportion of the non-overlapping areas of *F. virilis*' and the fish species niche width area multiplied by 100.



Reach	Species	*n*	SEAc (‰^{2})	% Overlap with *F. virilis*
Realized niche width				
BAP2 (a)	*Rhinichthys cataractae*	23	0.063	-
	Percopsis omiscomaycus	3	0.078	-
BEA1 (p)	*Faxonius virilis*	14	0.168	-
	Catostomus spp.	3	0.064	0 %
BMD2 (p)	*Faxonius virilis*	10	0.096	-
BMD3 (p)	*Couesius plumbeus*	26	0.052	0 %
	Catostomus spp.	3	0.051	< 0.1 %
	Faxonius virilis	6	0.134	-
COW1 (a)	*Couesius plumbeus*	6	0.024	-
	Catostomus spp.	9	0.064	-
POP1 (a)	*Catostomus spp.*	5	0.200	-
ROS2 (a)	*Rhinichthys cataractae*	26	0.080	-
SMO1(a)	*Lota lota*	7	0.026	-
	Rhinichthys cataractae	11	0.064	-
	Catostomus spp.	10	0.079	-
VER4 (p)	*Faxonius virilis*	10	0.061	-
	Catostomus spp.	3	0.168	0 %
WMD2 (p)	*Lota lota*	4	0.358	11.3 %
	Couesius plumbeus	4	0.063	0 %
	Rhinichthys cataractae	5	0.438	0 %
	Faxonius virilis	10	0.148	-
Fundamental niche width				
All	*Percopsis omiscomaycus*	3	0.079	13.8 %
	Catostomus spp.	35	0.394	23.3 %
	Lota lota	11	0.132	0 %
	Couesius plumbeus	38	0.243	0 %
	Rhinichthys cataractae	67	0.665	40.2 %
	Faxonius virilis	50	0.417	-

Table 3.3 – $\mathrm{SEA_B}$ means and 95% probability intervals by reach for each secondary consumer fish species. Superscript lowercase letters to the right of reach names indicate statistically significant differences between mean $\mathrm{SEA_B}$ values where different letters indicate significant differences with 95% confidence and like letters indicating statistically similar mean $\mathrm{SEA_B}$ values.

				95% Probability Interval	
Species	Reach	Presence/Absent of *F. virilis*	Mean $\mathrm{SEA_B}$	Upper	Lower
Couesius plumbeus					
	COW1^a	A	0.02	0.04	0.01
	BMD3^a	P	0.05	0.07	0.03
	WMD2^a	P	0.06	0.13	0.01
Rhinichthys cataractae					
	BAP2^a	A	0.07	0.09	0.04
	ROS2^a	A	0.08	0.11	0.05
	SMO1^a	A	0.06	0.11	0.03
	WMD2^b	P	0.45	0.88	0.12
Catostomus spp.					
	COW1^a	A	0.07	0.11	0.03
	POP1^a	A	0.26	0.52	0.07
	SMO1^a	A	0.08	0.13	0.03
	BEA1^a	P	0.17	0.43	0.02
	BMD3^a	P	0.17	0.40	0.02
	VER4^a	P	0.12	0.13	0.03

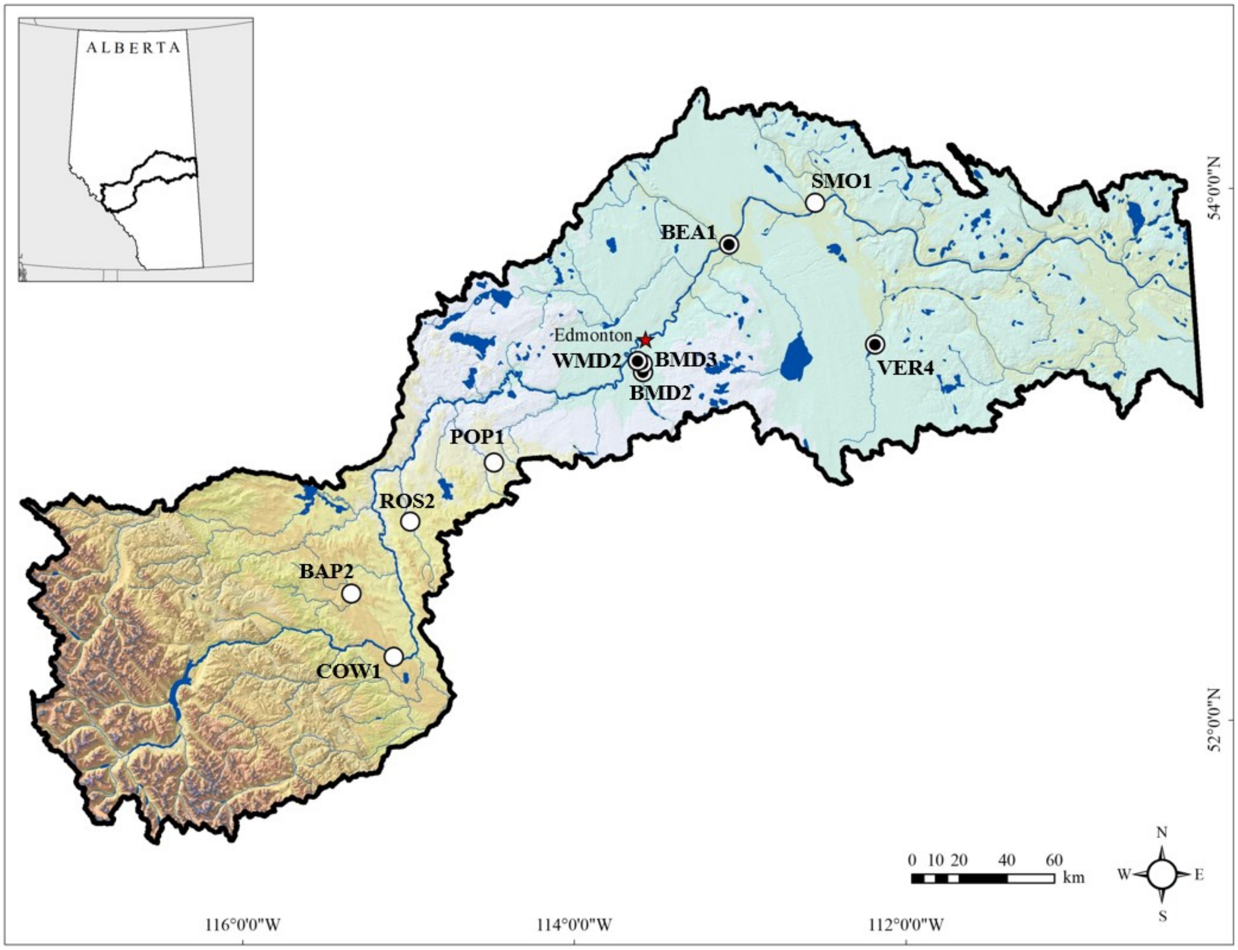


Figure 3.1 – Locations of ten study reaches in the North Saskatchewan River basin. Reaches absent of *F. virilis* are represented by empty white circles. Reaches occupied by *F. virilis* are represented by circles filled with a black solid circle. Unique reach identification codes are located near each reach's location marker.

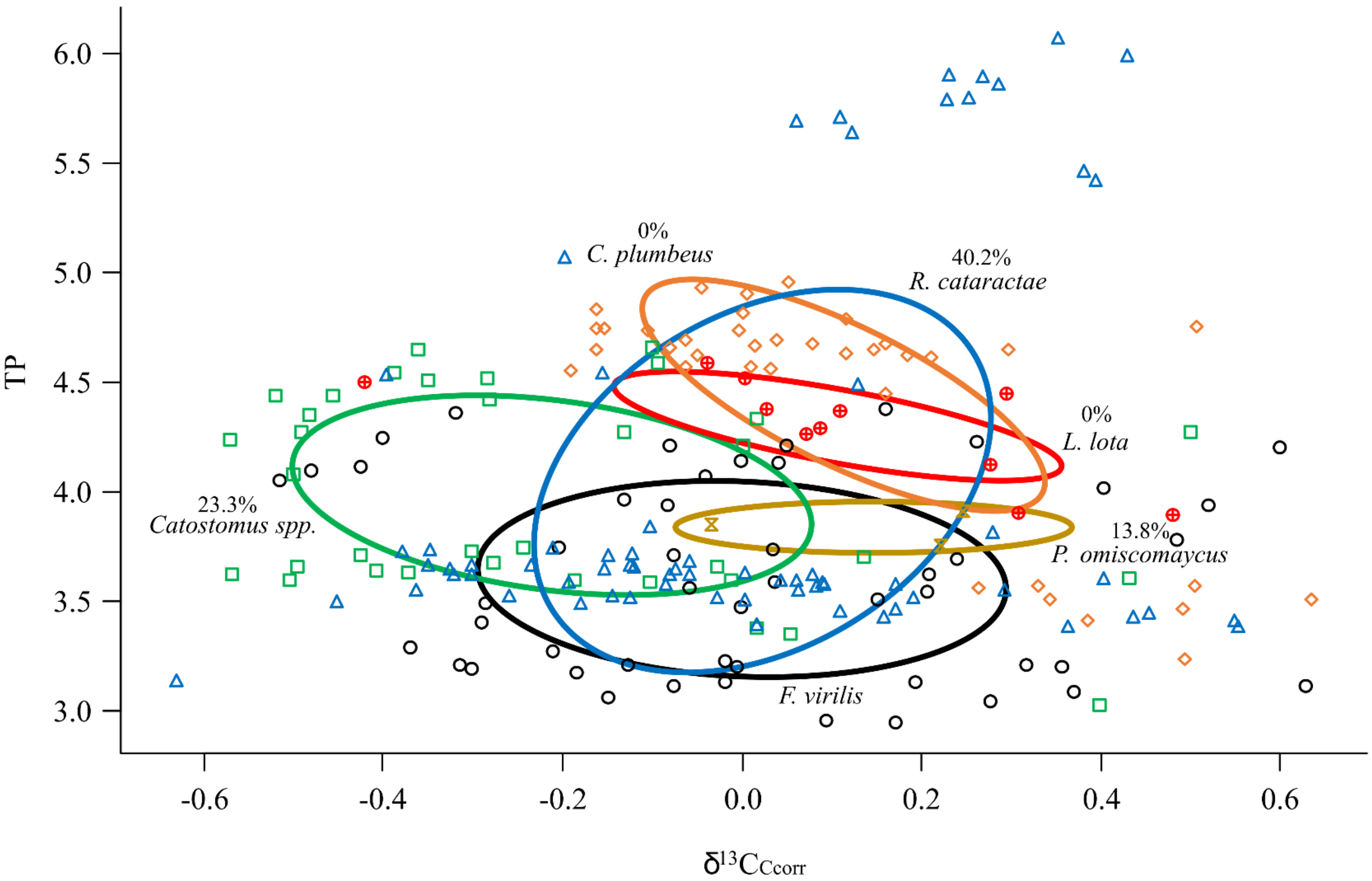


Figure 3.2 – Corrected isotopic carbon (δ^{13}Ccorr) and trophic position (TP) biplots showing each species' core fundamental isotopic niche width. Isotopic niche widths are expressed in ‰^{2} and were calculated using small sample size corrected standard ellipse area (SEAc) which contains 1 SD around the mean or approximately 40% of the data for each species. Isotopic niches are labeled with the corresponding species' shorthand name, ellipse color, and marker type. Black open circles = *F. virilis*; red crossed circles = *L. lota*; orange open diamonds = *C. plumbeus*; blue open triangles = *R. cataractae*; green open squares = *Catostomus spp.*; and gold hourglasses = *P. omiscomaycus*. Percentages above species names indicate the percent overlap of fish species' isotopic niche with that of *F. virilis*. Plotting of core isotopic niches and % overlap calculations were done using the *SIBER* R package.

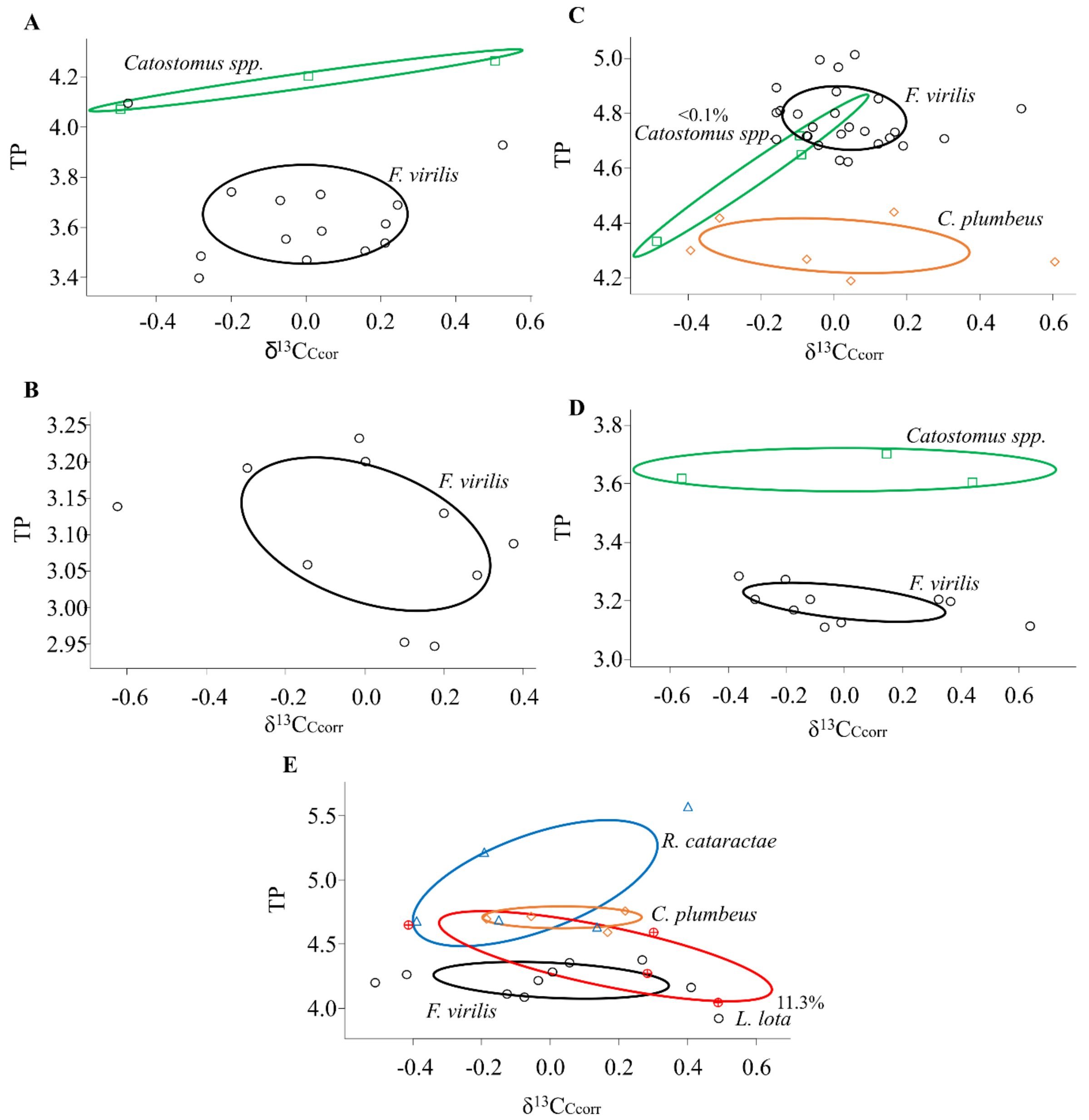


Figure 3.3 – Corrected isotopic carbon (δ^{13}Ccorr) and trophic position (TP) biplots showing the core realized isotopic niche width of each species within each reach where northern crayfish were found to be present. Panel letters indicate the specific reach as follows: (**A**) BEA1, (**B**), BMD2, (**C**) BMD3, (**D**) VER4, and (**E**) WMD2. Isotopic niche widths are expressed in ‰^{2} and were calculated using small sample size corrected standard ellipse area (SEAc) which contains 1 SD around the mean or approximately 40% of the data for each species. Isotopic niches are labeled with the corresponding species' shorthand name, ellipse color, and marker type. Black open circles = *F. virilis*; red crossed circles = *L. lota*; orange open diamonds = *C. plumbeus*; blue open triangles = *R. cataractae*; and green open squares = *Catostomus spp.*. Percentages above species names indicate the percent overlap of fish species' isotopic niche with that of *F. virilis*. Lack of a percentage above a fish species name indicates a lack of trophic niche overlap with *F. virilis*. Plotting of core isotopic niches and % overlap calculations were done using the *SIBER* R package.

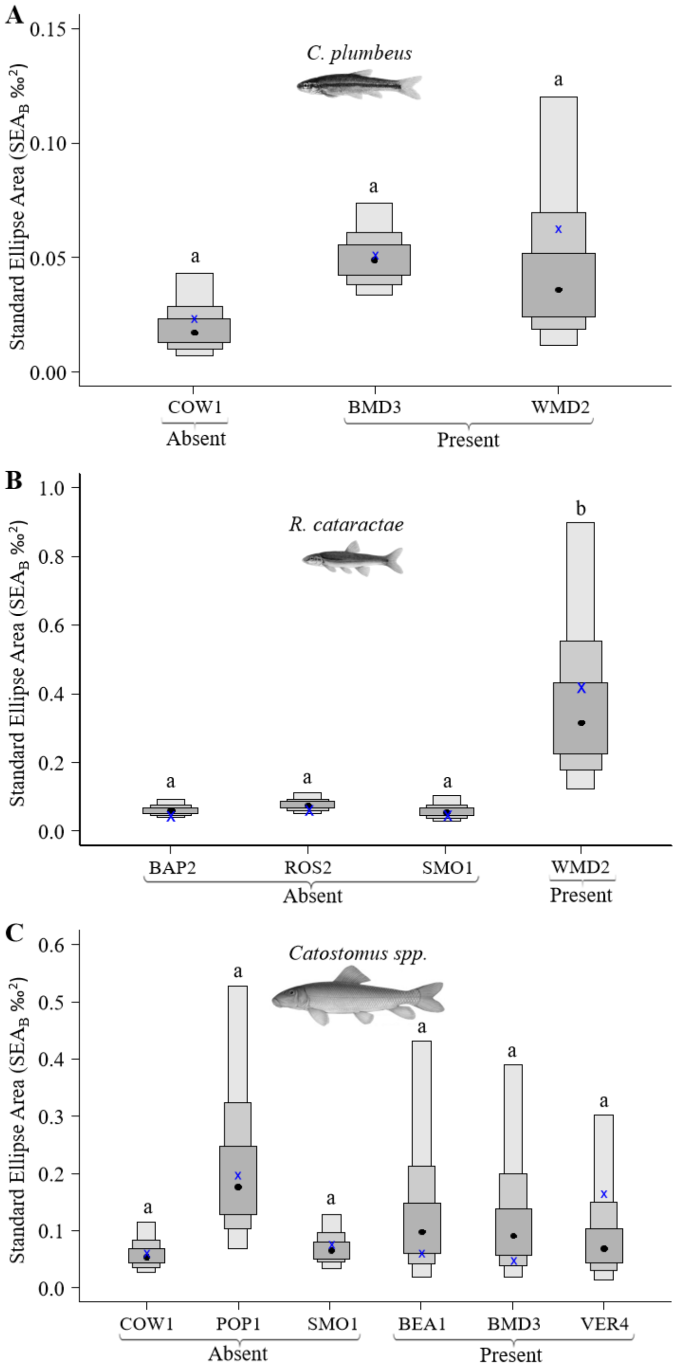


Figure 3.4 – Density plots of realized isotopic niche widths (SEA$_B$ ‰^{2}) of the three secondary consumer fish species ((**A**) *C. plumbeus* [*n*=35], (**B**) *R. cataractae* [*n*=65], and (**C**) *Catostomus spp*. [*n*=33]) compared where *F. virilis* are present vs. absent. Black dots represent the bootstrapped mean SEA$_B$ areas. Blue crosses represent the small sample size corrected standard ellipse area (SEAc). Boxes around means indicate the 95%, 75%, and 50% probability intervals of the SEA$_B$ area. Lower case letters indicate significant differences between mean SEA$_B$ values where different letters indicate significant differences with 95% confidence and like letters indicate statistically similar mean SEA$_B$ values. Unique reach codes appear below their respective bar.

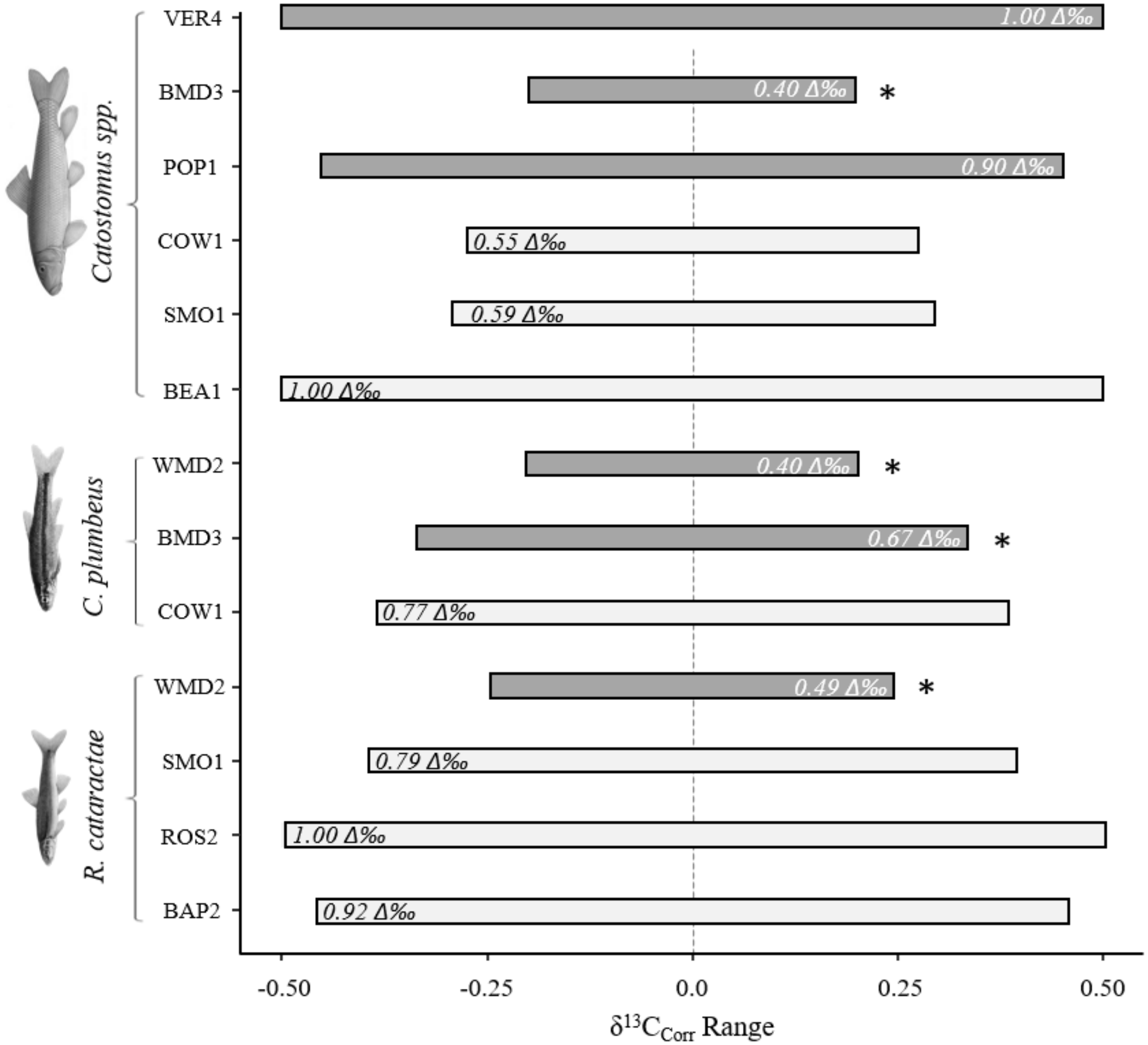


Figure 3.5 – Range plot comparing reach specific δ^{13}Ccorr ranges (Δ‰) which are reflective of dietary source richness of the three secondary consumer fish species (*R. cataractae*, *C. plumbeus*, and *Catostomus spp*.) compared between where *F. virilis* are present (grey bars) vs. absent (white bars). Unique reach codes appear on the y-axis for each respective bar. The * symbol to the right of a carbon range bar indicates that that reach is located in the highly urbanized core of Edmonton, Alberta.

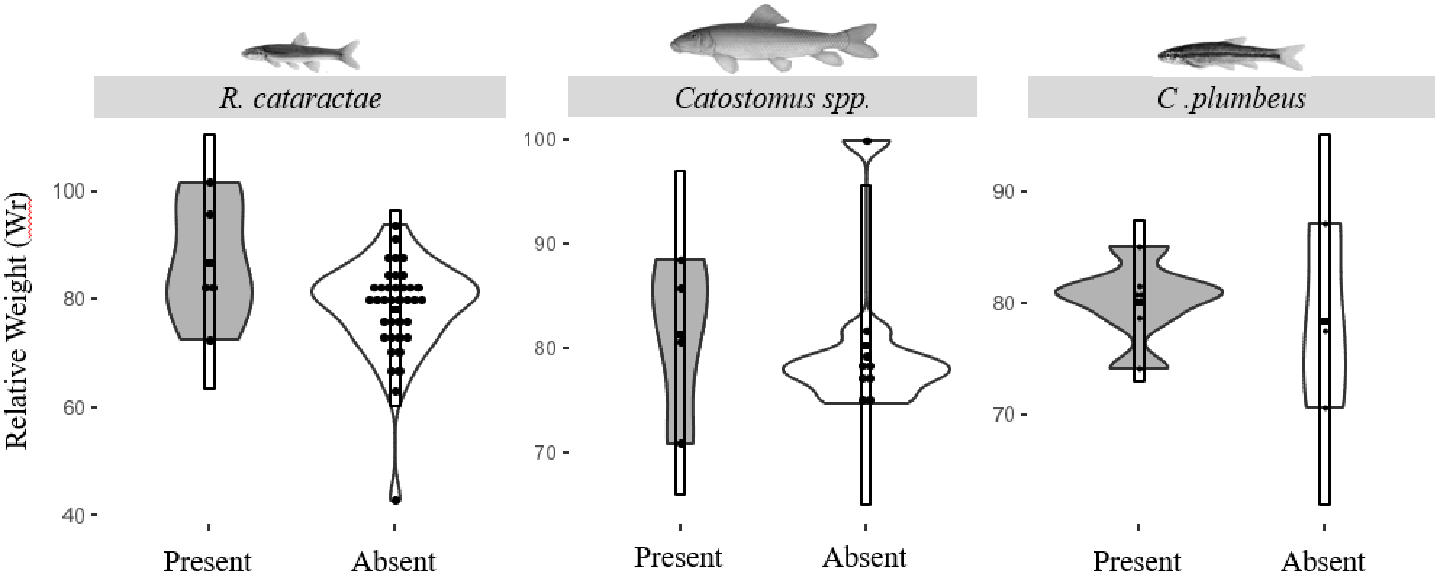


Figure 3.6 – Violin plots comparing mean body condition (as described by the relative weight condition metric [Wr] and reported in %) of *R. cataractae* (*n* = 43), *Catostomus spp*. (*n* = 9), and *C. plumbeus* (*n* = 13) over all reaches in which crayfish are present against all reaches where crayfish are absent. Significant difference between means is represented by an asterisk (*****).

Chapter 4: General Conclusions

The overarching goal of this thesis was to investigate the occupancy and impacts of *F. virilis* populations within the North Saskatchewan River basin. I hoped that my results would provide practical invasive species management advice as well as information on the trophic interactions and impacts of *F. virilis* on native fish in the basin's tributaries. To do this, I first synthesized knowledge about the importance of freshwater biodiversity and the negative impacts that invasive species can have on freshwater fishes, especially invasive crayfish species like *F. virilis* (Chapter 1). I then used occupancy modeling (Chapter 2) and stable isotope analysis (Chapter 3) to investigate aspects of *F. virilis* occupancy and effects on native fish species.

I created an occupancy model to determine which local environmental characteristic(s) were most associated with *F. virilis* occupancy of NSR tributaries (Chapter 2). I hypothesized that a suite of local environmental characteristics, namely a combination of water temperature, flow velocity, turbidity, and stream edge physical complexity would be strong drivers of *F. virilis* occupancy. In contrast to my hypothesis, analyses indicated that *F. virilis* occupancy is driven positively and solely by water temperature in NSR basin tributaries. This result was consistent with previous findings, where crayfish prefer and are most reproductively successful at water temperatures in the mid-twenty Celsius degree range (Bowman, 2019; Bugnot & López Greco, 2009; Jin et al., 2019; Oluoch, 1990; Parkyn et al., 2002; Whitmore & Huryn, 1999). In fact, occupancy was so strongly driven by water temperature that it appeared to mask the effect of instream physical complexity which was inversely related to water temperature along the basin's gradient. Model averaging of top occupancy models revealed that tributaries with mean summer water temperatures of 18°C have a 50% chance of being or becoming occupied by *F. virilis* and that the probability of occupancy approaches 100% when mean summer water temperatures is 21°C. These results indicate that cold-water streams may possess some natural protection from *F. virilis* occupancy while warm water streams are more vulnerable to occupation. Based on these results I provided the suggestion to watershed managers that streams with mean summer water temperatures ≥18°C should be prioritized for prevention measures that decrease the chance of *F. virilis* introduction. This suggestion can be extended to other river basins in Alberta that are similar to the NSR basin, however the effect of climate change on mean water temperatures should be closely monitored and considered if prioritization of tributaries each year is based on this study's findings.

I addressed the overarching research goal concerning the trophic interactions between *F. virilis* and six common-generalist native fishes by inspecting the overlap of fundamental and realized isotopic niches (Chapter 3). I hypothesized that crayfish omnivory and generality would result in the isotopic niche of *F. virilis* to be large and for it to overlap significantly with secondary consumer fish species due to nutritional resource sharing. Analyses partially supported the hypothesis in fundamental niche space in that the isotopic niche of *F. virilis* was broad and overlapped moderately with those of native fish species. In realized niche space however, results showed segregation of the *F. virilis* isotopic niche from those of native fishes indicating that although capable, *F. virilis* are not sharing nutritional resources with native fishes. Chapter 3 also examined the differences in standard ellipse area, carbon range, and body condition between *F. virilis* sympatric and allopatric native fish populations resulting from trophic interactions. I expected all three metrics to be lowered in *F. virilis* sympatric fish populations, however similar standard ellipse areas and body conditions were found between natural treatment groups. Carbon ranges were slightly narrowed in *F. virilis* sympatric populations, but this was likely a result of low nutritional resource richness at the reach level due to urbanization caused biodiversity loss rather than *F. virilis* sympatry. Taken together, the results of this chapter suggest that *F. virilis* may be utilizing dietary plasticity to avoid nutritional resource sharing and exploitative competition with these native fishes. This would be consistent with the crayfish's known omnivory and ability to be plastic in its nutritional resource use in response to stressors such as seasonal variability and sympatry with other species (Dorn & Wojdak, 2004; Jackson & Britton, 2014, 2014; Longshaw & Stebbing, 2016; Momot, 1967; Tran & Manning, 2019; Veselý et al., 2020). The ability of *F. virilis* to avoid resource sharing with native fishes could facilitate its establishment in currently unoccupied tributaries. Until further research is done to determine if and what other interactions/effects *F. virilis* has on other basin fish species, managers should continue to prevent further movement of the species in the NSR basin and in Alberta. This study is the first to provide information about the trophic interactions between *F. virilis* and native fish species in the NSR basin and will be a platform for future studies to build upon.

My findings successfully addressed the overall research goal which was to investigate the occupancy and impacts of *F. virilis* populations on native fishes of the North Saskatchewan River basin. Put simply, the occupancy of *F. virilis* in the NSR basin is driven solely by water temperature and *F. virilis* do not appear to share nutritional resources or to have exerted negative trophic impacts on the common-generalist native species that were studied. The information gained from this study is informative for future *F. virilis* management in Alberta as well as for future researchers as a spring board for further study.

References

Acosta, C. A., & Perry, S. A. (2000). Effective Sampling Area: A Quantitative Method for Sampling Crayfish Populations in Freshwater Marshes. *Crustaceana*, *73*(4), 425–431. JSTOR.

AEP. (2018). *Aquatic Invasive Species Pocket Guide*. 148.

AEP. (2021a). *2021 Alberta Guide to Sportfishing Regulations*. https://open.alberta.ca/dataset/dbf392f4-266f-4947-adc0-fa4bdf4e2c9c/resource/a4af881c-b069-41c1-84bc-a339f0880e30/download/alberta-guide-sportfishing-regulations-2021.pdf

AEP. (2021b). *Surface water quality data*. https://www.alberta.ca/surface-water-quality-data.aspx

Altman, D. G., & Bland, J. M. (2005). Standard deviations and standard errors. *BMJ : British Medical Journal*, *331*(7521), 903.

Anderson, C., & Cabana, G. (2007). Estimating the trophic position of aquatic consumers in river food webs using stable nitrogen isotopes. *Journal of the North American Benthological Society*, *26*(2), 273–285. https://doi.org/10.1899/0887-3593(2007)26[273:ETTPOA]2.0.CO;2

Baltensperger, A. P., Huettmann, F., Hagelin, J. C., & Welker, J. M. (2015). Quantifying trophic niche spaces of small mammals using stable isotopes (δ15N and δ13C) at two scales across Alaska. *Canadian Journal of Zoology*, *93*(7), 579–588. https://doi.org/10.1139/cjz-2015-0025

Bauer, R. T. (1998). Gill-Cleaning Mechanisms of the Crayfish Procambarus clarkii (Astacidea: Cambaridae): Experimental Testing of Setobranch Function. *Invertebrate Biology*, *117*(2), 129–143. https://doi.org/10.2307/3226965

Bearhop, S., Adams, C. E., Waldron, S., Fuller, R. A., & Macleod, H. (2004). Determining trophic niche width: A novel approach using stable isotope analysis. *Journal of Animal Ecology*, *73*(5), 1007–1012. https://doi.org/10.1111/j.0021-8790.2004.00861.x

Bister, T. J., Willis, D. W., Brown, M. L., Jordan, S. M., Neumann, R. M., Quist, M. C., & Guy, C. S. (2000). Proposed Standard Weight (W s) Equations and Standard Length Categories for 18 Warmwater Nongame and Riverine Fish Species. *North American Journal of Fisheries Management*, *20*(2), 570–574. https://doi.org/10.1577/1548-8675(2000)020<0570:PSWWSE>2.3.CO;2

Bolker, B. M., Brooks, M. E., Clark, C. J., Geange, S. W., Poulsen, J. R., Stevens, M. H. H., & White, J.-S. S. (2009). Generalized linear mixed models: A practical guide for ecology and evolution. *Trends in Ecology & Evolution*, *24*(3), 127–135. https://doi.org/10.1016/j.tree.2008.10.008

Botta, S., Secchi, E. R., Rogers, T. L., Prado, J. H. F., de Lima, R. C., Carlini, P., & Negrete, J. (2018). Isotopic niche overlap and partition among three Antarctic seals from the Western Antarctic Peninsula. *Deep Sea Research Part II: Topical Studies in Oceanography*, *149*, 240–249. https://doi.org/10.1016/j.dsr2.2017.11.005

Bowman, A. (2019). *Chemosensory behavior and temperature preferences of northern crayfish (Faxonius virilis) and rusty crayfish (Faxonius rusticus)*. https://summit.plymouth.edu/handle/20.500.12774/261

Brazo, D. C., Liston, C. R., & Anderson, R. C. (1978). Life History of the Longnose Dace, Rhinichthys cataractae, in the Surge Zone of Eastern Lake Michigan Near Ludington, Michigan. *Transactions of the American Fisheries Society*, *107*(4), 550–556. https://doi.org/10.1577/1548-8659(1978)107<550:LHOTLD>2.0.CO;2

Brewer, S. K., DiStefano, R. J., & Rabeni, C. F. (2009). The influence of age-specific habitat selection by a stream crayfish community (Orconectes spp.) on secondary production. *Hydrobiologia*, *619*(1), 1–10. https://doi.org/10.1007/s10750-008-9577-8

Buckland, S. T., Burnham, K. P., & Augustin, N. H. (1997). Model Selection: An Integral Part of Inference. *Biometrics*, *53*(2), 603–618. https://doi.org/10.2307/2533961

Bugnot, A. B., & López Greco, L. S. (2009). Sperm production in the red claw crayfish Cherax quadricarinatus (Decapoda, Parastacidae). *Aquaculture*, *295*(3), 292–299. https://doi.org/10.1016/j.aquaculture.2009.07.021

Bunte, K., & Abt, S. R. (2001). *Sampling surface and subsurface particle-size distributions in wadable gravel-and cobble-bed streams for analyses in sediment transport, hydraulics, and streambed monitoring* (RMRS-GTR-74; p. RMRS-GTR-74). U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station. https://doi.org/10.2737/RMRS-GTR-74

Burnham, K. P., & Anderson, D. R. (2004). Multimodel Inference: Understanding AIC and BIC in Model Selection. *Sociological Methods & Research*, *33*(2), 261–304. https://doi.org/10.1177/0049124104268644

Busst, G. M. A., & Britton, J. R. (2017). Comparative trophic impacts of two globally invasive cyprinid fishes reveal species-specific invasion consequences for a threatened native fish. *Freshwater Biology*, *62*(9), 1587–1595. https://doi.org/10.1111/fwb.12970

Caroffino, D. C., Sutton, T. M., Elliott, R. F., & Donofrio, M. C. (2010). Predation on Early Life Stages of Lake Sturgeon in the Peshtigo River, Wisconsin. *Transactions of the American Fisheries Society*, *139*(6), 1846–1856. https://doi.org/10.1577/T09-227.1

Carr, M., Li, L., Sadeghian, A., Phillips, I. D., & Lindenschmidt, K.-E. (2019). Modelling the possible impacts of climate change on the thermal regime and macroinvertebrate species of a regulated prairie river. *Ecohydrology*, *12*(6), e2102. https://doi.org/10.1002/eco.2102

Chambers, P. A., Hanson, J. M., & Prepas, E. E. (1991). The effect of aquatic plant chemistry and morphology on feeding selectivity by the crayfish, Orconectes virilis. *Freshwater Biology*, *25*(2), 339–348. https://doi.org/10.1111/j.1365-2427.1991.tb00496.x

Chandler, Kellner, Fiske, & Miller. (2021). *Package 'unmarked.'* https://cran.r-project.org/web/packages/unmarked/unmarked.pdf

David, P., Thébault, E., Anneville, O., Duyck, P.-F., Chapuis, E., & Loeuille, N. (2017). Chapter One - Impacts of Invasive Species on Food Webs: A Review of Empirical Data. In D. A. Bohan, A. J. Dumbrell, & F. Massol (Eds.), *Advances in Ecological Research* (Vol. 56, pp. 1–60). Academic Press. https://doi.org/10.1016/bs.aecr.2016.10.001

de Winter, J. C. F. (2013). *Using the Student's t-test with extremely small sample sizes*. https://doi.org/10.7275/E4R6-DJ05

Dionne, K., Dufresne, F., & Nozais, C. (2016). Variation in δ13C and δ15N trophic enrichment factors among Hyalella azteca amphipods from different lakes. *Hydrobiologia*, *781*(1), 217–230. https://doi.org/10.1007/s10750-016-2846-z

Donahou, D. A. (2020). *Faxonius virilis (Hagen, 1870)* [Database]. U.S. Geological Survey, Nonindigenous Aquatic Species Database. https://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=215

Dormann, C. F., Elith, J., Bacher, S., Buchmann, C., Carl, G., Carré, G., Marquéz, J. R. G., Gruber, B., Lafourcade, B., Leitão, P. J., Münkemüller, T., McClean, C., Osborne, P. E., Reineking, B., Schröder, B., Skidmore, A. K., Zurell, D., & Lautenbach, S. (2013).

Collinearity: A review of methods to deal with it and a simulation study evaluating their performance. *Ecography*, *36*(1), 27–46. https://doi.org/10.1111/j.1600-0587.2012.07348.x

Dorn, N. J., & Wojdak, J. M. (2004). The role of omnivorous crayfish in littoral communities. *Oecologia*, *140*(1), 150–159. https://doi.org/10.1007/s00442-004-1548-9

Dudgeon, D., Arthington, A. H., Gessner, M. O., Kawabata, Z.-I., Knowler, D. J., Lévêque, C., Naiman, R. J., Prieur-Richard, A.-H., Soto, D., Stiassny, M. L. J., & Sullivan, C. A. (2006). Freshwater biodiversity: Importance, threats, status and conservation challenges. *Biological Reviews*, *81*(02), 163. https://doi.org/10.1017/S1464793105006950

Elton, C. S. (2020). *The Ecology of Invasions by Animals and Plants*. Springer Nature.

Ficetola, G. F., Siesa, M. E., Manenti, R., Bottoni, L., Bernardi, F. D., & Padoa-Schioppa, E. (2011). Early assessment of the impact of alien species: Differential consequences of an invasive crayfish on adult and larval amphibians. *Diversity and Distributions*, *17*(6), 1141–1151. https://doi.org/10.1111/j.1472-4642.2011.00797.x

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

Fry, B. (2006). *Stable isotope ecology*. Springer.

Gale, K. S. P., & Proctor, H. C. (2011). Diets of two congeneric species of crayfish worm (annelida: Clitellata: branchiobdellidae) from western Canada. *Canadian Journal of Zoology*, *89*(4), 289–296.

Geiger, W., & Alcorlo, P. (2004). *Impact of an introduced Crustacean on the trophic webs of Mediterranean wetlands*. 25.

Giannetto, D., Carosi, A., Franchi, E., Pompei, L., Giovanni, P., & Lorenzoni, M. (2011). Proposed standard weight (W s) equations for Telestes muticellus (Bonaparte, 1837) in the Tiber River basin. *Cybium: International Journal of Ichthyology*, *35*, 141–147.

Giannetto, D., Franchi, E., Pompei, L., Lorenzoni, M., Porcellotti, S., & Tancioni, L. (2012). Proposed Empirical Standard Weight Equation for Brook Chub Squalius lucumonis. *North American Journal of Fisheries Management*, *32*(3), 428–435. https://doi.org/10.1080/02755947.2012.686006

Guzzo, M. M., Haffner, G. D., Legler, N. D., Rush, S. A., & Fisk, A. T. (2013). Fifty years later: Trophic ecology and niche overlap of a native and non-indigenous fish species in the western basin of Lake Erie. *Biological Invasions*, *15*(8), 1695–1711. https://doi.org/10.1007/s10530-012-0401-z

Haddaway, N. R., Mortimer, R. J. G., Christmas, M., & Dunn, A. M. (2015). Water chemistry and endangered white-clawed crayfish: A literature review and field study of water chemistry association in Austropotamobius pallipes. *Knowledge and Management of Aquatic Ecosystems*, *416*, Article 416. https://doi.org/10.1051/kmae/2014037

Hanson, J. M., Chambers, P. A., & Prepas, E. E. (1990). Selective foraging by the crayfish Orconectes virilis and its impact on macroinvertebrates. *Freshwater Biology*, *24*(1), 69–80. https://doi.org/10.1111/j.1365-2427.1990.tb00308.x

Hartig, & Lohse. (2021). *Package 'DHARMa.'* https://cran.r-project.org/web/packages/DHARMa/DHARMa.pdf

Hayden, B. (2021). *Atomic Ecology—Analyzing Stable Isotope Data*. https://sites.google.com/view/brianhayden/teaching/analyzing-stable-isotope-data

Hirsch, P. E., & Fischer, P. (2008). Interactions between native juvenile burbot (Lota lota) and the invasive spinycheek crayfish (Orconectes limosus) in a large European lake. *Canadian Journal of Fisheries and Aquatic Sciences*, *65*(12), 2636–2643. https://doi.org/10.1139/F08-162

Hobbs, H. H. Jr. (1972). *CRAYFISHES (ASTICIDAE) OF NORTH AND MIDDLE AMERICA*. Environmental Protection Agency (EPA).

Hobson, K. A., Gloutney, M. L., & Gibbs, H. L. (1997). Preservation of blood and tissue samples for stable-carbon and stable-nitrogen isotope analysis. *Canadian Journal of Zoology*, *75*(10), 1720–1723. https://doi.org/10.1139/z97-799

Holomuzki, J. R., Feminella, J. W., & Power, M. E. (2010). Biotic interactions in freshwater benthic habitats. *Journal of the North American Benthological Society*, *29*(1), 220–244. https://doi.org/10.1899/08-044.1

Hutchinson, G. E. (1957). *Concluding remarks cold spring harbor symposia on quantitative biology* (p. 22: 415-427).

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, A., & Parnell, A. C. (2020). *Package "SIBER."*

Jackson, M. C., & Britton, J. R. (2014). Divergence in the trophic niche of sympatric freshwater invaders. *Biological Invasions*, *16*(5), 1095–1103. https://doi.org/10.1007/s10530-013-0563-3

Jackson, M. C., Jones, T., Milligan, M., Sheath, D., Taylor, J., Ellis, A., England, J., & Grey, J. (2014). Niche differentiation among invasive crayfish and their impacts on ecosystem structure and functioning. *Freshwater Biology*, *59*(6), 1123–1135. https://doi.org/10.1111/fwb.12333

Jacobs, G. R., Madenjian, C. P., Bunnell, D. B., & Holuszko, J. D. (2010). Diet of lake trout and burbot in Northern Lake Michigan during spring: Evidence of ecological interaction. *Journal of Great Lakes Research*, *36*(2), 312–317. https://doi.org/10.1016/j.jglr.2010.02.007

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*. 45.

Jin, S., Jacquin, L., Huang, F., Xiong, M., Li, R., Lek, S., Li, W., Liu, J., & Zhang, T. (2019). Optimizing reproductive performance and embryonic development of red swamp crayfish Procambarus clarkii by manipulating water temperature. *Aquaculture*, *510*, 32–42. https://doi.org/10.1016/j.aquaculture.2019.04.066

Kennard, M. J., Arthington, A. H., Pusey, B. J., & Harch, B. D. (2005). Are alien fish a reliable indicator of river health? *Freshwater Biology*, *50*(1), 174–193. https://doi.org/10.1111/j.1365-2427.2004.01293.x

Kienzle, S. W., Nemeth, M. W., Byrne, J. M., & MacDonald, R. J. (2012). Simulating the hydrological impacts of climate change in the upper North Saskatchewan River basin, Alberta, Canada. *Journal of Hydrology*, *412–413*, 76–89. https://doi.org/10.1016/j.jhydrol.2011.01.058

Kracht, O. (2011). *Simultaneous N, C and S Isotope Ratio Determination on a DELTA V Isotope Ratio MS using a Flash Elemental Analyzer,*. Thermo Fisher Scientific. https://www.thermofisher.com/document-connect/document-connect.html?url=https%3A%2F%2Fassets.thermofisher.com%2FTFS-Assets%2FCMD%2FApplication-Notes%2FAN-30194-IRMS-Nitrogen-Carbon-Sulfur-Isotope-Ratios-AN30194-EN.pdf

Kurylyk, B. L., MacQuarrie, K. T. B., Linnansaari, T., Cunjak, R. A., & Curry, R. A. (2015). Preserving, augmenting, and creating cold-water thermal refugia in rivers: Concepts derived from research on the Miramichi River, New Brunswick (Canada). *Ecohydrology*, *8*(6), 1095–1108. https://doi.org/10.1002/eco.1566

Labconco. (2021). *FreeZone 1 Liter Benchtop Freeze Dry System*. Labconco. https://www.labconco.com/product/freezone-1-liter-benchtop-freeze-dry-system/261

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[42:CSIRPF]2.0.CO;2

Legendre, P., & Legendre, L. F. J. (2012). *Numerical Ecology*. Elsevier.

Lehner, B., Verdin, K., & Jarvis, A. (2008). *New global hydrography derived from spaceborne elevation data* (89(10): 93-94) [Map]. Eos, Transactions, AGU. https://www.hydrosheds.org/

Lehti-Koivunen, S. M., & Kivivuori, L. A. (1994). Effect of temperature acclimation in the crayfish Astacus astacus L. on the locomotor activity during a cyclic temperature change. *Journal of Thermal Biology*, *19*(5), 299–304. https://doi.org/10.1016/0306-4565(94)90065-5

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

Light, T. (2003). Success and failure in a lotic crayfish invasion: The roles of hydrologic variability and habitat alteration. *Freshwater Biology*, *48*(10), 1886–1897. https://doi.org/10.1046/j.1365-2427.2003.01122.x

Light, T. (2005). Behavioral effects of invaders: Alien crayfish and native sculpin in a California stream. *Biological Invasions*, *7*(3), 353–367. https://doi.org/10.1007/s10530-004-2510-9

Linzmaier, S. M., Musseau, C., Matern, S., & Jeschke, J. M. (2020). Trophic ecology of invasive marbled and spiny-cheek crayfish populations. *Biological Invasions*, *22*(11), 3339–3356. https://doi.org/10.1007/s10530-020-02328-z

Lockwood, J. L., Hoopes, M. F., & Marchetti, M. P. (2013). *Invasion Ecology*. John Wiley & Sons.

Longshaw, M., & Stebbing, P. (2016). *Biology and Ecology of Crayfish: Vol. 1st Edition*. CRC Press. https://doi-org.login.ezproxy.library.ualberta.ca/10.1201/b20073

Loughman, Z. J. (2010). Crayfishes of Western Maryland: Conservation and Natural History. *Southeastern Naturalist*, *9*(sp3), 33. https://doi.org/10.1656/058.009.s303

Loughman, Z. J., Simon, T. P., & Welsh, S. A. (2012). Occupancy rates of primary burrowing crayfish in natural and disturbed large river bottomlands. *Journal of Crustacean Biology*, *32*(4), 557–564. https://doi.org/10.1163/193724012X637339

Love, J., & Savino, J. F. (1993). Crayfish (Orconectes virilis) Predation on Zebra Mussels (Dreissena polymorpha). *Journal of Freshwater Ecology*, *8*(3), 253–259. https://doi.org/10.1080/02705060.1993.9664861

Mack, R. N., LONSDALE, W. M., SIMBERLOFF, D., EVANS, L. H., CLOUT, M., & BAZZAZ, F. A. (2000). *Biotic invasions. Causes, epidemiology, global consequences, and control.*

MacKenzie, D. I., & Bailey, L. L. (2004). Assessing the fit of site-occupancy models. *Journal of Agricultural, Biological, and Environmental Statistics*, *9*(3), 300–318. https://doi.org/10.1198/108571104X3361

MacKenzie, D. I., Nichols, J. D., Royle, J. A., Pollock, K. H., Bailey, L., & Hines, J. E. (2017). *Occupancy Estimation and Modeling: Inferring Patterns and Dynamics of Species Occurrence*. Elsevier.

Magnusson, Skaug, Nielsen, & Bolker. (2021). *Package 'glmmTMB.'* https://cran.r-project.org/web/packages/glmmTMB/glmmTMB.pdf

Magoulick, D. D., DiStefano, R. J., Imhoff, E. M., Nolen, M. S., & Wagner, B. K. (2017). Landscape- and local-scale habitat influences on occupancy and detection probability of stream-dwelling crayfish: Implications for conservation. *Hydrobiologia*, *799*(1), 217–231. https://doi.org/10.1007/s10750-017-3215-2

Maude, S. H., & Williams, D. D. (1983). Behavior of Crayfish in Water Currents: Hydrodynamics of Eight Species with Reference to Their Distribution Patterns in Southern Ontario. *Canadian Journal of Fisheries and Aquatic Sciences*, *40*(1), 68–77. https://doi.org/10.1139/f83-010

Mazerolle. (2020). *Package 'AICcmodavg.'* https://cran.r-project.org/web/packages/AICcmodavg/AICcmodavg.pdf

McAlpine, D. F., McAlpine, A. H. E., & Madden, A. (2007). *Occurrence of the potentially invasive crayfish, Orconectes virilis (Decapoda, Cambaridae) in eastern New Brunswick, Canada. 80*(4), 509–511.

McCutchan, J. H., Lewis, W. M., Kendall, C., & McGrath, C. C. (2003). Variation in trophic shift for stable isotope ratios of carbon, nitrogen, and sulfur. *Oikos*, *102*(2), 378–390. https://doi.org/10.1034/j.1600-0706.2003.12098.x

McKinney, M. L. (2002). Urbanization, Biodiversity, and Conservation: The impacts of urbanization on native species are poorly studied, but educating a highly urbanized human population about these impacts can greatly improve species conservation in all ecosystems. *BioScience*, *52*(10), 883–890. https://doi.org/10.1641/0006-3568(2002)052[0883:UBAC]2.0.CO;2

McRae, L., Deinet, S., & Freeman, R. (2017). The Diversity-Weighted Living Planet Index: Controlling for Taxonomic Bias in a Global Biodiversity Indicator. *PLOS ONE*, *12*(1), e0169156. https://doi.org/10.1371/journal.pone.0169156

Meador, M. R., Brown, L. R., & Short, T. (2003). Relations between introduced fish and environmental conditions at large geographic scales. *Ecological Indicators*, *3*(2), 81–92. https://doi.org/10.1016/S1470-160X(03)00013-X

Merritt, R. W., Cummins, K. W., & Berg, M. B. (2019). *An Introduction to the Aquatic Insects of North America* (5th ed.). Kendall Hunt Publishing.

METTLER TOLEDO. (2004). *Operating Instructions METTLER TOLEDO AX and MX/UMX Balances*. METTLER TOLEDO. https://www.mt.com/us/en/home/library/operating-instructions/laboratory-weighing/AX_MX_UMX_2xx-3xx.html

Momot, W. T. (1967). *Population dynamics and productivity of the crayfish, Orconectes virilis, in a marl lake.* 55–81.

Momot, W. T. (1995). Redefining the role of crayfish in aquatic ecosystems. *Reviews in Fisheries Science*, *3*(1), 33–63. https://doi.org/10.1080/10641269509388566

Nahon, S., Séité, S., Kolasinski, J., Aguirre, P., & Geurden, I. (2017). Effects of euthanasia methods on stable carbon (δ ^{13} C value) and nitrogen (δ ^{15} N value) isotopic compositions of fry and juvenile rainbow trout *Oncorhynchus mykiss*. *Rapid Communications in Mass Spectrometry*, *31*(20), 1742–1748. https://doi.org/10.1002/rcm.7958

Nelson, J. S., & Paetz, M. J. (1992). *The fishes of Alberta* (2nd ed). University of Alberta Press ; University of Calgary Press.

North Saskatchewan Watershed Alliance (NSWA). (2010). *Economic Activity and Ecosystem Services in the North Saskatchewan River Basin* (pp. 149–161). https://www.nswa.ab.ca/wp-content/uploads/2017/09/Economic-Activity.-in-NSR-Basin.pdf

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. JSTOR. https://doi.org/10.2307/3546704

Ogle, D. (2019). *Package "FSAdata."* https://cran.r-project.org/web/packages/FSAdata/FSAdata.pdf

Ogle, D. H. (2018). *Introductory Fisheries Analyses with R*. Chapman and Hall/CRC. https://doi.org/10.1201/9781315371986

Oksanen, J., Guillaume Blanchet, Friendly, Kindt, & Legendre,. (2020). *Package 'vegan.'* https://cran.r-project.org/web/packages/vegan/vegan.pdf

Olsson, K., & Nyström, P. (2009). Non-interactive effects of habitat complexity and adult crayfish on survival and growth of juvenile crayfish (Pacifastacus leniusculus). *Freshwater Biology*, *54*(1), 35–46. https://doi.org/10.1111/j.1365-2427.2008.02089.x

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

Oluoch, A. O. (1990). Breeding biology of the Louisiana red swamp crayfish Procambarus clarkii Girard in Lake Naivasha, Kenya. *Hydrobiologia*, *208*(1–2), 85–92. https://doi.org/10.1007/BF00008447

Parkyn, S. M., Collier, K. J., & Hicks, B. J. (2002). Growth and population dynamics of crayfish *Paranephrops planifrons* in streams within native forest and pastoral land uses. *New Zealand Journal of Marine and Freshwater Research*, *36*(4), 847–862. https://doi.org/10.1080/00288330.2002.9517137

Pennington, D. N., Hansel, J. R., & Gorchov, D. L. (2010). Urbanization and riparian forest woody communities: Diversity, composition, and structure within a metropolitan landscape. *Biological Conservation*, *143*(1), 182–194. https://doi.org/10.1016/j.biocon.2009.10.002

Pettitt-Wade, H., Wellband, K. W., Heath, D. D., & Fisk, A. T. (2015). Niche plasticity in invasive fishes in the Great Lakes. *Biological Invasions*, *17*(9), 2565–2580. https://doi.org/10.1007/s10530-015-0894-3

Ponce, T., Cubillos, L. A., Ciancio, J., Castro, L. R., & Araya, M. (2021). Isotopic niche and niche overlap in benthic crustacean and demersal fish associated to the bottom trawl fishing in south-central Chile. *Journal of Sea Research*, *173*, 102059. https://doi.org/10.1016/j.seares.2021.102059

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[0703:USITET]2.0.CO;2

Prestie, K., Phillips, I. D., Chivers, D. P., & Jardine, T. D. (2019). Effects of ontogeny and invasive crayfish on feeding ecology and mercury concentrations of predatory fishes. *Canadian Journal of Fisheries and Aquatic Sciences*, *76*(11), 1929–1939. https://doi.org/10.1139/cjfas-2018-0455

R Core Team. (2021). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria.* https://www.R-project.org/

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

Rodríguez, C. F., Bécares, E., Fernández-aláez, M., & Fernández-aláez, C. (2005). Loss of diversity and degradation of wetlands as a result of introducing exotic crayfish. *Biological Invasions*, *7*(1), 75. https://doi.org/10.1007/s10530-004-9636-7

Rogowski, D. L., Sitko, S., & Bonar, S. A. (2013). Optimising control of invasive crayfish using life-history information. *Freshwater Biology*, *58*(6), 1279–1291.

Rosenberg, D. M., Turner, M. A., Jansen, W., Mosindy, T., & Watkinson, D. A. (2010). *Threats to Lake of the Woods and the Winnipeg River by the rusty crayfish (Orconectes rusticus), an aquatic invader* (Technical Workshop Report TWR-005; p. 54). Ontario Ministry of Natural Resources.

Rosewarne, P. J., Svendsen, J. C., Mortimer, R. J. G., & Dunn, A. M. (2014). Muddied waters: Suspended sediment impacts on gill structure and aerobic scope in an endangered native and an invasive freshwater crayfish. *Hydrobiologia*, *722*(1), 61–74. https://doi.org/10.1007/s10750-013-1675-6

Rosinski, C. L., Vinson, M. R., & Yule, D. L. (2020). Niche Partitioning among Native Ciscoes and Nonnative Rainbow Smelt in Lake Superior. *Transactions of the American Fisheries Society*, *149*(2), 184–203. https://doi.org/10.1002/tafs.10219

Royle, J. A., & Nichols, J. D. (2003). ESTIMATING ABUNDANCE FROM REPEATED PRESENCE–ABSENCE DATA OR POINT COUNTS. *Ecology*, *84*(3), 777–790. https://doi.org/10.1890/0012-9658(2003)084[0777:EAFRPA]2.0.CO;2

Ruppert, J. L. W., Docherty, C., Neufeld, K., Hamilton, K., MacPherson, L., & Poesch, M. S. (2017). Native freshwater species get out of the way: Prussian carp (*Carassius gibelio*) impacts both fish and benthic invertebrate communities in North America. *Royal Society Open Science*, *4*(10), 170400. https://doi.org/10.1098/rsos.170400

Savino, J. F., & Miller, J. E. (1991). Crayfish (Orconectes virilis) Feeding on Young Lake Trout (Salvelinus namaycush): Effect of Rock Size. *Journal of Freshwater Ecology*, *6*(2), 161–170. https://doi.org/10.1080/02705060.1991.9665290

Schoener, T. W. (1968). The Anolis Lizards of Bimini: Resource Partitioning in a Complex Fauna. *Ecology*, *49*(4), 704–726. https://doi.org/10.2307/1935534

Scott, W. B., & Crossman, E. J. (1973). *Freshwater Fishes of Canada*.

Setzer, M., Norrgård, J. R., & Jonsson, T. (2011). An invasive crayfish affects egg survival and the potential recovery of an endangered population of Arctic charr. *Freshwater Biology*, *56*(12), 2543–2553. https://doi.org/10.1111/j.1365-2427.2011.02679.x

Simon, T., & Cooper, N. (2014). Habitat Suitability Index Relationships for the Northern Clearwater Crayfish,Orconectes Propinquus (Decapoda: Cambaridae). *Fisheries & Aquaculture Journal*, *5*, 1–7. https://doi.org/10.4172/2150-3508.1000098

Smith, G. R. T., Learner, M. A., Slater, F. M., & Foster, J. (1996). Habitat features important for the conservation of the native crayfish Austropotamobius pallipes in Britain. *Biological Conservation*, *75*(3), 239–246. https://doi.org/10.1016/0006-3207(95)00073-9

Smith, K. R., Roth, B. M., Jones, M. L., Hayes, D. B., Herbst, S. J., & Popoff, N. (2019). Changes in the distribution of Michigan crayfishes and the influence of invasive rusty crayfish (Faxonius rusticus) on native crayfish substrate associations. *Biological Invasions*, *21*(2), 637–656. https://doi.org/10.1007/s10530-018-1852-7

Styrishave, B., Bojsen, B. H., Witthøfft, H., & Andersen, O. (2007). Diurnal variations in physiology and behaviour of the noble crayfish ***Astacus astacus*** and the signal crayfish ***Pacifastacus leniusculus*** . *Marine and Freshwater Behaviour and Physiology*, *40*(1), 63–77. https://doi.org/10.1080/10236240701241538

Svanbäck, R. (2015). *Individuals in food webs: The relationships between trophic position, omnivory and among-individual diet variation*. 12.

Svobodová, J., Douda, K., Fischer, D., Lapšanská, N., & Vlach, P. (2017). Toxic and heavy metals as a cause of crayfish mass mortality from acidified headwater streams. *Ecotoxicology*, *26*(2), 261–270. https://doi.org/10.1007/s10646-017-1760-0

Thermo Scientific. (2009). *Precision Compact Gravity Convection Ovens*. Thermo Scientific. https://www.nist.gov/system/files/documents/ncnr/Oven_ThermoScientific_Precision_658.pdf

Thomas, C. L., & Taylor, C. A. (2013). Scavenger or predator? Examining a potential predator–prey relationship between crayfish and benthic fish in stream food webs. *Freshwater Science*, *32*(4), 1309–1317. https://doi.org/10.1899/12-169.1

Tilman, D. (2020). Resource Competition and Community Structure. (MPB-17), Volume 17. In *Resource Competition and Community Structure. (MPB-17), Volume 17*. Princeton University Press. https://www.degruyter.com/document/doi/10.1515/9780691209654/html

Tran, M. V., & Manning, A. (2019). *Seasonal Diet Shifts in the Rusty Crayfish, Faxonius rusticus (Girard)*. 5.

Vaudo, J., & Heithaus, M. (2011). Dietary niche overlap in a nearshore elasmobranch mesopredator community. *Marine Ecology Progress Series*, *425*, 247–260. https://doi.org/10.3354/meps08988

Veselý, L., Ercoli, F., Ruokonen, T. J., Bláha, M., Kubec, J., Buřič, M., Hämäläinen, H., & Kouba, A. (2020). The crayfish distribution, feeding plasticity, seasonal isotopic variation and trophic role across ontogeny and habitat in a canyon-shaped reservoir. *Aquatic Ecology*, *54*(4), 1169–1183. https://doi.org/10.1007/s10452-020-09801-w

Wege, G. J., & Anderson, R. O. (1978). Relative weight (Wr): A new index of condition for largemouth bass. New approaches to the management of small impoundments. *American Fisheries Society, North Central Division, Special Publication*, *5*, 79–91.

Wetzel, J. E., & Brown, P. B. (1993). Growth and Survival of Juvenile Orconectes virilis and Orconectes immunis at Different Temperatures1. *Journal of the World Aquaculture Society*, *24*(3), 339–343. https://doi.org/10.1111/j.1749-7345.1993.tb00165.x

Whitmore, N., & Huryn, A. D. (1999). Life history and production of Paranephrops zealandicus in a forest stream, with comments about the sustainable harvest of a freshwater crayfish. *Freshwater Biology*, *42*(3), 467–478. https://doi.org/10.1046/j.1365-2427.1999.00482.x

Williams, B. W. (2012). *A molecular assessment of range expansion of the northern or virile crayfish (Orconectes virilis), crayfish-based community co-structure, and phylogeny of crayfish-affiliated symbionts*.

Williams, B. W., Gelder, S. R., & Proctor, H. (2009). Distribution and First Reports of Branchiobdellida (Annelida: Clitellata) on Crayfish in the Prairie Provinces of Canada. *Western North American Naturalist*, *69*(1), 119–124. https://doi.org/10.3398/064.069.0104

Williams, B. W., Proctor, H. C., & Clayton, T. (2011). Range extension of the northern crayfish, Orconectes virilis (Decapoda, Cambaridae), in the western Prairie Provinces of Canada. *Crustaceana*, 451–460.

Williams, B. W., Williams, K. L., Gelder, S. R., & Proctor, H. C. (2011). Distribution of Entocytheridae (Crustacea: Ostracoda) in the Northern Prairies of North America and Reports of Opportunistic Clitellate Annelids on Crayfish Hosts. *Western North American Naturalist*, *71*(2), 276–282. https://doi.org/10.3398/064.071.0215

Yarra, A. N., & Magoulick, D. D. (2018). Stream permanence is related to crayfish occupancy and abundance in the Ozark Highlands, USA. *Freshwater Science*, *37*(1), 54–63. https://doi.org/10.1086/696020

Zale, A. V., Parrish, D. L., Sutton, T. M., & editors. (2012). *Fisheries Techniques* (3rd Edition). American Fisheries Society.

Zambrano, L., Valiente, E., & Vander Zanden, M. J. (2010). Food web overlap among native axolotl (Ambystoma mexicanum) and two exotic fishes: Carp (Cyprinus carpio) and tilapia (Oreochromis niloticus) in Xochimilco, Mexico City. *Biological Invasions*, *12*(9), 3061–3069. https://doi.org/10.1007/s10530-010-9697-8

Zanden, M. J. V., & Rasmussen, J. B. (2001). Variation in δ15N and δ13C trophic fractionation: Implications for aquatic food web studies. *Limnology and Oceanography*, *46*(8), 2061–2066. https://doi.org/10.4319/lo.2001.46.8.2061

Zuur, A. F., Ieno, E. N., Walker, N., Saveliev, A. A., & Smith, G. M. (2009). *Mixed effects models and extensions in ecology with R*. Springer New York. https://doi.org/10.1007/978-0-387-87458-6

Appendices

Table A2.1 – Global and best occupancy models for water temperature standardized (≥18°C) dataset ranked by QAICc weights within ΔQAICc < 2 and corresponding covariates relationship estimates. Flow velocity (flow), turbidity (turb), physical complexity (comp), and water temperature (temp) were predictor variables for the occupancy parameter (λ). Water depth (dep) and flow velocity (flow) were predictor variables for the detection probability parameter (p). Significant relationships appear in bold.

	Detection estimates			Occupancy estimates				Ranking statistics		
Model	Intercept	depth (cm)	flow (ms^{-1})	flow (ms^{-1})	turbidity (NTU)	physical complexity	water temperature (C°)	QAICc	ΔQAICc	weight
λ(.),p(.)	0.67	-	-	-	-	-	-	38.15	0	0.566
λ (temp),p(.)	0.62	-	-	-	-	-	**0.472**	38.68	0.53	0.434

Table A3.1 – Means and standard deviations for raw $\delta^{13}C$ and $\delta^{15}N$ muscle content as well as means and standard deviations for baseline corrected $\delta^{13}C$ (δ^{13}Ccorr) and baseline calculated trophic position (TP) for each species, within each reach sampled. Letter in parentheses beside unique reach code indicates *F. virilis* occupancy of that reach: p = present, a = absent. CPUE is the *F. virilis* catch per unit effort of each reach reported in mean number of individuals caught per single 24-hour overnight trapline survey. The number of individuals of each species within each reach (*n*) is provided. Species with $n < 3$ within each reach were excluded from realized niche width analyses with the exception of species of the genus *Catostomus*. If the sum of individuals of species of the genus *Catostomus* was $n \geq 3$, these individuals were combined into a group called *Catostomus spp.*. This *Catostomus spp. g*roup was included in realized niche width analyses. All individuals, including those with $n < 3$ within each reach were retained and included in fundamental niche width analysis.

				Muscle δ^{13}C (‰)		Muscle δ^{15}N (‰)		Muscle δ^{13}Ccorr (‰)		Trophic Position (TP)	
Reach	CPUE	Species	*n*	Mean	St. Dev	Mean	St. Dev	Mean	St. Dev	Mean	St. Dev
BAP2 (a)	0.00	*Couesius plumbeus*	1	-29.21	-	7.30	-	0.64	-	3.51	-
		Rhinichthys cataractae	23	-31.95	1.06	7.44	0.34	-0.05	0.26	3.56	0.10
		Percopsis omiscomaycus	3	-31.18	0.63	8.41	0.27	0.14	0.16	3.84	0.08
		Catostomus commersonii	1	-31.69	-	6.84	-	0.02	-	3.38	-
BEA1 (p)	3.25	*Faxonius virilis*	14	-28.25	0.93	9.91	0.64	< -0.01	0.26	3.65	0.19
		Catostomus commersonii	3	-31.81	0.14	11.73	0.34	< -0.01	0.50	4.19	0.10
BMD2 (p)	4.00	*Faxonius virilis*	10	-27.60	0.39	10.89	0.34	< 0.01	0.30	3.10	0.10
BMD3 (p)	7.00	*Couesius plumbeus*	26	-27.40	0.33	12.21	0.36	0.03	0.15	4.72	0.10
		Catostomus catostomus	1	-27.66	-	11.78	-	-0.09	-	4.59	-
		Faxonius virilis	6	-27.08	0.09	10.64	0.33	< -0.01	0.36	4.26	0.10
		Catostomus commersonii	2	-28.10	0.61	11.36	0.92	-0.30	0.28	4.48	0.27
COW1 (a)	0.00	*Couesius plumbeus*	6	-29.20	0.41	8.65	0.23	0.39	0.09	3.52	0.07
		Rhinichthys cataractae	1	-30.85	-	8.62	-	< -0.01	-	3.51	-
		Catostomus catostomus	4	-31.85	0.82	9.05	0.10	-0.23	0.19	3.63	0.03
		Catostomus commersonii	5	-32.08	0.92	9.08	0.56	-0.28	0.21	3.64	0.16
POP1 (a)	0.00	*Couesius plumbeus*	1	-28.23	-	8.13	-	0.49	-	3.24	-
		Rhinichthys cataractae	1	-30.59	-	9.33	-	0.09	-	3.59	-
		Catostomus commersonii	5	-31.80	1.98	9.01	0.90	-0.12	0.34	3.50	0.27
ROS2 (a)	0.00	*Rhinichthys cataractae*	26	-30.39	0.69	7.18	0.37	< -0.01	0.25	3.62	0.11
SMO1(a)	0.00	*Lota lota*	7	-29.28	0.93	10.90	0.75	0.08	0.11	4.33	0.22
		Rhinichthys cataractae	11	-27.90	0.96	15.90	0.58	0.25	0.12	5.80	0.17
		Catostomus commersonii	10	-32.67	1.42	11.23	0.44	-0.33	0.17	4.43	0.13
VER4 (p)	8.75	*Faxonius virilis*	10	-28.77	0.57	10.52	0.21	< -0.01	0.33	3.20	0.06
		Catostomus commersonii	3	-30.22	0.40	12.06	0.18	< -0.01	0.51	3.65	0.05
WMD2 (p)	9.50	*Lota lota*	4	-27.48	0.68	10.98	0.97	0.16	0.40	4.24	0.28
		Couesius plumbeus	4	-27.70	0.32	12.01	0.24	0.03	0.19	4.55	0.07
		Rhinichthys cataractae	5	-27.83	0.53	12.92	1.42	-0.05	0.31	4.81	0.42
		Catostomus catostomus	1	-28.64	-	11.65	-	-0.52	-	4.44	-
		Faxonius virilis	10	-27.89	0.32	10.33	0.46	< 0.01	0.32	4.05	0.13

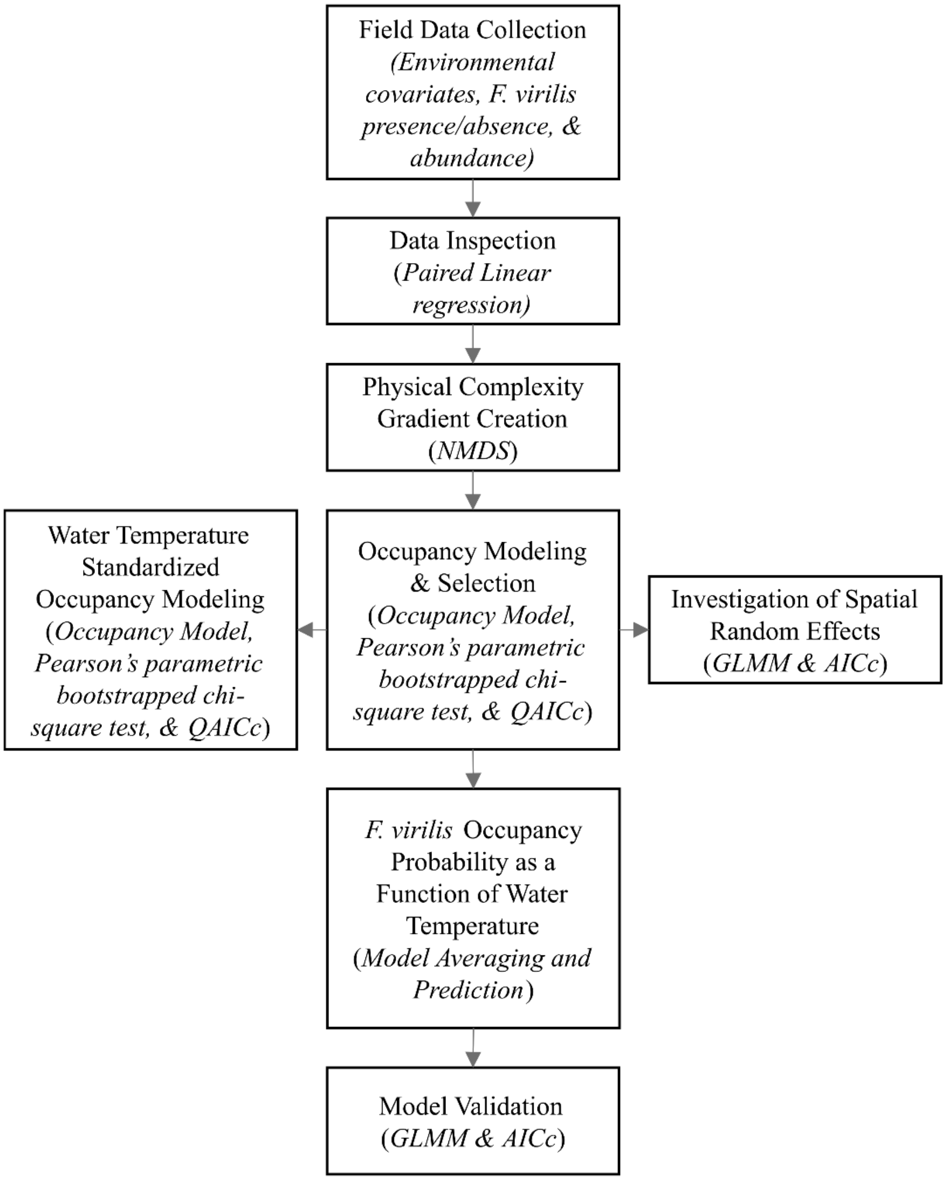


Figure A2.1 – Work flow of all statistical analyses conducted in this study. Individual boxes represent results section headings. Within each box, headings indicate the purpose of the analyses conducted while the italicized text in brackets list the statistical tools/analyses used to achieve the goal of each section. Boxes lined up vertically make up the analysis work flow that resulted in the study's main findings. Boxes to either side contain analyses that were complimentary to the main results.

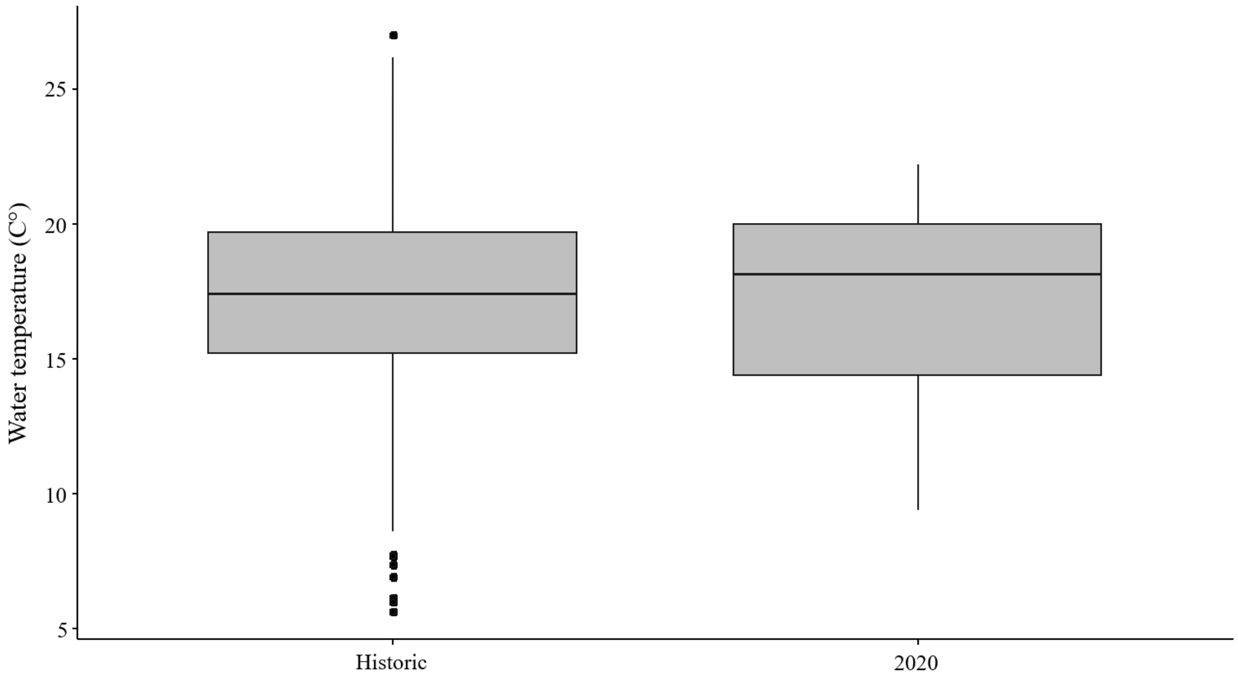


Figure A2.2 – Box and whisker plots comparing historic summer (June-August) water temperatures from 1954-2019 in the NSR basin (*n*=111) to the water temperatures sampled during this study in 2020 (*n* =37). No significant difference was found (p = n.s).

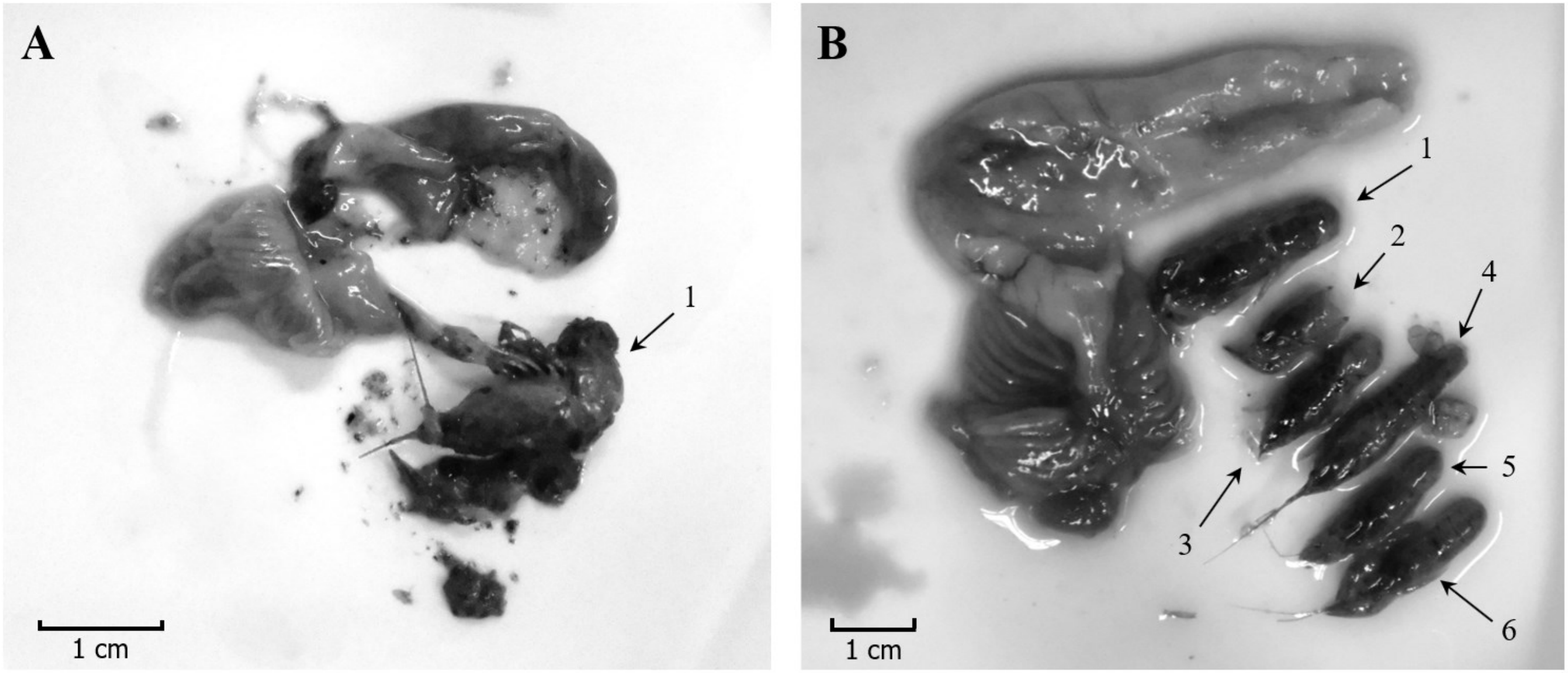


Figure A3.1 – Dissection images of two *L. lota* stomach contents which consisted of juvenile *F. virilis*. Both *L. lota* specimens were captured from reach WMD2. Arrows and numbers indicate the location and number of juvenile *F. virilis* in each stomach. Panel **A** shows that only one single *F virilis* juvenile was found in the stomach contents. Panel **B** shows six individual juvenile *F. virilis'* present in the *L. lota* stomach contents.