

Research Article

Assessing differences in food web metrics in freshwater ecosystems after the invasion of Northern crayfish (*Faxonius virilis*)

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Abstract

Aquatic invasive species are among the greatest threats to freshwater biodiversity. Crayfish are especially robust freshwater invaders that can compete on various trophic levels simultaneously. The Northern Crayfish (*Faxonius virilis*) was introduced to the North Saskatchewan River basin circa 1990. Their impact on Alberta's native fish communities remains unknown. We sampled 10 North Saskatchewan River basin tributaries for *F. virilis* and six common native fishes. We used stable isotope analysis to investigate if there exists resource partitioning and/or competition between *F. virilis* and native fishes and whether *F. virilis* sympatry is related to differences in isotopic metrics/body condition of native fishes. Overlap (0.14–31.2%) of *F. virilis* and native species basin-wide isotopic niches indicated that *F. virilis* can potentially consume the same dietary resources as secondary consumer fishes. However, segregation of realized isotopic niches indicated no actual consumption of the same resources. Similarity in isotopic metrics/body condition of allopatric and sympatric native fish populations indicated that *F. virilis* sympatry did not have detectable negative trophic effects on native fishes. Thus, *F. virilis* may be using dietary plasticity to exploit a different trophic niche than native fishes, ergo, avoiding interspecific competition through resource partitioning.



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Key words: Aquatic invasive species, dietary plasticity, North Saskatchewan River, northern ecosystems, niche segregation, rivers, stable isotopes

Introduction

Invasive species are those that have established self-sustaining and expanding populations outside of their native range (Falk-Petersen et al. 2006). All invasive species have some effect on the native community that they are introduced to (Falk-Petersen et al. 2006) with a number of invasions resulting in negative ecological, social, and/or economic impacts (Mack et al. 2000). Invasive species can

negatively impact native species through resource competition (Ricciardi et al. 2013), with a multitude of examples recorded in the body of scientific literature (Hänfling et al. 2011). When dietary resources are limiting, the indirect effect of one species exploiting resources to the detriment of the other is a form of indirect competition, referred to as resource competition (Tilman 1982; Holomuzki et al. 2010). Trophic niches can be used to assess resource competition between native and invasive species, because the trophic niche space within the ecological niche (sensu Hutchinson 1957) is explicitly based on the dietary resources that are available and consumed by a species (Bearhop et al. 2004). Controlling for habitat-level interspecific interactions such as predation and competition by considering the trophic niche of a species over a broad geographic scale (e.g., at the basin-wide scale) approximates a component of the species' fundamental niche (Hutchinson 1957), representing its potential scope of dietary resource use (Baltensperger et al. 2015). Conversely, the realized trophic niche represents the actual dietary resource use of species in the presence of habitat-level interspecific interactions (Hutchinson 1957). When the realized trophic niches of two species overlap sufficiently, competition for dietary resources can result in either resource partitioning that causes a shift in trophic niche space allowing co-existence, or extirpation of the less competitive species, if resources are limiting (Tilman 1982).

Stable isotope ratios of carbon ($\delta^{13}\text{C}$) and nitrogen ($\delta^{15}\text{N}$) in tissues can be used to estimate trophic niches because these ratios have predictable relationships with diet (Bearhop et al. 2004; Fry 2006). Specifically, $\delta^{13}\text{C}$ concentration changes very little between food sources and consumers, usually enriching around 0–1‰ (Post 2002). Consequently, $\delta^{13}\text{C}$ reflects the diversity of food sources in a given species' diet (Post 2002). In contrast, $\delta^{15}\text{N}$ reflects the trophic position and shows enrichment of 2.2–3.4‰ from resource to consumer (Zanden and Rasmussen 2001; Post 2002). When represented in a biplot, these isotopic values represent the isotopic niche, which is tightly correlated with a species' trophic niche (Newsome et al. 2007; Jackson et al. 2011). Many studies have used stable isotopes to estimate species' dietary habits, location/size of trophic niches, and degree of overlap between species' trophic niches (e.g. Olsson et al. 2009; Jackson and Britton 2014; Baltensperger et al. 2015). Stable isotopes are useful in determining species' trophic positions and interactions under natural conditions (Botta et al. 2018) and exposure to stressors, such as climatic change (Baltensperger et al. 2015) and invasion events (Zambrano et al. 2010).

The negative impacts of invasive crayfish in aquatic ecosystems have been documented worldwide (Phillips et al. 2009b; Reynolds 2011). A particularly robust invader species of crayfish is the Northern Crayfish (*Faxonius virilis* [Hagen, 1870]), which has a history of negatively affecting macrophyte communities (e.g. Dorn and Wojdak 2004; Rodríguez et al. 2005; Edgar et al. 2025), changing invertebrate assemblages due to predation (e.g. (Hanson et al. 1990; Phillips et al. 2009a), and competing with native fishes (e.g. Savino and Miller 1991; Dorn and Mittelbach 2004; Carpenter 2005) and crayfishes when found outside of its native range (e.g. Loughman 2010; Rozansky et al. 2021). This invader was introduced to Alberta, Canada's North Saskatchewan River basin in the early 1990s and has since established populations in central and eastern tributaries of the basin (Williams 2012; Van Mierlo et al. 2022). The North Saskatchewan River has no native crayfish species (Williams 2012). *Faxonius virilis*, thus represents a novel invader in the basin and could have pronounced effects on the basin's native communities (Ricciardi and Atkinson 2004). While some studies have investigated the trophic ecology of invasive crayfish in North America, studies in river systems entirely lacking native crayfish species are scarce.

Here we used stable isotope analyses to determine whether *F. virilis* is (1) potentially competing for and/or partitioning dietary resources consumed by native fishes in tributaries of the North Saskatchewan River, and (2) associated with differences in the isotopic metrics (proxies for resource use) and/or body condition of native species due to sympatry. We hypothesized that omnivorous *F. virilis* consume similar dietary resources as native secondary consumer fish and that this would be demonstrated by overlap of the basin-wide and realized standard ellipse area (SEA) of *F. virilis* with native secondary consumer fish species (NSCFS). Crayfish have been shown to have wide isotopic trophic niches due to their omnivory which, when resources are limiting, 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, we also hypothesized reduction in the realized trophic niche space (estimated by SEA), carbon ranges, and body condition of NSCFS due to resource competition when sympatric with *F. virilis*.

Methods

Study area & tributary selection

The study area comprised ten tributaries (Strahler streams order of 4 to 6) of the North Saskatchewan River basin in Alberta, Western Canada (Fig. 1). Three reaches (WMD2, BMD2, and BMD3) are located in the highly developed and populated Edmonton Metro Area, 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. Of the latter group, COW1, BAP2, ROS2, and POP1 are tributaries that originate in the montane and foothills regions of the basin and feature colder mean summer water temperatures. In contrast, BEA1, SMO1, and VER4 are warmer water streams that originate in the prairies (Fig. 1). It is important to note that, to investigate potential competition for and/or partitioning of dietary resources between *F. virilis* and native species in this study, we assume that dietary resources are limited in all 10 reaches.

Crayfish sampling

We sampled *F. virilis* during the summer (June-August) of 2020 in each tributary along a 200–300 m reach using modified minnow traps with 5.7 cm diameter openings (Mangan et al. 2009; De Palma-Dow et al. 2020). Individual traps were tied to nylon rope 3 m apart in sets of six to form a trapline (Rosewarne et al. 2014). Individual traps in each trapline were baited using salmon-based cat food-filled perforated film canisters (one canister Purina Friskies® per trap) (Mangan et al. 2009). Each trapline was then affixed to the riverbank by a loop and rebar stake on the upstream end. A total of four traplines were deployed at each reach for a total of 24 traps per reach. 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. All captured crayfish were enumerated and humanely euthanized using a 15-minute ice bath and pithing in accordance with animal handling and ethics regulations (Animal Use Protocol No.: AUP00003578). Whole specimens were frozen at -20 °C prior to sample processing and analysis. *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) (Suppl. material 1: table S1).

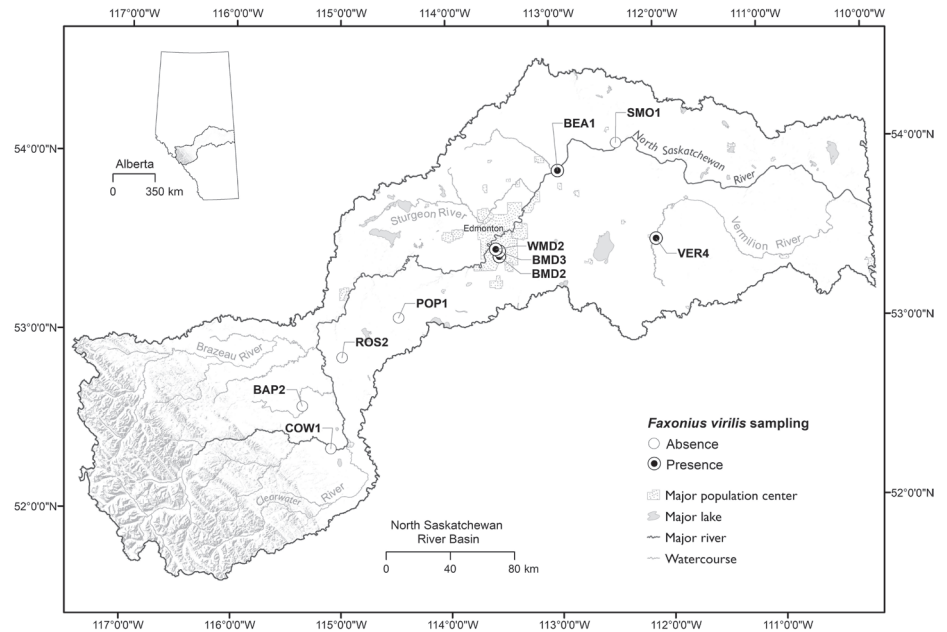


Figure 1. Locations of ten study reaches in the North Saskatchewan River basin. Reaches where *F. virilis* are absent are represented by empty white circles. Reaches occupied by *F. virilis* are represented by circles filled with a black solid circle. Unique reach identification codes are located near each reach's location marker.

Fish and benthic macroinvertebrate sampling

Fish and benthic invertebrates were sampled within the same week as crayfish were sampled in each tributary to minimize effects of any changes in flow conditions over time. Each 300 m reach was subdivided into six 50 m transects within which fish were sampled via backpack electrofishing in a sweeping systematic pattern. All fish captured were identified to species, fork length measured, and released back into the river from which they were caught, except for 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]). Target species were selected due to their ubiquity in the basin so that direct species comparisons could be made across all sampled sites. 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). Whole specimens were frozen at $-20\text{ }^{\circ}\text{C}$ prior to sample processing and analysis. All fish sampling and euthanasia was conducted in accordance with the 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 triangular kick net (400 μm mesh) in a single zig-zag pattern, sweeping over erosional zones (riffles and runs) a minimum of 3 m of where traplines were set and standardized to three-minute sampling effort. Collected material was placed into enamel pans where invertebrates were separated from other material using forceps

and wash water bottles. All samples were preserved in 70% ethanol (Hobson et al. 1997). 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).

Laboratory specimen & sample processing

During dissections, a sample of dorsal muscle tissue was collected from each fish specimen. Also recorded were specimen wet weight (g), fork length (mm), total length (mm) and sex (m/f/juv) based on presence of female or male gonads. Additionally, the stomach contents of *L. lota* (a larger predator species) were inspected for evidence of *F. virilis* consumption. A sample of abdominal (tail) muscle tissue was collected from each of the 52 crayfish specimens. Weight (g), carapace length (mm), total length (mm), and sex (m/f) were also recorded.

Prior to stable isotope analysis, all fish and crayfish tissue samples were preserved at -20 °C, while all benthic macroinvertebrate samples were preserved whole, in 70% ethanol. Fish dorsal muscle and crayfish abdominal muscle tissue samples were freeze dried at -55 °C and 0.015 Bar for 24 hours in a LABCONO® Free-Zone 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 homogenous powder and weighed into a 6X8 mm tin capsule to 0.4000–0.4999 mg using the UMX2 Ultra-microbalance (Mettler Toledo 2004). All samples were analyzed for $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ ratios at the Natural Resources Analytical Lab in Alberta, Canada using ThermoScientific DeltaV Advantage isotope ratio mass spectrometer (IRMS) coupled to the ThermoScientific FlashSmart Organic Elemental Analyzer and ConFloIV. All values are reported in delta notation (‰) relative to international standards: Pee Dee Belemnite (vPDB) for $\delta^{13}\text{C}$ and atmospheric N_2 , Vienna Air (VAIR) for $\delta^{15}\text{N}$. Calibration was performed using seven-point normalization which includes six certified reference material and two international reference standard material LSVEC and IAEA-N2 with known values of $\delta^{13}\text{C} = -46.6\text{‰}$ and $\delta^{15}\text{N} = 20.3\text{‰}$, respectively. In-house pea grain standard with $\delta^{13}\text{C} = 8.94\text{‰}$ and $\delta^{15}\text{N} = 3.234\text{‰}$ were analyzed every 12 samples to monitor drift and ensure accuracy. Accuracy and precision are in relative standard deviation and were better than $\pm 0.20\text{‰}$ $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$. Accuracy was within error for secondary reference material. Blanks and empty capsules were included at the beginning of each run cycle to monitor for contamination.

Data preparation

Prior to analysis, raw isotope data were inspected for carbonate contamination using multiple linear regression analysis to compare $\delta^{13}\text{C}$ and percent carbon of samples of each reach (Jardine et al. 2003). Next, we investigated carbon depletion caused by lipid richness. Samples with C:N ratios > 4 were considered lipid rich and in need of correction (Post et al. 2007). We detected no lipid richness. Further, because ethanol fixation is known to have a measurable effect on stable isotope carbon values (e.g. Ventura and Jeppesen 2009; Hogsden and McHugh 2017; Blechinger et al. 2024) we corrected for the effect of preservation in 70% ethanol on our benthic invertebrate $\delta^{13}\text{C}$ signatures by applying the

mass balance approach as described by Ventura and Jeppesen, (2009) for sample sets where the original C:N is unknown. Specifically, we calculated each corrected $\delta^{13}\text{C}$ value using the equation:

$$\delta^{13}\text{C}_{\text{EtOHCorr}} = \delta^{13}\text{C}_{\text{EtOHfixed}} - [D ((\text{C:N}_{\text{EtOHmean}} - \text{C:N}_{\text{EtOHfixed}}) / \text{C:N}_{\text{EtOHmean}})]$$

Where $\delta^{13}\text{C}_{\text{EtOHfixed}}$ is the original ethanol contaminated $\delta^{13}\text{C}$ signature of the given sample, D is equal to 4.65 – the difference coefficient for ethanol preserved freshwater benthic invertebrate samples that was determined by Ventura and Jeppesen (2009), $\text{C:N}_{\text{EtOHfixed}}$ is the C:N ratio of the given sample, $\text{C:N}_{\text{EtOHmean}}$ is the mean C:N value of all samples, and $\delta^{13}\text{C}_{\text{EtOHCorr}}$ is the final $\delta^{13}\text{C}$ signature of the given sample after ethanol preservation correction. No ethanol preservation correction was applied to $\delta^{15}\text{N}$ values as the balance of literature indicates this effect is negligible (e.g. Hobson et al. 1997; Hogsden and McHugh 2017; Hajisafarali et al. 2023). Finally, *C. commersonii* and *C. catostomus* samples were combined into a single group called *Catostomus* spp. because the sample size of *C. catostomus* ($n = 6$ over all sampled reaches) was insufficient for subsequent stable isotope analysis. This combination was based on the two species sharing a close taxonomic lineage (same genus) and similar life history, morphology, and diet (Scott and 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. We used primary consumer benthic invertebrates for baseline source estimates rather than long-lived primary consumers such as clams and snails because these long-lived organisms were unavailable. Benthic invertebrates have been demonstrated as reasonable indicators of baseline $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values in previous studies (e.g. Anderson and Cabana 2007; Jackson and Britton 2014). Baseline $\delta^{15}\text{N}$ values were calculated following the suggestions of Anderson and Cabana (2007) to convert raw $\delta^{15}\text{N}$ and $\delta^{13}\text{C}$ values into trophic position (TP) and corrected $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{Corr}}$), respectively. The family Elmidae was the most nitrogen depleted benthic invertebrate family and present in 60% of sampled reaches. Where Elmidae did not occur, the next collector family with the lowest $\delta^{15}\text{N}$ value was selected and corrected to Elmidae (Anderson and Cabana 2007). We used $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values from the most nitrogen depleted benthic invertebrate families within each reach to calculate the mean $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values, pooled across all reaches. The pooled $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ baseline values were then used to calculate TP and $\delta^{13}\text{C}_{\text{Corr}}$.

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

Trophic position was calculated for each consumer's muscle tissue sample using the pooled baseline $\delta^{15}\text{N}$ value and individual raw $\delta^{15}\text{N}$ values by substituting them into the single source trophic position model described by Post (2002):

$$\text{TP}_{\text{con}} = \lambda + (\delta^{15}\text{N}_{\text{con}} - \delta^{15}\text{N}_{\text{base}}) / \Delta_n$$

Where TP_{con} is the trophic position of the consumer, λ is the trophic position of baseline organisms ($\lambda = 2$ for herbivorous benthic invertebrates), $\delta^{15}\text{N}_{\text{con}}$ is the isotopic nitrogen value of the consumer, $\delta^{15}\text{N}_{\text{base}}$ is the calculated pooled baseline $\delta^{15}\text{N}$ value, and Δ_n is the trophic enrichment factor (TEF) equal to $3.4 \pm 1\%$ which is the applicable

fractionation value first determined by Post (2002). 3.4‰ is used as the fixed nitrogen TEF for studies conducted on wild populations (Dionne et al. 2016), where trophic TEFs specific to species and diet (McCutchan et al. 2003) are not available.

We corrected $\delta^{13}\text{C}$ consumer values based on the pooled $\delta^{13}\text{C}$ benthic invertebrate baseline value as was used for calculation of trophic position using the equation described by (Olsson et al. 2009).

$$\delta^{13}\text{C}_{\text{corr}} = (\delta^{13}\text{C}_{\text{con}} - \delta^{13}\text{C}\mu_{\text{baseline}}) / \text{CR}_{\text{baseline}}$$

Where $\delta^{13}\text{C}_{\text{corr}}$ is the basal isotopic corrected $\delta^{13}\text{C}$ value of the consumer, $\delta^{13}\text{C}_{\text{con}}$ is the raw $\delta^{13}\text{C}$ value of the consumer, $\delta^{13}\text{C}\mu_{\text{baseline}}$ is the pooled $\delta^{13}\text{C}$ value, and $\text{CR}_{\text{baseline}}$ is the range of source $\delta^{13}\text{C}$ values across all reaches. Trophic position and corrected $\delta^{13}\text{C}$ values were used in all subsequent statistical analyses. Summary statistics of trophic position and $\delta^{13}\text{C}_{\text{corr}}$ are provided by species (Table 1) and by reach (Suppl. material 1: table S1).

Basin-wide and realized niche interactions

To determine the size and position of each species' basin-wide and realized trophic niches, 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 and Parnell 2020 *Package "SIBER"*). 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 basin-wide trophic niche of each species, we pooled samples by species across all reaches before plotting and calculating the niche width of each species. 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 evened (Baltensperger et al. 2015). Therefore, the standard ellipse area of conspecific individuals that are pooled over all study reaches is representative of that species' basin-wide niche width.

In contrast, when the species' standard ellipse areas of individual reaches are plotted separately, they represent the realized niche of the species present in that reach. Here, we assume that all individuals within a reach are subjected to similar interspecific interactions and resource availability. Therefore, we plotted the standard ellipse areas within each reach to inspect the realized niche widths of each species. Niche widths were reported in units of ‰² area.

To detect if dietary resources were being consumed by both native fish and *F. virilis*, the basin-wide 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. 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 unit-less 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 an overlap of 0% indicating completely unique ellipses and an overlap of 100% indicating complete overlap.

Table 1. Means and standard deviations of raw $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as well as means and standard deviations for baseline corrected $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{corr}}$) and baseline calculated trophic position (TP) for each species. The number of individuals of each species over all reaches (n) is provided. Mean, standard deviation, maximum and minimum body lengths are provided as carapace length (mm) for *F. virilis*, total length (mm) for *L. Lota*, and fork length (mm) for all other fish species.

Species	n	Body Length		Muscle $\delta^{13}\text{C}$ (‰)		Muscle $\delta^{15}\text{N}$ (‰)		Muscle $\delta^{13}\text{C}_{\text{corr}}$ (‰)		Trophic Position (TP)	
		Mean \pm St. Dev	Max, Min	Mean	St. Dev	Mean	St. Dev	Mean	St. Dev	Mean	St. Dev
<i>Faxonius virilis</i>	52	31 \pm 7	43, 7	-28.01	0.78	10.40	0.56	0.51	0.13	3.93	0.17
<i>Catostomus</i> spp.	35	93 \pm 48	226, 31	-31.54	1.78	10.38	1.45	-0.06	0.29	3.92	0.43
<i>Couesius plumbeus</i>	38	78 \pm 16	125, 36	-27.78	0.77	11.39	1.60	0.55	0.13	4.22	0.47
<i>Lota lota</i>	11	123 \pm 67	317, 86	-28.62	1.21	10.93	0.79	0.41	0.20	4.08	0.23
<i>Percopsis omiscomaycus</i>	3	65 \pm 5	69, 60	-31.18	0.63	8.41	0.27	-0.01	0.10	3.34	0.08
<i>Rhinichthys cataractae</i>	67	62 \pm 10	85, 46	-30.33	1.76	9.18	3.39	0.12	0.28	3.57	1.00

Impacts of *F. virilis* sympatry on NSCFs

The following analyses were conducted on three native secondary consumer fish species (NSCFs) (*R. cataractae*, *C. plumbeus*, and *Catostomus* spp.) only. *Percopsis 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 in each reach, we calculated the Bayesian estimate of realized standard ellipse area (SEA_B) using the R *SIBER* Package. We used a null prior distribution 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. We 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).

In addition to differences in SEA_B , the $\delta^{13}\text{C}_{\text{corr}}$ carbon range of NSCFs were calculated and compared between *F. virilis* sympatric populations and allopatric populations to determine if the richness of consumed dietary resources was reduced (narrowed carbon range) when sympatric with *F. virilis*. Carbon range was calculated as the difference between the greatest individual $\delta^{13}\text{C}_{\text{corr}}$ value and smallest $\delta^{13}\text{C}_{\text{corr}}$ value and was expressed in units of $\Delta\text{‰}$ (Layman et al. 2007).

To determine if *F. virilis* sympatry and/or trophic niche overlap may be related to reduced body condition of native secondary consumer fish, the relative weight (W_r) fish condition metric was calculated as described by Wege and Anderson (1978). W_r was calculated using the R *FSA* Package (Ogle 2019, Package “FSAdata.”), 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 an 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

underweight or overweight individual for its size and species, respectively (Wege and Anderson 1978; Ogle 2018). Mean relative weight and standard deviation of each NSCFS in *F. virilis* occupied and unoccupied reaches were calculated and a two-sided t-test was used to determine if there were significant differences between the mean relative weight of each species dependent on the presence of *F. virilis*. The two-sided t-test is sufficiently accurate to determine significant difference in means with sample sizes $n \geq 5$ (de Winter 2013).

Results

A total of 52 crayfish were captured from five of ten reaches (Fig. 1) (mean = 10 ± 3 individuals per reach). Among the five *F. virilis* occupied reaches, maximum and minimum relative abundances were 9.50 indv/trapline at reach WMD2 and 3.25 indv/trapline at reach BEA1, respectively (Suppl. material 1: table S1). Captured *F. virilis* were in even sex ratios with 26 males and 26 females caught over all reaches and maximum and minimum % female catch within individual reaches being 64% and 27%, respectively. Mean *F. virilis* carapace length was 31 mm over all reaches with 83% of captured *F. virilis* being sexually mature adults with carapace lengths ≥ 25 mm (Weagle and Ozburn 1972) (Table 1) (Suppl. material 1: table S2). 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 1). Mean *L. lota* total length was 123 mm and mean fork length was 93, 78, 65, and 62 mm for *Catostomus* spp., *C. plumbeus*, *P. omiscomaycus*, and *R. cataractae*, respectively. The majority (71%) of all sampled fish were sexually mature adults (Suppl. material 1: table S2). Sex ratios of fishes varied considerably among sites and species (Suppl. material 1: table S2). Dissection and stomach content analysis of *L. lota* revealed that the two largest individuals (total length = 317.00 mm & 145.00 mm) found in sympatry with *F. virilis* had evidence of *F. virilis* consumption, with individual's stomachs containing one and six juvenile *F. virilis*, respectively (Suppl. material 1: fig. S1).

After baseline correction, mean $\delta^{13}\text{C}_{\text{corr}}$ values ranged from -0.06 to 0.55‰ in fish and was 0.51‰ in *F. virilis*. Trophic position ranged from 3.34 to 4.22 in fish with *P. omiscomaycus* having the lowest mean trophic position and *C. plumbeus* having the highest mean trophic position of fish species (Table 1). *F. virilis* had the third highest mean trophic position overall (mean TP = 3.93) (Table 1).

Basin-wide niche interactions

Percopsis omiscomaycus possessed the smallest basin-wide niche (SEA_c) width of all fish species with an area of 0.051‰^2 (Table 2). The largest basin-wide niche width of all fish species belonged to *R. cataractae* with an area of 0.610‰^2 (Table 2). The basin-wide niche width of *F. virilis* was found to be 0.067‰^2 , having the second smallest basin-wide niche of all sampled species (Table 2). *Faxonius virilis*' basin-wide niche occupied a moderate trophic position and showed a $\delta^{13}\text{C}_{\text{corr}}$ component nearly triple that of the TP component, which was reflective of the species omnivory (Fig. 2). The basin-wide niche of *F. virilis* overlapped with three out of five native fish species: *L. lota*, *C. plumbeus*, and *R. cataractae* (Fig. 2). Two of these were moderate overlaps with the basin-wide niche of *L. lota* overlapping 31.2% with that of *F. virilis* and the basin-wide niche of *C. plumbeus* overlapping 23.8% with that of *F. virilis* (Fig. 2, Table 2). The basin-wide niche of *R. cataractae* was nearly independent of that of *F. virilis* with an overlap value of 0.14% (Fig. 2, Table 2).

Table 2. Core isotopic niche widths, defined as the small sample size corrected standard ellipse area (SEA_c) of each species within in each reach (realized niche width), among reaches (basin-wide 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 basin-wide niche widths (SEA_c) 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. Percent overlap of “0%” is provided where there was a possibility of the species’ niche overlapping with that of *F. virilis*, but no overlap occurred and “–” is provided where there was no possibility of niche overlap.

Reach	Species	<i>n</i>	SEA_c (‰ ²)	% Overlap with <i>F. virilis</i>
Realized niche width				
BEA1 (p)	<i>Faxonius virilis</i>	14	0.098	–
	<i>Catostomus</i> spp.	3	0.003	0%
BMD2 (p)	<i>Faxonius virilis</i>	10	0.020	–
BMD3 (p)	<i>Couesius plumbeus</i>	26	0.018	0%
	<i>Catostomus</i> spp.	3	0.019	0%
	<i>Faxonius virilis</i>	6	0.005	–
VER4 (p)	<i>Faxonius virilis</i>	10	0.017	–
	<i>Catostomus</i> spp.	3	0.021	0%
WMD2 (p)	<i>Lota lota</i>	4	0.099	2.57%
	<i>Couesius plumbeus</i>	4	0.018	0%
	<i>Rhinichthys cataractae</i>	5	0.121	0%
	<i>Faxonius virilis</i>	10	0.024	–
COW1 (a)	<i>Couesius plumbeus</i>	6	0.017	–
	<i>Catostomus</i> spp.	9	0.044	–
POP1 (a)	<i>Catostomus</i> spp.	5	0.189	–
ROS2 (a)	<i>Rhinichthys cataractae</i>	26	0.036	–
SMO1(a)	<i>Lota lota</i>	7	0.034	–
	<i>Rhinichthys cataractae</i>	11	0.085	–
	<i>Catostomus</i> spp.	10	0.105	–
BAP2 (a)	<i>Rhinichthys cataractae</i>	23	0.041	–
	<i>Percopsis omiscomaycus</i>	3	0.051	–
Basin-wide niche width				
All	<i>Percopsis omiscomaycus</i>	3	0.051	0%
	<i>Catostomus</i> spp.	35	0.393	0%
	<i>Lota lota</i>	11	0.134	31.2%
	<i>Couesius plumbeus</i>	38	0.103	23.8%
	<i>Rhinichthys cataractae</i>	67	0.610	0.14%
	<i>Faxonius virilis</i>	50	0.067	–

Realized niche interactions

Core realized niches (SEA_c) were plotted for each species in the five reaches where crayfish were present. Realized niches were mostly segregated in isotopic space (Fig. 3, Table 2). Out of a total of seven potential overlap events with native fish species, the realized niches of *F. virilis* overlapped with those of native fish only once (Fig. 3E). This overlap occurred in reach WMD2 with a minor overlap of 2.57% with *L. lota* (Table 2).

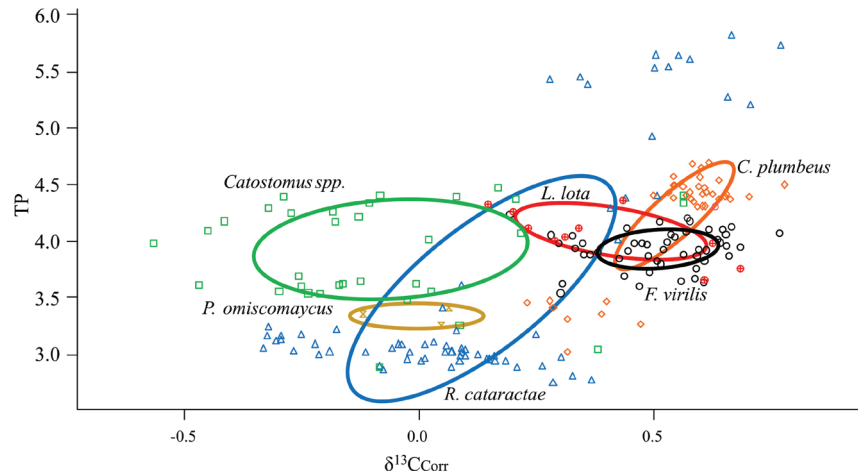


Figure 2. Corrected isotopic carbon ($\delta^{13}\text{C}_{\text{Corr}}$) and trophic position (TP) biplots showing each species' core basin-wide isotopic niche width. Isotopic niche widths are expressed in $\%o^2$ and were calculated using small sample size corrected standard ellipse area (SEAc) which 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*. Plotting of core isotopic niches were done using the *SIBER* R package.

Impacts of *F. virilis* sympatry on NSCFS

The mean Bayesian estimated core realized niche width area (SEA_B) of *C. plumbeus* and *R. cataractae* in *F. virilis* sympatric reaches were statistically similar (95% probability intervals overlapping) to those of their conspecifics in *F. virilis* allopatric reaches (Fig. 4A, B). However, the realized niche of *Catostomus* spp that were in sympatry with *F. virilis* were significantly smaller in two reaches (reaches BEA1 & VER4) compared with conspecifics found in two *F. virilis* absent reaches (POP1 & SMO1) (Fig. 4C). Additionally, all three mean SEA_B estimates of *Catostomus* spp were lowered in *F. virilis* present reaches compared to those where *F. virilis* was absent.

For all three NSCFS, carbon ranges were neither consistently broader nor narrower in *F. virilis* occupied and absent reaches, indicating no detectable effect of *F. virilis* on the richness of diet consumed by NSCFS (Fig. 5).

Finally, the mean relative weights of *C. plumbeus*, *R. cataractae*, and *Catostomus* spp. were $79.56 \pm 5.11\%$, $79.23 \pm 9.66\%$, and $80.59 \pm 7.35\%$, respectively. There was also no significant difference in mean relative weight between NSCFS sympatric and allopatric populations 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) (Fig. 6).

Discussion

Our study aimed to investigate the potential trophic overlap and impacts of invasive *F. virilis* on the native fish community of the North Saskatchewan river basin, a system that possesses no native crayfishes. In contrast with our hypotheses, our results suggest no negative effect of *F. virilis* on the isotopic metrics or body condition of common native fishes in the basin. We contend that *F. virilis* may be using dietary plasticity to avoid competition for dietary resources with these fishes, although we note that the invasion time period is relatively new and not all fish species were included in the analyses.

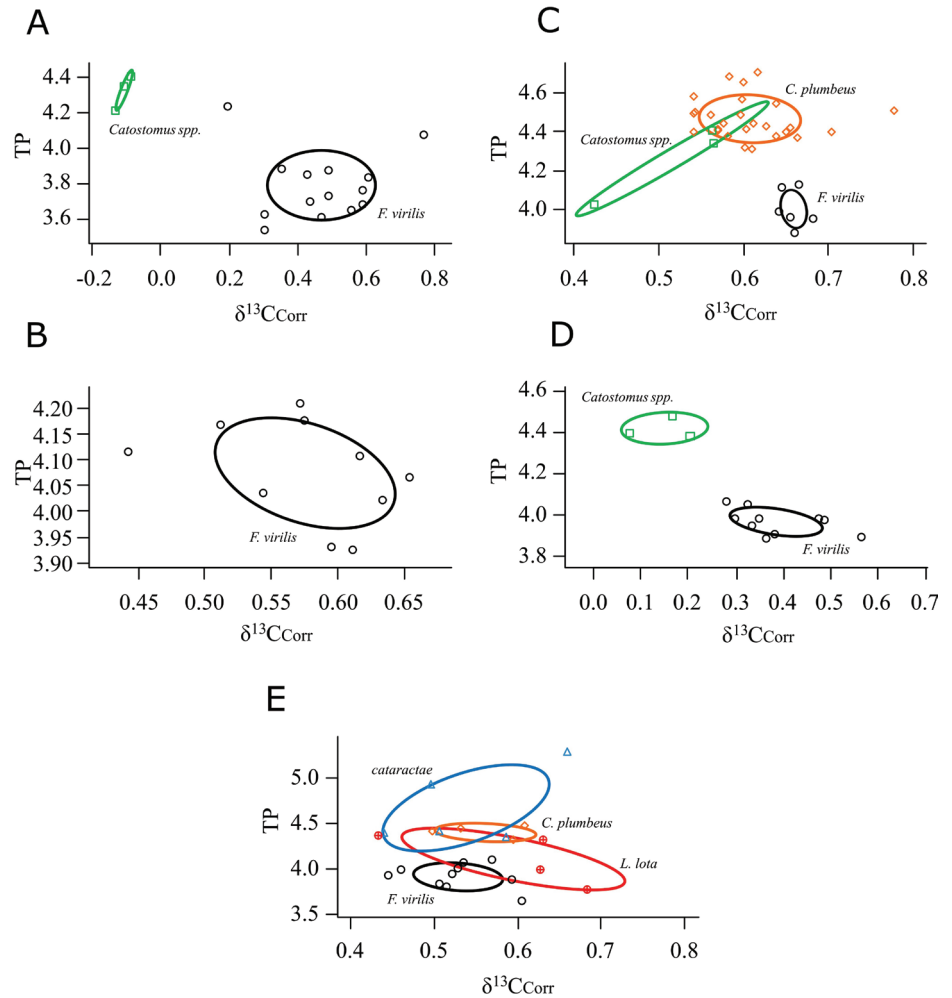


Figure 3. Corrected isotopic carbon ($\delta^{13}\text{C}_{\text{Corr}}$) and trophic position (TP) biplots showing the core realized isotopic niche width of each species within each reach where northern crayfish were found to be present. Panel letters indicate the specific reach as follows: (A) BEA1, (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.. Plotting of core isotopic niches was done using the *SIBER* R package.

Basin-wide and realized niche interactions

Overlap of basin-wide niches between *F. virilis* and three native fishes suggested that *F. virilis* have the potential to consume the same dietary resources as native fish species. Overlap at the basin scale seen between *F. virilis* and *C. plumbeus* and *R. cataractae* is consistent with diet studies showing that these fish species, like crayfish, are known benthic feeders who readily consume benthic macroinvertebrates, macrophytes, and/or benthic detritus (Scott and Crossman 1973; Brazo et al. 1978). Additionally, the overlap between *L. lota* and *F. virilis* is reflective of the carnivorous diets of these species. *Lota lota* are known carnivorous fish which feed on benthic invertebrates such as mayfly nymphs and crayfish when small (51- 305 mm) (Scott and Crossman 1973). The overlap of the basin-wide niche of *F. virilis* with the lower TP portion of that of *L. lota* suggests that *F. virilis* are consuming similar benthic macroinvertebrates as small *L. lota* in the system – further reflecting *F. virilis*' omnivory.

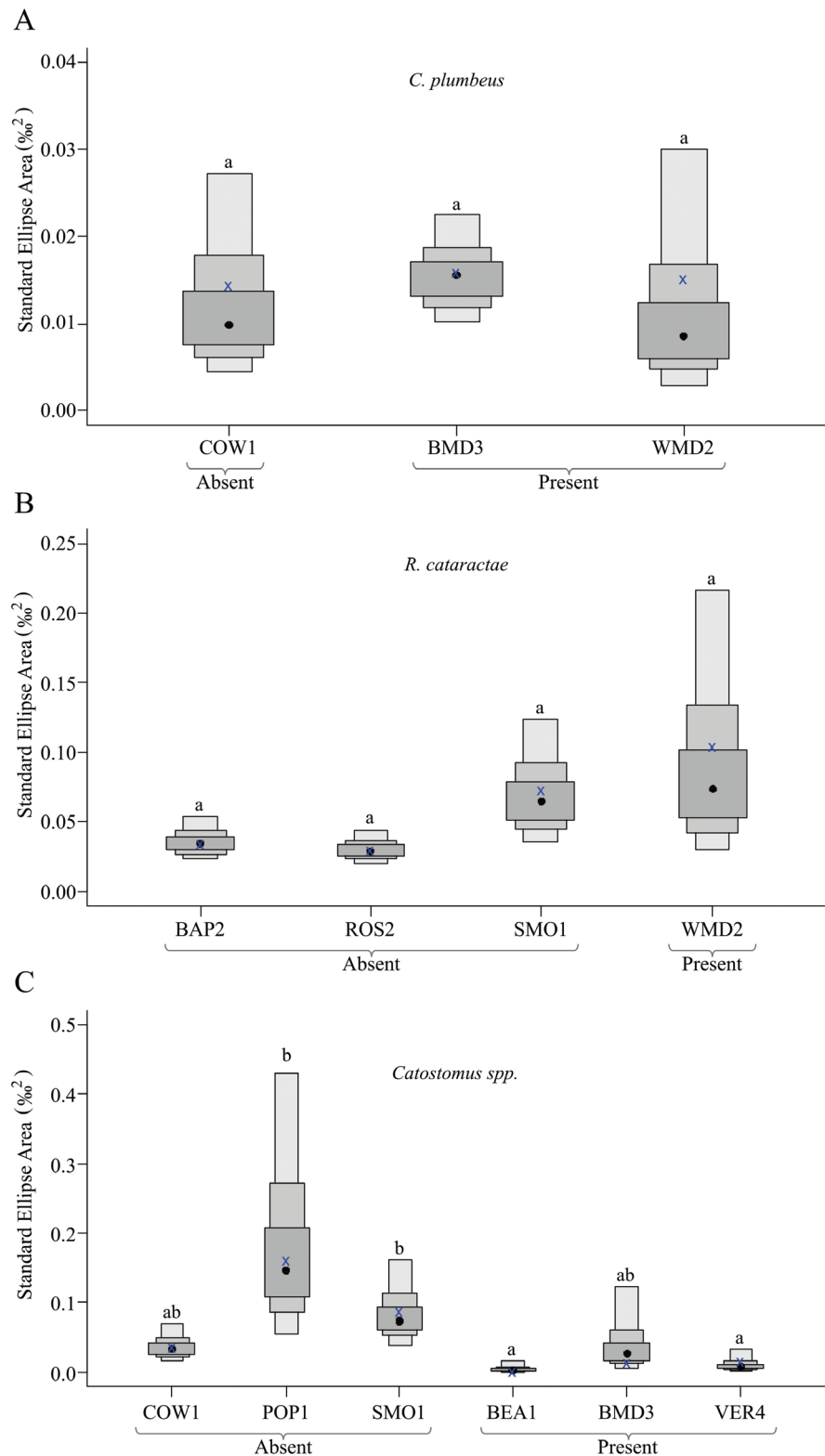


Figure 4. Density plots of realized isotopic niche widths (SEA_B %²) of the three secondary consumer fish species ((**A**) *C. plumbeus* [$n = 35$], (**B**) *R. cataractae* [$n = 65$], and (**C**) *Catostomus* spp. [$n = 33$]) compared where *F. virilis* are present vs. absent. Black dots represent the 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.

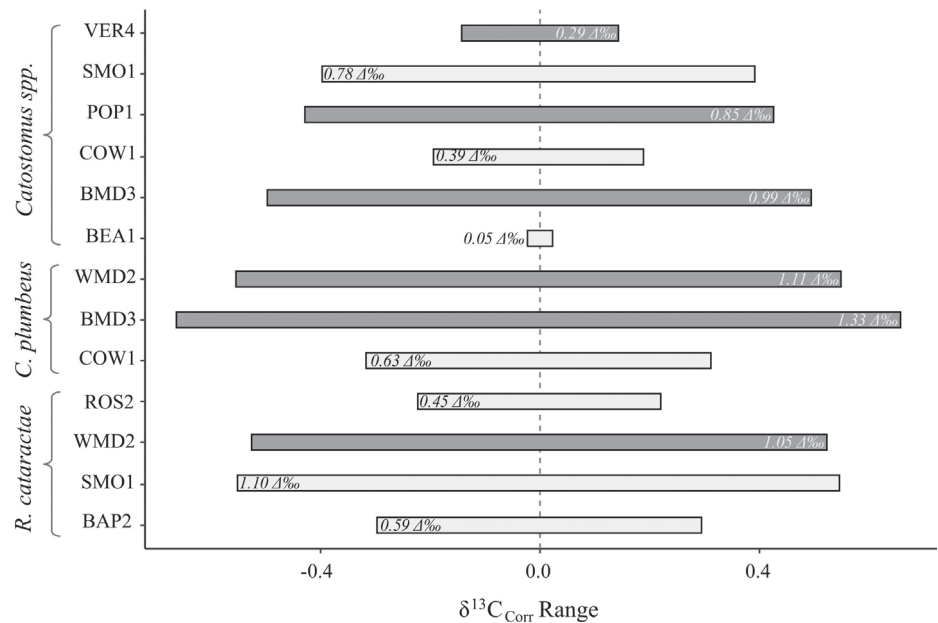


Figure 5. Range plot comparing reach specific $\delta^{13}\text{C}_{\text{Corr}}$ ranges ($\Delta\text{‰}$) which are reflective of dietary source richness of the three secondary consumer fish species (*R. cataractae*, *C. plumbeus*, and *Catostomus* spp.) compared between where *F. virilis* are present (grey bars) vs. absent (white bars). Unique reach codes appear on the y-axis for each respective bar.

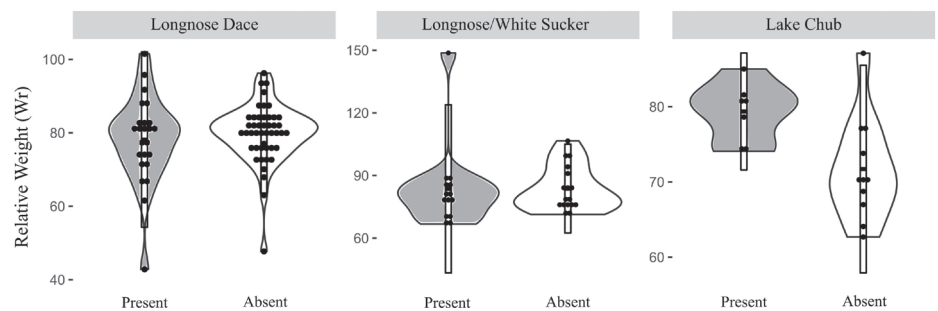


Figure 6. Violin plots comparing mean body condition (as described by the relative weight condition metric [Wr] and reported in %) of *R. cataractae* ($n = 43$), *Catostomus* spp. ($n = 9$), and *C. plumbeus* ($n = 13$) over all reaches in which crayfish are present (grey) against all reaches where crayfish are absent (white). Significant difference between means is represented by an asterisk (*).

Realized niche interactions

In contrast with the basin-wide niche analyses and our original hypothesis, within individual reaches, the realized niche of *F. virilis* was largely segregated from those of native fishes. Lack of overlap of realized niches suggested that while crayfish do consume the same resources as native fish at the basin scale, *F. virilis* and native fishes are not consuming a significant amount of the same dietary resources when in sympatry. This lack of overlap suggests that *F. virilis* is not competing for dietary resources with our study's native fish species. A possible explanation for this unexpected lack of realized trophic overlap is dietary plasticity. Specifically, *F. virilis* may be using plasticity in food resource selection to avoid competition for dietary resources with sympatric native fishes, such as *C. plumbeus* and *R. cataractae*. Omnivory and the ability of crayfish to exercise dietary plasticity are well documented in the literature (e.g. Momot 1995; Veselý et al. 2020). While *F. virilis* show a preference for consuming animal tissues (Momot 1995), they are able to successfully subsist on less preferred dietary resources by readily consuming macrophytes (Momot 1995), macroinvertebrates (Hanson et

al. 1990), and detritus. The ability of *Faxonius* genus crayfish, specifically *Faxonius rusticus* and *Faxonius limosus*, to be highly plastic in their diet has been seen in response to environmental stimuli such as seasonal changes in dietary resource availability and over-invasions – when a formerly successful invader is supplanted by a new one (Tran and Manning 2019; Linzmaier et al. 2020). During times of high animal tissue abundance crayfish preferentially consumed animal tissue whereas crayfish increased their consumption of diatoms and detritus significantly during times of low animal tissue abundance (Tran and Manning 2019). Veselý et al. found similar patterns of dietary plasticity through time and among populations with different dietary resources (Veselý et al. 2020). The use of dietary plasticity to avoid trophic overlaps with native species is also shown in studies of crayfish invasion. In mesocosm experiments, dietary plasticity facilitated niche differentiation between native and non-native crayfishes (Jackson et al. 2014). Further, invasive crayfish seem to use dietary plasticity to occupy a different trophic niche than sympatric invasive fish species (Jackson and Britton 2014).

Impacts of *F. virilis* sympatry on NSCFs

Our trophic metric and body condition results are consistent with the segregation of *F. virilis*' realized trophic niche from those of native fishes. In all but two cases, the niche widths (SEA_B) and body condition of sympatric NSCFs populations were statistically similar to conspecific allopatric populations. The two cases where niche widths were significantly reduced in the presence of *F. virilis* were seen in *Catostomus* spp. which had no overlap with either the basin-wide or realized niches of *F. virilis*. Therefore, we conclude that the observed reduction in niche widths is likely not associated with *F. virilis* sympatry. Additionally, the overall similarity of realized SEA_B niche widths, carbon ranges, and body condition of *F. virilis* sympatric and allopatric native fish populations indicates that *F. virilis* have had no detectable detrimental trophic effects on sympatric NSCFs. This finding is consistent with the results of our first objective that indicated *F. virilis* and native fishes are consuming different dietary resources and not participating in resource competition when in sympatry.

Implications

The ability of *F. virilis* to use 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. Our study indicates that *F. virilis* are currently not competing with common native fishes *R. cataractae*, *C. plumbeus*, or *Catostomus* spp.. However, these species are generalist and generally robust (Scott and Crossman 1973). Currently unoccupied tributaries with 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 negative effects on sensitive species by way of indirect and/or direct competition, predation, habitat modification, etc. Further, of all Canadian provinces, excluding the Maritimes and Territories, Alberta has the lowest native fish richness (Scott and Crossman 1973). This is due to dispersion barriers for routes from glacial refugia after the last (Late Wisconsinian) glaciation (Nelson and Paetz 1992). As biotic resistance to invasion is positively correlated with biodiversity (Elton 2020), species-poor North Saskatchewan River 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 positive in all cases (Levine and 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 North Saskatchewan River basin and other Alberta watersheds.

Limitations

Our study has two potential limitations. First, in order to evaluate potential competition between *F. virilis* and native fishes, we assume that dietary resources are limited in all sampled reaches. However, we did not explicitly quantify resource availability. As limited resources are a requirement for competition to occur, it is possible that, rather than using dietary plasticity, *F. virilis* may be co-occurring without competition due to ample resources in the sampled reaches. However, we contend that if the latter was the case, we would expect there to be more instances and greater percentages of overlap between realized niches of *F. virilis* and native fishes. As our results stand, we feel that the most plausible explanation is that *F. virilis* is using dietary plasticity to exploit a different trophic niche than native fishes. Second, we used ethanol to preserve benthic invertebrate samples prior to stable isotope analysis and baseline calculation. While this was done to preserve samples collected in the more remote western sites where freezing was not a feasible preservation method and applied the mass balance approach to correct for the effect of fixation on carbon signatures, we recognize that this could still have introduced some error into our results. However, comparison of our results with and without the mass balance correction indicate that the patterns gleaned were consistent and likely are robust to ethanol preservation-induced error.

Future directions

Our study evaluated the trophic effects of *F. virilis* on three common and generally robust native species. While we found little evidence that *F. virilis* is competing with these native fishes for dietary resources, our study does not exclude the possibility that *F. virilis* may be competing for resources with other North Saskatchewan River 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 we studied. For example, crayfish have been shown to compete with benthic carnivorous fish species for spatial resources (Reynolds 2011). Crayfish have been shown to force juvenile *L. lota* to leave preferred shelter habitats which can make juveniles more vulnerable to predators (Hirsch and Fischer 2008), while sculpins are displaced from shelters and spend increased time fleeing in the presence of crayfish which resulted in reduced growth rates and lowered body condition (Light 2005).

Our 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 and Taylor 2013) as well as the eggs and fry of threatened fish species (Fitzsimons et al. 2002). Considering predation in the other direction, our stomach content analysis revealed that *F. virilis* are being preyed upon and consumed by at least one piscivorous fish species in the North Saskatchewan River basin: *L. lota*. *Lota lota* has been documented to prey upon crayfish as a natural prey item in their native range (Jacobs et al. 2010).

The impacts of *F. virilis* in the North Saskatchewan River basin may not be limited to fishes. *F. virilis* have been known to change the species assemblages of benthic macroinvertebrates drastically and decimate native snail and clam

biomass (e.g. Hanson et al. 1990; Rodríguez et al. 2005). Future studies would do well to investigate *F. virilis*' behavioral interactions with juvenile piscivorous fish; if North Saskatchewan River 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.

Lastly, while our study investigates the potential competition for trophic resources between NSCFS and invasive *F. virilis*, we acknowledge that physical and physiological resources such as habitat and preferred temperature can be limiting factors for crayfishes and could impact the interpretation of our results. All five of our sites where *F. virilis* is present possess a similar, moderate degree of habitat complexity and are centrally located within the North Saskatchewan River basin and therefore have similar summer water temperature regimes (Van Mierlo et al. 2022). Therefore, we contend that the sites that we evaluated are likely similar enough in habitat and preferred temperature to have a negligible influence on the patterns seen in this study. However, investigation of how limited physical or physiological resources interact with and/or affect the potential competition for trophic resources between NSCFS and *F. virilis* is an interesting area of study that should be pursued in the future.

In conclusion, overlap of *F. virilis*' basin-wide 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 realized niches showed a lack of resource competition within communities of the North Saskatchewan River basin. Our results suggest that rather than participate in resource competition, *F. virilis* may be using dietary plasticity to exploit a slightly different trophic niche than those occupied by native fishes and in doing so, avoid competition for dietary resources through resource partitioning. 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.

Author Contributions

VVM, SJG, CAE, CB, FRW, RDV, and MSP conceptualized the study. CB, FRW, CAE, MSP, RDV, and SJG were responsible for funding acquisition. VVM, SJG, CAE, RDV, and MSP developed investigation (field sampling) and methodology (statistical analyses). VVM and BRS led field data collection. VVM led data curation, formal analysis, implementation of R code and supporting algorithms, visualization, wrote the original draft of the manuscript, coordinated manuscript contributions, and led manuscript review and editing. CAE and MN performed additional formal analyses. SJG, CAE and MSP verified all aspects of the study. All authors reviewed, provided feedback, and edited all versions of the manuscript. CAE, CB, SJG, RDV, and MSP provided project administration and resources.

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Data availability statement

All data from this study and from related studies of the Alberta Innovates Water Innovation Program (Project No. 2614) will be made available publicly available.

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Supplementary material 1

Additional information

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Data type: docx

Explanation note: **table S1.** Means and standard deviations for raw $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ muscle content, baseline corrected $\delta^{13}\text{C}$ ($\delta^{13}\text{C}_{\text{corr}}$), and baseline calculated trophic position (TP) for each species, within each reach sampled. **table S2.** Counts of male, female, and juvenile individuals used for stable isotope analysis within each sampled reach. **fig. S1.** Dissection images of two *L. lota* stomach contents which consisted of juvenile *E. virilis*.

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