# Invasive Round Goby (*Neogobius melanostomus*) impacts on native fishes in tributaries of the Great Lakes

by

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# Author's Declaration

This thesis consists of material all of which I authored or co-authored: see Statement of Contributions included in the thesis. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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# Statement of Contributions

**Chapter 2:** Round Goby (*Neogobius melanostomus*) impacts on benthic fish communities in two tributaries of the Great Lakes

Keith McAllister, D. Andrew R. Drake, Michael Power

Sampling was performed by Fisheries and Oceans Canada and the data obtained from sampling were provided. KM completed data analyses and wrote the paper. Each author contributed to the idea for the study and provided editorial comments. Funding was provided through MP and DARD. This chapter was published as:

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**Chapter 3:** Ecological resource use and overlap of invasive Round Goby (*Neogobius melanostomus*) and native fishes in a Lake Erie tributary

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Sampling was performed by Fisheries and Oceans Canada and the data obtained from sampling were provided. KM completed data analysis and wrote the paper. MP contributed to data analysis. Each author contributed ideas for the study and funding was provided through MP and DARD.

# Abstract

Many invasive species have established in the Laurentian Great Lakes basin and have caused substantial impacts to native species and the lacustrine ecosystems within the basin. The establishment of Round Goby (*Neogobius melamostomus*) in the Great Lakes and its subsequent effects on native species have been well documented. However, after its secondary invasion into tributaries of the Great Lakes, there is limited study of how Round Goby has affected the native fishes within these ecosystems. Therefore, the overall objective of this research was to increase understanding of how Round Goby has affected the relative abundance, diversity, and resource use of native fishes in tributary ecosystems of the Great Lakes.

The catch per unit area (CPUA) of Round Goby in both the Ausable River (a tributary of Lake Huron) and Big Otter Creek (a tributary of Lake Erie) was highest in the downstream reaches located closest to lake habitats, but CPUA rapidly decreased upstream from each lake and approached zero after 18 and 14 river km upstream in the Ausable River and Big Otter Creek, respectively. A negative relationship between the CPUA of Round Goby and several darter species was detected along the tributaries, with moderately negative association between Round Goby and Rainbow Darter (*Etheostoma caeruleum*) in the Ausable River and Johnny Darter (*Etheostoma nigrum*) and overall Percidae species in Big Otter Creek. The negative relationship between the CPUA of Round Goby and these darter species was found over greater spatial scales than reported in previous studies of Round Goby in Great Lakes tributaries and highlights how impacts from Round Goby likely vary both temporally and spatially.

To better understand resource use by Round Goby, stable isotope values of Round Goby in Big Otter Creek were compared to values of Round Goby from around the Great Lakes. Round Goby displayed high niche plasticity across the Great Lakes basin, but were generally more depleted in $\delta^{13}C$ in Big Otter Creek than in Great Lakes populations. Additionally, the resource use of benthic and benthopelagic fishes was compared between sites where Round Goby was present and absent in Big Otter Creek to determine whether Round Goby may have altered trophic relationships. Benthopelagic species appeared to shift their resource use in the presence of Round Goby, whereas significant resource overlap was evident between benthic species (including Blackside Darter (*Percina maculata)* and White Sucker (*Catostomus commersonii*)) and Round Goby. The effects of niche compression on benthic species were reflected by reduced mean fish condition, which benthopelagic species appeared to have avoided due to the larger isotopic niche shift away from that of Round Goby. Round Goby was also associated with reduced relative abundance of native fishes, suggesting a resource-based competitive effect. Collectively, results show that Round Goby has affected the ecological resource use of native fish communities in a Great Lakes tributary and that benthic species (and those unable to shift their resource use) are likely most susceptible to competition pressure from Round Goby.

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# Chapter 1: General Introduction

## 1.1 Impacts of Invasive Species

Invasive species cause significant, measurable changes to the ecosystems they invade (Vitousek et al. 1996; Ricciardi et al. 2013). As populations of non-native species grow within an ecosystem, impacts may initially accumulate but can thereafter decline over longer time scales (Strayer et al. 2006). Inter- or intra-specific regulation (e.g., adaptation by predators, abiotic constraints on population growth, other alterations to the community) may eventually weaken the effects of non-native species on native communities (Strayer et al. 2006). However, aquatic ecosystems already experiencing disturbance (e.g., habitat loss and fragmentation, hydrologic alteration, climate change, overexploitation, and pollution) may be more susceptible to the impacts that accompany non-native species (Pimm and Hyman 1987; Baltz and Moyle 1993; Dextrase and Mandrak 2006; Olden et al. 2010). Thus, the effects of invasive species vary and are expected to depend on the state of the invaded ecosystem.

Trophic alterations (i.e., those caused by competitive exclusion or disruptions in resource partitioning leading to shifts in resource use by native species) are the most commonly reported consequence of species invasions (Dextrase and Mandrak 2006). Several theories have been suggested to account for how invasive species alter trophic dynamics within invaded ecosystems. In ecosystems where resources are not completely used, invasive species may fill empty dietary niches with their occupancy facilitating establishment by limiting competition between them and other native species (Shea and Chesson 2002; Jackson et al. 2013; Tran et al. 2015). In cases where interspecific competition is present, species can become more specialized, leading to strong resource partitioning (i.e., niche divergence) (van Valen 1965). For example, the introduction of Smallmouth Bass (*Micropterus dolomieui*) in two Ontario lakes led to alterations in the trophic linkages of native prey fishes and Lake Trout (*Salvelinus namaycush*) (Vander Zanden et al. 1999; Cucherousset and Olden 2011). Lake Trout were forced to shift their dietary niche toward planktivory specialization due to the high consumption levels of native littoral prey fish by Smallmouth Bass (Vander Zanden et al. 1999). Conversely, an increase in resource competition in an ecosystem where prey resources are limited may lead to weakened niche partitioning (i.e., niche convergence) (Svanbäck & Bolnick 2007). For example, resource partitioning between Alewife (*Alosa pseudoharengus*), Rainbow Smelt (*Osmerus mordax*), and Slimy Sculpin (*Cottus cognatus*) weakened over time due to food-web alterations by dreissenids in Lake Ontario, resulting in niche convergence (Paterson et al. 2014).

## 1.2 Round Goby

The Round Goby (*Neogobius melanostomus*) originated from the Ponto-Caspian region in Europe but was likely transported to North America via the ballast water of commercial ships. After its first detection in 1990 in the St. Clair River (Jude et al. 1992), it spread to all five of the Laurentian Great Lakes within five years (Corkum et al. 2004), reached high abundances, and affected local native fish (Lauer et al. 2004; Kornis et al. 2012) and invertebrate communities (Lederer et al. 2008). Specifically, the establishment of Round Goby has led to the decline of Mottled Sculpin (*Cottus bairdii*), Logperch (*Percina caprodes*), Johnny Darter (*Etheostoma nigrum*), Rainbow Darter (*Etheostoma caeruleum*), and Tessellated Darter (*Etheostoma olmstedi*) populations (French and Jude 2001; Lauer et al. 2004; Balshine et al. 2005; Krakowiak and Pennuto

2008; Bergstrom and Mensinger 2009; Morissette et al. 2018). The traits thought to have driven its success include tolerance to a wide range of environmental conditions, a broad dietary niche, aggressive behaviour, repeated spawning, male parental care (which facilitates recruitment success), and a large body size relative to other benthic fish species (Charlebois et al. 1997). Concern regarding the proliferation of Round Goby also exists due to their ability to transfer contaminants through the food web (Charlebois et al. 1997) and the potential for further dispersal (Corkum et al. 2004). Thus, it has been suspected that expansion of Round Goby into connected waterways would further contribute to their overall ecological and economic consequences (Ricciardi and MacIssac 2000).

The initial invasion of the Great Lakes by Round Goby was followed by a secondary invasion that saw Round Goby move upstream into Great Lakes tributaries as a result of natural dispersal (Bronnenhuber et al. 2011) and human-mediated transport via angler bait buckets (Janssen and Jude 2001; Carman et al. 2006; Drake and Mandrak 2014). Tributaries to the Great Lakes contain a high diversity of fish species (Staton and Mandrak 2005), including several species protected under conservation legislation (e.g., Eastern Sand Darter (*Ammocrypta pellucida*) and Northern Madtom (*Noturus stigmosus*)), and so the establishment of Round Goby has generated concern over how it might affect native fish communities. Due to similar diet and habitat preferences, it was thought that Round Goby would outcompete small native benthic fishes, specifically darters (Percidae spp.) (Jude et al. 1992; Poos et al. 2010). In support of that claim, a study in the Sydenham River found diet overlap between Round Goby and numerous darter species, including: Greenside Darter

(*Etheostoma blennioides*), Johnny Darter (*Etheostoma nigrum*), Blackside Darter (*Percina maculate*), and the Threatened Eastern Sand Darter (Firth et al. 2020).

Despite the finding of significant dietary overlap, and therefore probable competition between Round Goby and several native fishes (e.g., Firth et al. 2020), the overall effects of Round Goby on the fish communities in Great Lakes tributaries appear to have been variable. For example, a 10.8-fold increase in Round Goby abundance in 23 Lake Michigan tributaries had no apparent effect on the abundance of five native benthic fish species over a four-year period (Kornis et al. 2013). Additionally, Blackside Darter had similar densities at riverine sites regardless of whether Round Goby was present or absent within two Lake Michigan tributaries (Malone 2016). However, both Kornis et al. (2013) and Malone (2016) suggested the limited impacts observed on benthic fish abundance may have been due to the recency of invasion within the chosen study locations. Conversely, another study found Round Goby to be associated with declines in darter abundance. In New York tributaries of Lake Erie, Rainbow Darter (*Etheostoma caeruleum*) and Johnny Darter (*Etheostoma nigrum*) were not detected in any sampled streams recently invaded by Round Goby despite the darter species having been historically present in the streams (Krakowiak and Pennuto 2008). While these prior studies have provided important information for understanding the initial impacts of Round Goby in Great Lakes tributaries, their limited spatial breadth (< 20 km upstream from the Great Lakes where Round Goby is known to occur at high densities) limits their ability to describe the potential extent of invasion impacts.

Studies of Round Goby invasions in Europe have evaluated impacts across large spatial scales (up to 250 km) of riverine habitat and found that Round Goby populations vary across temporal and longitudinal gradients. For example, the distribution and abundance of Round Goby populations varied across years and locations along the Danube River (Cerwenka et al. 2018) due to their ability to rapidly disperse (up to 17 river km/year) (Brandner et al. 2013). At the invasion front, Round Goby populations also displayed female-biased sex ratios, with adults having greater body condition, and juveniles being less prevalent than in established populations (Brandner et al. 2018). Collectively, these findings emphasize the need to study Round Goby impacts in North American tributaries at broader spatial scales.

### 1.3 Stable Isotope Analyses

Stable isotope analyses (SIA), particularly carbon and nitrogen, are useful for inferring the effects of invasive species on aquatic food webs with respect to shifts in resource use and diet overlap (Vander Zanden et al. 1999; Britton et al. 2010; Cucherousset et al. 2012; Paterson et al. 2014; Coulter et al. 2019). Overlap in isotopic signatures among species can indicate the use of shared resources and provides the ability to make inferences regarding competition and resource partitioning (Post 2002; Jackson et al. 2012). Diet overlap between Round Goby and native fishes in the Sydenham River has been detected using gut content analysis (Firth et al. 2020), but because gut content analysis reflects more recent diets, they have misrepresented the longer-term impacts of Round Goby on the use of ecosystem resources in the Great Lakes in past studies (Barton et al. 2005; Brush et al. 2012). In contrast, SIA represent diets as assimilated over longer periods of time (weeks to months), thereby providing a greater understanding of long-term resource use by Round Goby relative to native fishes (Grey 2006; Rybczynski et al. 2008). Although SIA has been widely used to determine the resource use of Round Goby in the Great Lakes (Barton et al. 2005; Pettitt-Wade et al. 2015; McCallum et al. 2017; Mumby et al. 2018; Miano et al. 2021), resource use relationships between Round Goby and native fishes in Great Lakes tributaries have not been similarly evaluated with SIA.

## 1.4 Objectives

Due to the limited understanding of how Round Goby may affect native fish species in the tributary ecosystems of the Great Lakes, the following research objectives were addressed:

Chapter 2 determined how Round Goby may have affected the relative abundance of native fishes (specifically Percidae species) in Great Lakes tributaries (Ausable River and Big Otter Creek) using spatial analyses, linear regression, and co-occurrence relationships. The impact of Round Goby on overall fish communities within these streams was also assessed using diversity indices (species diversity, evenness, overlap), species accumulation curves, and local richness estimators. Findings were compared to other studies of Round Goby in Great Lakes tributaries and other riverine ecosystems in Europe (Danube River and River Rhine).

Chapter 3 used stable isotope analyses to identify ecological resource use by Round Goby in Big Otter Creek, compare its resource use to other Round Goby populations in the Great Lakes, and evaluate how the isotopic niche, relative abundance, and condition of native fishes in Big Otter Creek may have been altered by the presence of Round Goby.

The overall objective of this thesis was to better understand how Round Goby has affected native fishes within Great Lakes tributaries. While past studies have evaluated the impacts of Round Goby in tributaries, this study aimed to increase the within-stream spatial scales over which impacts were evaluated and to use SIA to determine the resource use of Round Goby relative to native fishes to infer whether competition might be occurring between Round Goby and native species.

# Chapter 2: Round Goby (*Neogobius melanostomus*) impacts on benthic fish communities in two tributaries of the Great Lakes

## 2.1 Introduction

Aquatic invasive species (AIS) can drastically affect the ecosystems they invade with many AIS having led to significant declines in native fishes (Chick et al. 2020; Cucherousset and Olden, 2011; Fetterolf Jr., 1980; Hermoso et al. 2011; Ogutu-Ohwayo, 1990). Generally, ecological impacts increase as the density of the invader increases, with impacts to native species occurring through a variety of ecological mechanisms that include competition, predation, behavioural effects, and food web changes (Bradley et al. 2019; Gallardo et al. 2016). At high invader densities, increased intraspecific interactions may lead to diminished ecological impacts, as has been experimentally demonstrated for invasive Round Goby (*Neogobius melanostomus*) (Kornis et al. 2014). AIS are often more aggressive and grow larger than native species, which may prevent native species from accessing optimal habitat and dietary resources as a result of interference competition (Persson, 1985; Pimm et al. 1985; St-Pierre et al. 2006; Volpe et al. 2001). Exploitative competition for food resources may also simultaneously occur between trophically similar invasive and native species, resulting in lower growth in native species compared to allopatric conspecifics (Seiler and Keeley, 2009). Abundance declines in numerous native fish species have been linked to competitive interactions with AIS that have negatively affected population vital rates (e.g., growth and fecundity) (Cucherousset and Olden, 2011). AIS may also alter food web structure, often by increasing food chain length or modifying basal trophic levels (Cucherousset et al. 2012).

The Round Goby is native to the Ponto-Caspian region, but was transported to North America in the ballast water of commercial ships. It was first detected in the St. Clair River in 1990 (Jude et al. 1992) and subsequently spread to all five Great Lakes within five years (Corkum et al. 2004). Round Goby quickly attained high abundance in nearshore lake habitats and its occurrence has been linked to reduced abundance of several benthic fishes in the Great Lakes basin, including Mottled Sculpin (*Cottus bairdii*), Logperch (*Percina caprodes*), Johnny Darter (*Etheostoma nigrum*), Rainbow Darter (*Etheostoma caeruleum*), and Tessellated Darter (*Etheostoma olmstedi*) (Balshine et al. 2005; Bergstrom and Mensinger, 2009; French and Jude, 2001; Krakowiak and Pennuto, 2008; Lauer et al. 2004; Morissette et al. 2018).

The initial establishment of Round Goby in the Great Lakes was followed by secondary expansion into numerous Great Lakes tributaries (e.g., Ontario: Big Otter Creek in 2002, Trent River in 2003, Thames River in 2003, Grand River in 2005, Ausable River in 2007) (Poos et al. 2010; Raab et al. 2018; Raby 2010). Round Goby impacts on the native fish communities in North American rivers have varied despite the expectation that Round Goby would outcompete small native benthic fishes such as darters and other Percidae species (Jude et al. 1992; Poos et al. 2010). For example, Rainbow Darter and Johnny Darter were not detected in any of the sampled streams within studied New York state tributaries of Lake Erie after Round Goby establishment despite the historical presence of the darter species (Krakowiak and Pennuto, 2008). Conversely, an approximately 11-fold increase in Round Goby in numerous Lake Michigan tributaries had no detectable negative effects on the abundance of several native benthic species, including: Johnny Darter, Blackside Darter (*Percina maculata*), and Fantail Darter (*Etheostoma flabellare*) over a four-year study period, although the result may have been linked to the recency of the Round Goby invasion at the selected study sites (Kornis et al. 2013). Similarly, Round Goby had no apparent effect on Blackside Darter abundance in two Lake Michigan tributaries (Silver Creek and Pigeon River), which was also attributed to the recency of invasion (Malone, 2016). Thus, Round Goby impacts appear to be context-dependent and may vary widely depending on ecosystem factors, including native community composition, food web dynamics, time since invasion, and Round Goby density.

The few studies that have examined Round Goby impacts in North American rivers have been limited with respect to their spatial breadth as studied sites have typically been located near invaded lacustrine environments, e.g., < 20 km (Kornis et al. 2013; Krakowiak and Pennuto, 2008; Malone, 2016). Conversely, several European studies have investigated Round Goby impacts in riverine environments at greater spatial scales (up to 250 km) and have observed differences in Round Goby impacts when compared to North American studies. In European rivers, Round Goby impacts vary spatially across longitudinal river gradients (Borcherding et al. 2011; Brandner et al. 2018; Cerwenka et al. 2018). For example, Round Goby has become the dominant fish species in terms of relative abundance in the upper Danube River (Cerwenka et al. 2018) and directly contributed to the declines of many specialized native species (Mueller et al. 2018). When comparing along the invasion gradient in the upper Danube, Round Goby populations located near the invasion front are composed of more females, larger and better-conditioned adults, and have a lower proportion of juveniles than in areas where Round Goby has been established for longer time periods

(Brandner et al. 2018). Given these differences, it is important to further examine the variation in Round Goby impacts on fish communities in North American lotic environments at greater longitudinal scales.

In the Great Lakes basin, numerous Percidae species are facing population declines due to anthropogenic threats (e.g., pollution, excess nutrients, sedimentation, impoundment effects) and many are protected under Canadian federal and provincial conservation legislation (e.g., Eastern Sand Darter (*Ammocrypta pellucida*), River Darter (*Percina shumardi*), Channel Darter (*Percina copelandi*)) (Pratt et al. 2016). Round Goby expansion and establishment in the tributaries of the Great Lakes, therefore, is believed to pose further threats to these and other darter species due to probable competition for similar dietary and habitat resources, and via egg predation by Round Goby (French and Jude 2001; Raab et al. 2018). For example, Percidae species have displayed increased specialized feeding on Chironomidae in tributaries also occupied by Round Goby (Firth et al. 2020), likely because Round Goby deplete Ephemeroptera, Plecoptera, and Trichoptera (EPT) and other grazers and shredders that would otherwise constitute important food resources for native fish species (Krakowiak and Pennuto 2008; Pennuto et al. 2018). There is also evidence of Round Goby displacing darters to different microhabitats (Abbett et al. 2013; Reid 2019), with experimental studies indicating that Round Goby can outcompete Logperch for their preferred habitat (Balshine et al. 2005; Leino and Mensinger 2017). Collectively, previous studies have provided sound evidence that darter species are the most likely fishes to be impacted by the establishment of Round Goby in tributaries of the Great Lakes (Raab et al. 2018; Firth et al. 2020).

Past studies investigating Round Goby in small tributaries flowing directly into the Great Lakes have mostly focused on fish community impacts at limited spatial scales (i.e., downstream reaches < 20 km upstream from the river mouth). Analyzing the effects of Round Goby at broader spatial scales within invaded tributaries, however, is critical to develop a broader understanding as to how invaded riverine ecosystems will eventually be impacted. Thus, the main objective of this study is to evaluate whether the presence of Round Goby is associated with lower relative abundance of Percidae species (specifically darters and Logperch) and lower diversity, evenness, and species richness of the fish communities in invaded tributaries of the Great Lakes. Specifically, we hypothesize that: [1] sites with higher relative abundance of Round Goby will be associated with lower relative abundance of other benthic Percidae species (Greenside Darter (*Etheostoma blennioides*), Blackside Darter, Johnny Darter, Rainbow Darter, and Logperch) and [2] tributary reaches with high Round Goby abundance will exhibit lower diversity, evenness, and species richness.

## 2.2 Methods

### 2.2.1 Field Sampling

Forty-five sites in the Ausable River (a tributary of Lake Huron, river mouth: 43°23′N, 81°91′W) and fifty sites in Big Otter Creek (a tributary of Lake Erie, river mouth: 42°64′N, 80°81′W) were sampled by Fisheries and Oceans Canada (DFO) field crews for this study (Barnucz et al. 2020). Sampling in the Ausable River was completed August 15th – September 28th, 2017 (36 sites) and July 24th – July 26th, 2018 (9 sites) using multiple gears: a siamese trawl (3.0 m tow ropes, two 6 kg 0.5 x 0.3 m otter doors, 3.0 mm mesh size, 2.4 m wide, 4.3 m length), a straight seine with chain (3.0 mm mesh, 6.0 m length), and a bag seine (3.0 mm mesh, 9.1 m length). The 9 sites from 2018 spanned the upstream-most (117 to 126 km upstream from the river mouth) sampled section in the Ausable River. Portions of the Ausable River consisted of habitats inaccessible by wading or dominated by large physical obstructions (e.g., woody debris). Thus, gear selection in the Ausable River was adapted to the site-specific conditions to optimize sampling efficiency. In Big Otter Creek, sampling occurred between July 9 - 19th and September 24 - 26th, 2018 using only a bag seine (3.0 mm bag mesh, 3.0 mm wing mesh, 9.1 m length). Seining in both tributaries was completed in a downstream direction at each site with three consecutive hauls. A time of roughly 5 minutes between hauls was designated to allow fish to repopulate the fished area. To minimize disturbance, survey crews began sampling in the downstream-most sampling unit and then worked upstream towards the next unit (allowing for the release of captured fishes downstream of the site to avoid recapture in sites upstream). Captured fishes were kept in bankside aquaria, identified to species, and enumerated for each haul. A subset of fishes was kept and preserved in a 10% formalin solution to confirm species identification and for future analyses. Additional sampling details can be found in Barnucz et al. (2020).

### 2.2.2 Aquatic Habitat Sampling

Aquatic habitat variables were measured at the midpoint of each sampling site after fishes were collected. Water temperature (°C), conductivity (μS), turbidity (NTU), and dissolved oxygen (mg/L) were measured roughly 0.1 m below the water surface with a YSI EX02 Multiparameter Sonde (Xylem Inc., White Plains, NY). Substrate composition was determined by taking a grab sample of bed material to record percent composition of the sample based on median particle diameter (clay: 0-0.002 mm, silt: 0.02-2 mm, gravel: 2-40 mm, cobble: 40-256 mm, and boulder: >256 mm). Channel depth was measured at three separate locations within the boundaries of the seined area (shallow, mid-depth, and deep) with a metre stick. Stream velocity (m/s) was similarly measured in three separate locations (slowest, mid-velocity, fastest) with a Swoffer 2100 current velocity meter (Swoffer Instruments, Sumner, WA) deployed at roughly 50% of the stream depth. Wetted stream channel width (m) was measured at the midpoint of the seining site (Ausable River) or river reach (Big Otter Creek) perpendicular to the bank with the use of a Nikon Laser 1200S waterproof laser range finder (Nikon Canada Inc., Mississauga, ON). Site latitude and longitude were recorded using a Garmin Montana 600 handheld GPS unit (Garmin Ltd., Olathe, KS).

### 2.2.3 Statistical Analysis

Catch per unit area sampled (CPUA) was determined as the aggregate number of captured fish × seined area $(m^2)^{-1}$. Broken-stick regression was used to compare CPUA of Round Goby and other Percidae species (Johnny Darter, Blackside Darter Greenside Darter, Rainbow Darter, and Logperch in the Ausable River and Johnny Darter, Blackside Darter, and Logperch in Big Otter Creek) with site distance from the river mouth (measured along the river channel using the linear measuring tool from ArcGIS Online® software (Esri Canada, Toronto, ON, Canada)) as follows:

$$Y = a + b_1\,(X) + \; b_2\,(Z)(X - T)$$

where *a* is the intercept, $b_1$ is the initial slope coefficient, $b_2$ is the slope modifying coefficient, *T* is the breakpoint of the line where the slope changes from $b_1$ to ($b_1 + b_2$), and *Z* defines where the breakpoint occurs ($Z = 0$ if $X < T$ and $Z = 1$ if $X > T$). The breakpoint was determined following methods for estimating piecewise regression models with unknown breakpoints using the 'segmented' package in R (Hudson 1966; Muggeo 2021). All statistical analyses were completed using R version 4.0.4 (R Core Team 2021).

Only species present at greater than 5% of sites were included in the statistical analyses as the focus of the study was on determining how Round Goby may be affecting the more common Percidae species along upstream/downstream tributary gradients. While Round Goby impacts may be most severe on rare Percidae species, it would be difficult to determine patterns in relative abundance when a species is detected in < 5% of sampled sites as rarity can obscure the ability to detect biologically significant differences between sites (Hawkins et al. 2000). Similarly, Kornis et al. (2013) restricted analyses to non-Round Goby species detected at > 5% of sites when testing associations between various species relative abundance and environmental data. Linear regressions were performed to test for the significance of correlations between the CPUA of Round Goby (excluding sites where Round Goby CPUA = 0) and the Percidae species in both tributaries. Additionally, Phi coefficients (Yule 1912) were calculated to test for relationships between the presence/absence of Round Goby and Percidae species as follows (Alofs and Jackson 2015, Jackson et al. 1989):

$$\varphi = \frac{ad - bc}{\sqrt{(a+b)(a+c)(c+d)(b+d)}}$$

Where *a*, *b*, *c*, and *d* are the entries from two-by-two contingency tables which define the number of sites where Round Goby and the Percidae species are both absent, Round Goby is absent and the Percidae species are present, Round Goby is present and the Percidae species are absent, and Round Goby and the Percidae species are both present, respectively. Phi coefficients represent pairwise associations between species independent from relative abundance and range from negative one (perfect negative association) to one (perfect positive association).

#### 2.2.3.1 Fish Community Indices

To analyze whether the impact of Round Goby differed spatially within each tributary, sampling sites were grouped into lower, middle, and upper sites based on longitudinal distance from the river mouth. In Big Otter Creek, lower, middle, and upper sites were 13.1 – 14.1 (n=13), 35.8 – 49.9 (n=9), and 71.2 – 83.6 km (n=5) upstream from Lake Erie, respectively. In the Ausable River, lower, middle, and upper sites were 11.2 – 17.7 (n=16), 32.2 – 75.6 (n=12), and 91.6 – 126.4 km (n=17) upstream from Lake Huron, respectively. Within each grouping, sites in the middle of the designated section were selected for use in statistical comparisons to ensure sufficient distance between each site grouping. Distances between the site groupings thus exceeded the linear home range distance typically observed for small benthic fishes (Minns 1995; Woolnough et al. 2009). Sites in both tributaries were grouped into 15 km clusters with CPUA of Round Goby and Percidae species averaged among the clusters to test for spatial autocorrelation using Moran's I from the package 'ape' (Paradis 2022). Sites in close proximity to low-head dams or the Great Lakes were excluded as Round Goby is known to exist at high densities in these locations (Krakowiak and Pennuto 2008; Kornis et al. 2013; Malone 2016b; Raab et al. 2018; May et al. 2020). Thus, in analyses comparing the three site groupings, 23 sites from Big Otter Creek were excluded and no sites in the Ausable River were excluded. Additionally, differences in mean habitat conditions of site groupings in each tributary were examined using one-way ANOVAs with post-hoc Tukey tests used to compare means between site groupings where necessary.

Diversity and evenness were used to characterize and compare the lower, middle, and upper river fish communities in both tributaries (Table 2.1), with species diversity determined using the widely used Shannon-Wiener diversity index (Shannon and Weaver 1949; Spellerberg and Fedor 2003). Evenness was calculated using Smith and Wilson's Index of Evenness as it is independent of species richness and sensitive to both rare and common species in the community (Smith and Wilson 1996). Several CPUA categories (CPUA of all fishes excluding Round Goby, CPUA of all fishes including Round Goby, and CPUA of Round Goby) were also compared between site groupings in each tributary. Additionally, the Morisita-Horn index (Horn 1966), modified from Morisita (1959) and noted as one of the more robust measures of overlap (Smith and Zaret 1982), was also calculated by adding CPUA of species in each site grouping to determine the similarity in species composition between site groupings.

#### 2.2.3.2 Species Accumulation Curves

Species accumulation curves were generated to compare the accumulation of fish species with an increasing number of sites in each tributary. The method involved using an exact calculation for site-based species richness (Ugland et al. 2003; Colwell et al. 2004; Kindt et al. 2006), given by:

$$\widehat{S_n} = \sum_{i=1}^{S}(1 - p_i), \qquad \text{where } p_i = \frac{\binom{N-f_i}{n}}{\binom{N}{n}},$$

and where $f_i$ is the frequency of species *i*, *S* is the number of species, with the expected number of species in a community rarefied from *N* to *n* individuals. Species accumulation curves for each site grouping were created using randomization to compute the curves and corresponding 95% confidence intervals with the ‘vegan’ and ‘BiodiversityR’ packages (Oksanen 2020; Kindt 2021).

#### 2.2.3.3 Local Richness Estimators

To quantitatively estimate the theoretical upper limit of the number of species present for each site grouping, local richness estimates were calculated (Chao and Chiu 2016). For comparative purposes, the function ‘specpool’ from the package ‘vegan’ was used to generate estimates of local species using the non-parametric and abundance-based Chao1 (Oksanen 2020). The ‘specpool’ function assumes that the number of undetected species is related to the number of rare species (i.e., those only seen once or twice) (Oksanen 2020).

## 2.3 Results

### 2.3.1 Round Goby Impacts on Relative Abundance of Percidae Species

In both rivers, Round Goby CPUA declined sharply with upstream distance from the tributary mouth (Figs. 2.1, 2.2) whereas the CPUA of the Percidae species increased. In the Ausable River, an overall increasing then decreasing trend in CPUA of Percidae species was largely driven by Greenside Darter, Blackside Darter, and Johnny Darter in sites between 44 and 52 km upstream from Lake Huron (Fig. 2.3). Logperch and Rainbow Darter CPUA remained consistently low as distance from the mouth of the Ausable River increased (Fig. 2.3). The overall increasing trend in the relative abundance of Percidae species in Big Otter Creek as a function of distance from the river mouth was largely driven by Johnny Darter while the CPUA of the other species remained consistently low (Fig. 2.3). In Big Otter Creek, an anomaly from the low occurrence of Round Goby at distances greater than 14 km from the river mouth occurred 58 km upstream where 32 Round Goby were caught (Fig. 2.1, 2.2). Round Goby were not detected after 18 and 62 km upstream, respectively, in the Ausable River or Big Otter Creek.

In the Ausable River, CPUA of Round Goby was not significantly correlated with CPUA of Blackside Darter, Greenside Darter, Logperch, or Johnny Darter (Table 2.2). Similarly, no significant associations between Round Goby CPUA and other Percidae species were noted in Big Otter Creek. Phi coefficients showed a similar lack of strong association between Round Goby and other studied fish species. In the Ausable River, Round Goby showed a moderately positive association with Logperch (0.26), Johnny Darter (0.21), and Greenside Darter (0.3) and a moderately negative association with Rainbow Darter (-0.25). Blackside Darter (-0.06) and Percidae species overall (0.06) showed no meaningful associations with Round Goby. In Big Otter Creek, there were weak associations with Logperch (0.18) and Blackside Darter (-0.16), and moderately negative associations with Johnny Darter (-0.31) and Percidae species overall (-0.28).

### 2.3.2 Aquatic Habitat Conditions

Stream physio-chemical conditions (conductivity (μS), dissolved oxygen (mg/L), pH, and turbidity (NTU)) varied along the length of the rivers (Table 2.3). Temperature did not differ meaningfully across site groupings in the Ausable River but was marginally cooler in the middle reach of Big Otter Creek (2.2-2.4 °C). The Ausable River became slower, deeper, and wider moving from the upstream to downstream sites. In Big Otter Creek, there were no meaningful differences in water velocities, depths, or widths. Organics, silts, and clays in the substrate of both rivers were consistent across site groupings, whereas % gravel increased from upstream to downstream in the Ausable River and decreased in the same direction in Big Otter Creek. Sands dominated the substrates of all sites in both rivers, with % sand tending to be higher in the lower site grouping of Big Otter Creek and consistent across site groupings in the Ausable River.

### 2.3.3 Round Goby Influence on Fish Community Metrics

In the Ausable River, evenness was lowest in the lower zone sites, and higher in the upper zone sites, but followed the reverse pattern in Big Otter Creek with the highest evenness in the lower zone sites and lowest in the upper zone sites (Fig. 2.4: a, f). In the Ausable River, the Shannon-Wiener index was highest in the middle zone sites, whereas in Big Otter Creek the index was highest in the lower and upper zone (Fig. 2.4: b, g). The CPUA of all fishes (including and excluding Round Goby) was highest in sites in the middle zone of the Ausable River, but highest in the upper zone sites of Big Otter Creek (Fig. 2.4: d, i). As seen in Fig. 2.1, 2.2, and 2.3, Round Goby

CPUA was highest in the lower sites and lowest in the middle and upper zones of both tributaries (Fig. 2.4: e, j).

The species accumulation curve for the upper zone of the Ausable River displayed a higher trajectory and contained higher species richness than the lower and middle zones (Fig. 2.5). Both the lower and middle zones of the Ausable River followed a similar trajectory with similar observed species richness. The species accumulation curve for the upper and lower zones of Big Otter Creek were similar and displayed slightly higher observed species richness than the middle zone, but overall, site groupings appeared to have a similar number of observed species (Fig. 2.5). In the Ausable River and Big Otter Creek, the middle zone had higher estimated species richness than the lower and upper zones (Table 2.5). The middle zone contained the second lowest and lowest total CPUA of Round Goby in the Ausable River and Big Otter Creek, respectively. The lowest estimated richness occurred in the lower zone of both tributaries where total Round Goby CPUA was highest. Fish communities in Big Otter Creek showed low similarity between site groupings (lower and middle $C_H = 0.31$, lower and upper $C_H = 0.26$, middle and upper $C_H = 0.05$). In the Ausable River, site groupings showed high similarity between the lower and middle ($C_H = 0.88$) and the lower and upper ($C_H = 0.97$) zones, but moderate similarity between the middle and upper ($C_H = 0.38$) zones. There was no evidence for spatial autocorrelation between sites at the analyzed spatial scale in Big Otter Creek: Round Goby ($I = -0.30$, standard deviation = 0.09, $p = 0.71$), Percidae species ($I = -0.13$, standard deviation = 0.11, $p = 0.06$) or the Ausable River: Round Goby ($I = -0.19$, standard deviation = 0.03, $p = 0.62$), Percidae species ($I = -0.23$, standard deviation = 0.05, $p = 0.57$).

In the Ausable River, Round Goby represented 7.36% of the CPUA in the lower grouping but was not captured elsewhere (Table 2.4). The dominance in the lower reaches of Leuciscidae remained stable (79.4 to 72.4%) as sampling moved to the upper reaches. Percidae (7.3 to 15.1%) and Centrarchidae (0.2 to 9.5%) both increased in importance when moving from the lower to the upper reaches. Similar declines in Round Goby were observed in Big Otter Creek, with percentage importance in the catch declining from 8.6% to 0% as sampling moved upstream. Leuciscidae showed no noticeable trend in relative abundance, remaining between 60.2 and 69.6% of the total catch along the length of the creek. Catostomidae, which had an only minor but stable presence in the Ausable River, were a significant portion of the catch at all sites (9 to 19.3%). Percidae, which peaked in percentage importance in the middle reaches (26.4%) showed no discernable trend and was a significant proportion of the catch in both the lower (11.8%) and upper (13.3%) reaches of Big Otter Creek. The most notable trend was the increase in Leuciscidae from 5.3% in the lower reaches to 11.6% in the upper reaches, a trend that was the direct opposite of that observed in the Ausable.

## 2.4 Discussion

We found evidence to suggest that Round Goby has negatively affected the relative abundance of several darter species and the overall fish community structure in invaded riverine ecosystems of the Great Lakes. However, overwhelming evidence of negative associations with darter species was not found. Round Goby relative abundance was highest proximate to the Great Lakes but sharply decreased thereafter, with upstream reaches in both tributaries having higher relative abundances of darter species: Greenside Darter, Blackside Darter, and Johnny Darter in the Ausable River, and Johnny Darter in Big Otter Creek. Significant negative correlations of CPUA between all darter species and Round Goby were not observed, with co-occurrence patterns indicating negative associations only for a subset of species (Ausable River: Rainbow Darter, Big Otter Creek: Johnny Darter and Percidae overall). Overall diversity, species richness, evenness, relative abundance, and species accumulation curves for fishes in the studied tributaries demonstrated variation across site groupings despite the similarity of stream habitat conditions, particularly substrate, between site groupings. The observed patterns of effect also differed between the rivers.

The general pattern of low darter relative abundance in sites with high Round Goby relative abundance suggests that Round Goby may have reduced the populations of several darter species, as has been noted for other tributaries of the Great Lakes (Krakowiak and Pennuto 2008; Raab et al. 2018). Alternatively, Round Goby may have triggered a habitat redistribution effect for darters (e.g., Greenside, Blackside, and Johnny Darters within the studied tributaries, as has been shown to occur elsewhere as a consequence of invasive fishes (Habit 2010). For example, the seasonal dispersal of non-native Brown Trout (*Salmo trutta*) from Czech reservoirs alters the spatial distribution of native fishes in tributary streams (Pfauserová et al. 2021). Furthermore, experimental studies have demonstrated the redistributive effect, having shown that benthic fish species can display significant shifts in riverine habitat use when co-occurring with invasive gobiids, moving from preferred shelter habitat to less preferred and riskier habitats subject to predation (Van Kessel et al. 2011). Thus, the rise in the relative abundance of Greenside, Blackside, and Johnny Darters in the Ausable River and Johnny Darter in Big Otter Creek may have been an artefact of redistribution caused by avoidance behaviour (Ayala et al. 2007).

Invasive species initially negatively affect the more abundant native species before affecting rarer native species (Powell et al. 2013). Based on the correlated reduction in abundant darter species along the tributary gradients, both tributaries may still be experiencing the early-stage effects of a Round Goby invasion given that Round Goby CPUA values in this study were comparable to those found shortly after introduction in the Flint River between 1998 and 2002 (0.29 – 0.78 individuals/m$^2$, Carman et al. 2006; Jude et al. 2018) and in Lake Michigan tributaries between 2007 and 2010 (0.07 – 0.36 individuals/m$^2$, Kornis et al. 2013). Similar to other invasive species, Round Goby has been noted to rapidly attain high densities during the early stages of invasion before stabilizing or declining in numbers as the invasion progresses (Young et al. 2010; Kornis et al. 2012; Burkett and Jude 2015; Kornis et al. 2014). For example, in Hamilton Harbour, it was proposed that declines in Round Goby abundance resulted from the exceedance of their ecological carrying capacity (Young et al. 2010).

Round Goby populations at higher densities have also been stabilized through predatory control. In Lake Erie, after dramatic population increases, Round Goby experienced population declines following the development of predatory control by Burbot (*Lota lota*) (Madenjian et al. 2011). In the St. Clair River, the diets of large Rock Bass (*Ambloplites rupestris*) and large Smallmouth Bass (*Micropterus dolomieu)* have been shown to consist of 56-67% and 100% Round Goby, respectively (Burkett and Jude 2015), with similar results reported from the River Dyje in the Czech Republic (Mikl et al. 2017). Predation control, however, appears unlikely to be occurring in either of the streams studied here, with cumulative catches of predatory or partially predatory fishes (e.g., Rock Bass, Smallmouth Bass, Northern Pike, Yellow Perch) in both rivers never exceeding 8.7% of the total CPUA and most reaches never exceeding 2% of the total CPUA.

The low relative abundance of darter species in areas of high Round Goby relative abundance observed along the tributary gradients may also be driven by competitive interactions. For example, one recent study assessing the trophic impacts of Round Goby on native benthic fishes in the Sydenham River (also a tributary of the Great Lakes) found diet overlap between Round Goby and several darter species including: Greenside Darter, Blackside Darter, Johnny Darter, and the Threatened Eastern Sand Darter (Firth et al. 2020). In addition to competition for similar food resources, Round Goby has been shown to outcompete other species for habitat due to their aggressive behaviour and ability to achieve greater body sizes than other similar species (Charlebois et al. 2001; Balshine et al. 2005) and, as has been noted in laboratory experiments, has the capacity to displace native benthic fishes from more sheltered, desirable habitats (Van Kessel et al. 2011). Thus, interspecific competition for habitat and/or dietary resources may account for the low relative abundance of darter species observed in the downstream reaches with high Round Goby relative abundance and the moderately negative co-occurrences between Round Goby and several darter species. Continued competition and/or displacement of native darter species by Round Goby as the invasion front advances would contribute to further declines in riverine darter populations (Poos et al. 2010; Firth et al. 2020).

Increases in Round Goby populations have led to declines to native fishes in other freshwater environments, most notably in several major European rivers (Danube River and River Rhine) and their tributaries. The continued dispersal and increase in abundance of Round Goby is expected to further increase competition with native fishes in those systems (Borcherding et al. 2011; Cerwenka et al. 2018; Dashinov and Uzunova 2020). In North America, Round Goby in the Great Lakes has also caused declines to numerous native benthic fishes (e.g., *Etheostoma spp*., *Percina spp*., and Cottus *spp.*) via competition for food, habitat, and spawning sites (French and Jude 2001; Lauer et al. 2004; Reid and Mandrak 2008; Abbett et al. 2013). Accordingly, Round Goby is renowned for its ability to outcompete other fishes through both interference and exploitative competition. Laboratory studies have provided evidence that Round Goby can outcompete Spoonhead sculpin (*Cottus ricei),* Slimy sculpin (*Cottus cognatus*), and Logperch for optimal habitat and food resources (Balshine et al. 2005; Bergstrom and Mensinger 2009). However, without direct evidence of overlap in resource use between Round Goby and Percidae species in the Ausable River and Big Otter Creek (i.e., through gut content analysis or stable isotope analysis), uncertainty exists as to the mechanisms through which Round Goby may be contributing to the negative co-occurrences with some darters and the observed low darter abundances in downstream reaches.

While several studies have investigated the impacts of Round Goby invasion in tributaries flowing directly into the Great Lakes, their scope has been confined to assessing impacts at limited spatial scales and in areas proximate to the lakes. For example, Krakowiak and Pennuto (2008) found that darter species (Johnny Darter and

Rainbow Darter) historically present in streams before Round Goby establishment were absent after Round Goby establishment at sites located 2-3 km from Lake Erie where Round Goby were already known to occur in high nearshore densities. Similarly, in both the Ausable River and Big Otter Creek, abundances of Johnny Darter increased upstream of sites proximate to the lakes, with the study of resident fish communities at greater longitudinal scales demonstrating a Round Goby density gradient effect on native darter abundances. In addition, effects found here may be related to the longer post-invasion intervals (11-16 years) considered in our study. Studies that have investigated Round Goby impacts in tributaries shortly after establishment may not accurately reflect impacts to native fish communities over prolonged time periods. Long-term studies across large spatial scales in European rivers have demonstrated considerable variability in the observed impacts of Round Goby on native fish communities. Round Goby became the most abundant fish species in a 248 km stretch of the upper Danube River ten years after its introduction (Cerwenka et al. 2018). However, the effects from Round Goby populations in the Danube River differ across spatial gradients depending on invasion stage. Individuals at the invasion fronts have larger bodies and greater body condition than those in already established areas due to weaker intraspecific competition and greater food availability at the invasion fronts (Brandner et al. 2018). Although invasion impacts to aquatic ecosystems may be apparent after short time periods (Janssen and Jude 2001; Lauer et al. 2004; Britton et al. 2007; Connelly et al. 2007), it is necessary to assess impacts at longer post-invasion intervals to ultimately understand both how the invader will influence the ecosystem and how impacts are related to the invasion stage (Downing et al. 2013; Pelicice et al. 2014; Havel et al. 2015).

Fish community diversity metrics and abundances of Round Goby and Percidae species will have been influenced by habitat conditions, which are integral in shaping fish communities within streams (Moyle and Light 1996). Abiotic conditions (e.g., watershed area and temperature) were best at predicting Round Goby abundance in Lake Michigan tributaries (Kornis et al. 2013) and thus variation in Round Goby abundance may have been reflective of differences in habitat suitability along the lengths of the studied rivers. Round Goby has shown preference for a wide variety of habitat conditions in tributaries, occupying sites dominated by cobble, gravel, sand, or silt substrate (Pennuto et al. 2018; Reid 2019), slow to moderate water velocity (< 0.4 m/s; Raab et al. 2018; Reid 2019), and a wide range of water depths (0.2 – 2.2 m; Raab et al. 2018; Reid 2019). The preference for slow water velocity may explain why Round Goby density was high in the lower reach of the Ausable River where mean water velocity was lowest (0.04 m/s). Slow water velocity may also account for the anomaly in the middle grouping site 58 km upstream in Big Otter Creek where there is a higher relative abundance of Round Goby (CPUA = 0.32) than nearby sites. The notably slower water velocity (0.07 m/s) in that site than typically seen in the middle site grouping (mean = 0.25 m/s) of Big Otter Creek may have provided more favourable conditions for Round Goby. Water velocity and other habitat features likely play a role in shaping Round Goby populations in streams, particularly as Round Goby has shown preferences for volitional movement under low flow conditions (Tierney et al. 2011).

Several populations of Round Goby in the Great Lakes (Lake Erie, Lake Ontario) undergo seasonal migration into tributaries in the summer months followed by migration back to the lake in winter months (Blair et al. 2019). Tributary streams connected to large, invaded waterbodies (i.e., the Great Lakes) could be used primarily for reproduction and recruitment and Round Goby may not actually reside in the tributaries year-round (Pennuto et al. 2010, 2021; Blair et al. 2019). The high relative abundance of Round Goby observed in tributary sites adjacent to the Great Lakes is consistent with a pattern of seasonal migration into the Ausable River and Big Otter Creek and may explain the predominantly lower reach effects observed here. Indeed, seasonal pulse effects of non-native species on native species have been observed elsewhere where migration from lake habitats to tributaries is possible (Pfauserová et al. 2021). Nevertheless, Round Goby detections in Big Otter Creek 62 km upstream are suggestive of permanent, year-round populations. If populations of Round Goby permanently reside in lower reaches of the tributaries and dispersal and expansion continue to upstream reaches (Kornis et al. 2012, 2013), then native fishes will likely experience more sustained and consistent competition with Round Goby. Based on effects observed here, increasing Round Goby densities along greater expanses of the studied tributaries have the potential to lead to further darter and native benthic fish population declines and associated reductions in fish community diversity.

The assessment of the longitudinal changes in the relative abundance of Round Goby and native fish species and the associated impacts of Round Goby on fish community metrics in our tributaries provides evidence of the negative effects of Round Goby invasions on the relative abundance of key darter species. However, significant negative correlations between the CPUA of darter species and Round Goby were not observed, with co-occurrence patterns indicating moderately negative associations only for a subset of species (Ausable River: Rainbow Darter, Big Otter Creek: Johnny Darter and Percidae overall). Findings corroborate earlier studies but increase understanding of the longitudinal aspects of invasion impacts. As sampling did not directly address issues of inter-annual variability, temporal trends involving the relative abundances of fishes and fish community diversity metrics remain unknown in these particular tributaries. Accordingly, further sampling in Great Lakes tributaries is suggested as necessary for better evaluating Round Goby impacts across time and space and to gain greater understanding of how native fish communities will be ultimately influenced by this invader.

## 2.5 Figures and Tables

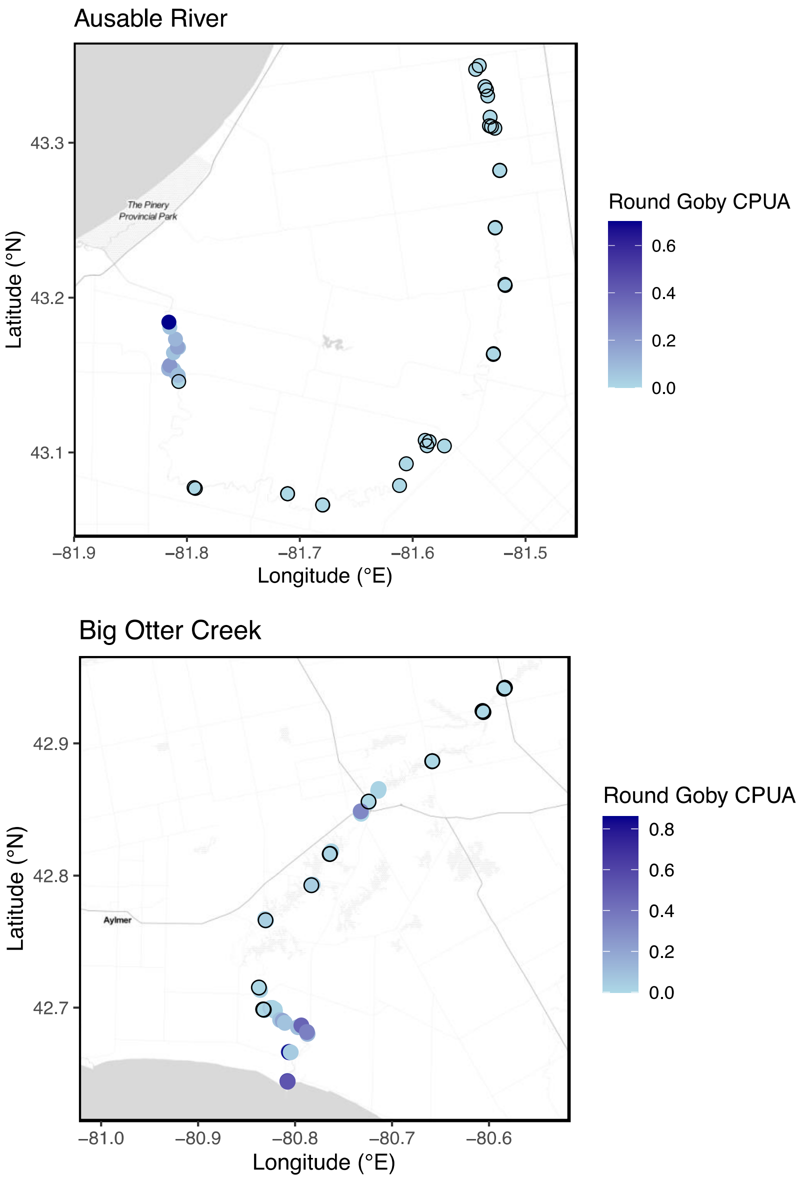

Figure 2.1 Round Goby CPUA (Number of fish × $(m^2)^{-1}$) in sampling locations along the Ausable River in 2017 and 2018 (n = 45 sites) and Big Otter Creek in 2018 (n = 50 sites). Sites where Round Goby was not detected (CPUA = 0) are outlined in black.

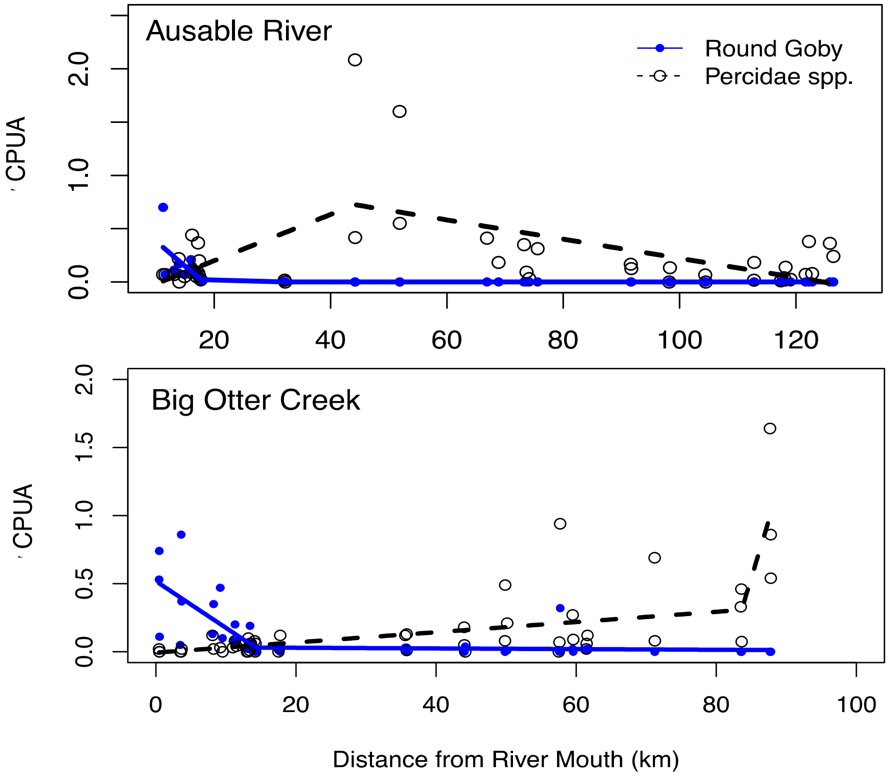

Figure 2.2 Broken-stick regression models and CPUA for Round Goby (solid blue line, blue dots; Adj. $R^2 = 0.51, 0.53$ for the Ausable River and Big Otter Creek, respectively) and Percidae species (dashed black line, open dots; Adj. $R^2 = 0.26, 0.57$ for the Ausable River and Big Otter Creek, respectively) at sampling locations along the Ausable River and Big Otter Creek in relation to the distance from river mouth (km). Percidae species from the Ausable River used in the model included: Johnny Darter (*Etheostoma nigrum*), Blackside Darter (*Percina maculata*), Greenside Darter (*Etheostoma blennioides*), Rainbow Darter (*Etheostoma caerulum*), and Logperch (*Percina caprodes*), whereas Percidae species from Big Otter Creek used in the model included: Johnny Darter, Blackside Darter, and Logperch. Percidae species collected in < 5% of sites within a tributary were excluded from analyses.

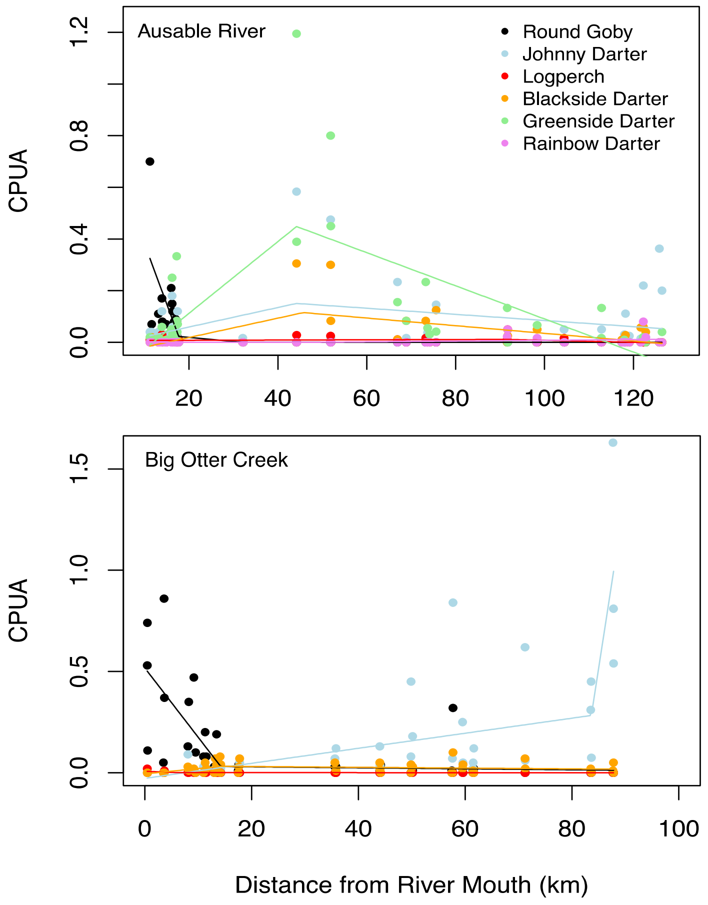

Figure 2.3 Broken-stick regression models and CPUA for Round Goby and individual Percidae species in sampling locations along the Ausable River (top panel) and Big Otter Creek (bottom panel) in relation to the distance from river mouth (km). Percidae species displayed for the Ausable River included: Johnny Darter (*Etheostoma nigrum*), Blackside Darter (*Percina maculata*), Greenside Darter (*Etheostoma blennioides*), Rainbow Darter (*Etheostoma caerulum*), and Logperch (*Percina caprodes*), whereas Percidae species displayed for Big Otter Creek included: Johnny Darter, Blackside Darter, and Logperch. Percidae species collected in < 5% of sites within a tributary were excluded from analyses.

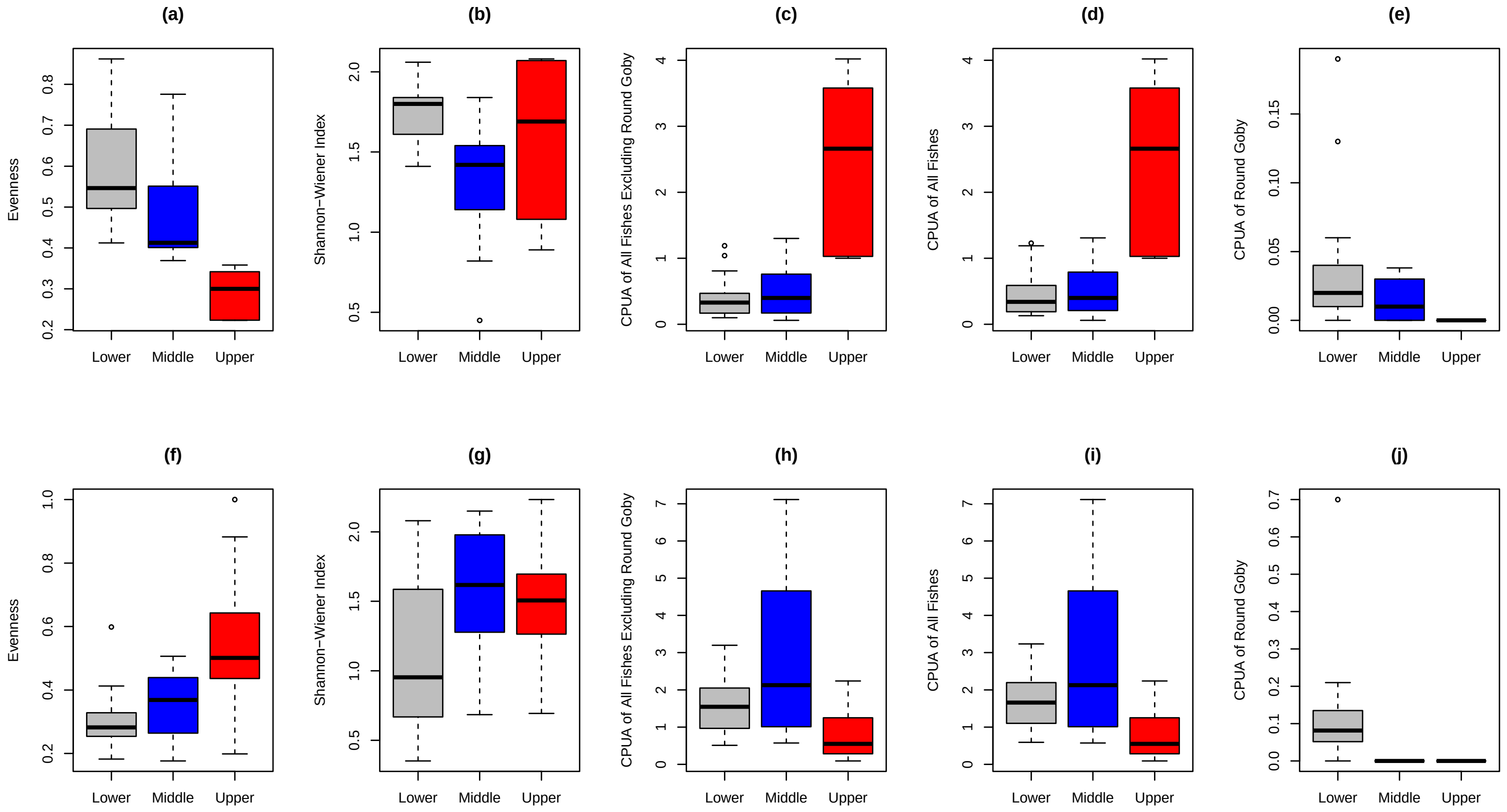

Figure 2.4 Boxplots comparing diversity metrics between site groupings in Big Otter Creek (top, a-e; lower sites: 13.1 - 14.1 km from river mouth, n = 13; middle sites: 35.8 - 49.9 km from river mouth, n = 9; upper sites: 71.2 - 83.6 km from river mouth, n = 5), and the Ausable River (bottom, f-j; lower sites: 11.2 - 17.7 km from river mouth, n = 16; middle sites: 32.1 - 75.6 km from river mouth, n = 12 sites; upper sites: 91.6 - 126.4 km from river mouth, n = 17). Diversity metrics

include: Evenness (a,f), Shannon-Wiener Index (b,g), CPUA of all sampled fishes excluding Round Goby (c,h), CPUA of all sampled fishes including Round Goby (d,i), and CPUA of Round Goby. Boxplots represent the median, minimum, maximum, and first and third quartiles of sites in each site grouping.

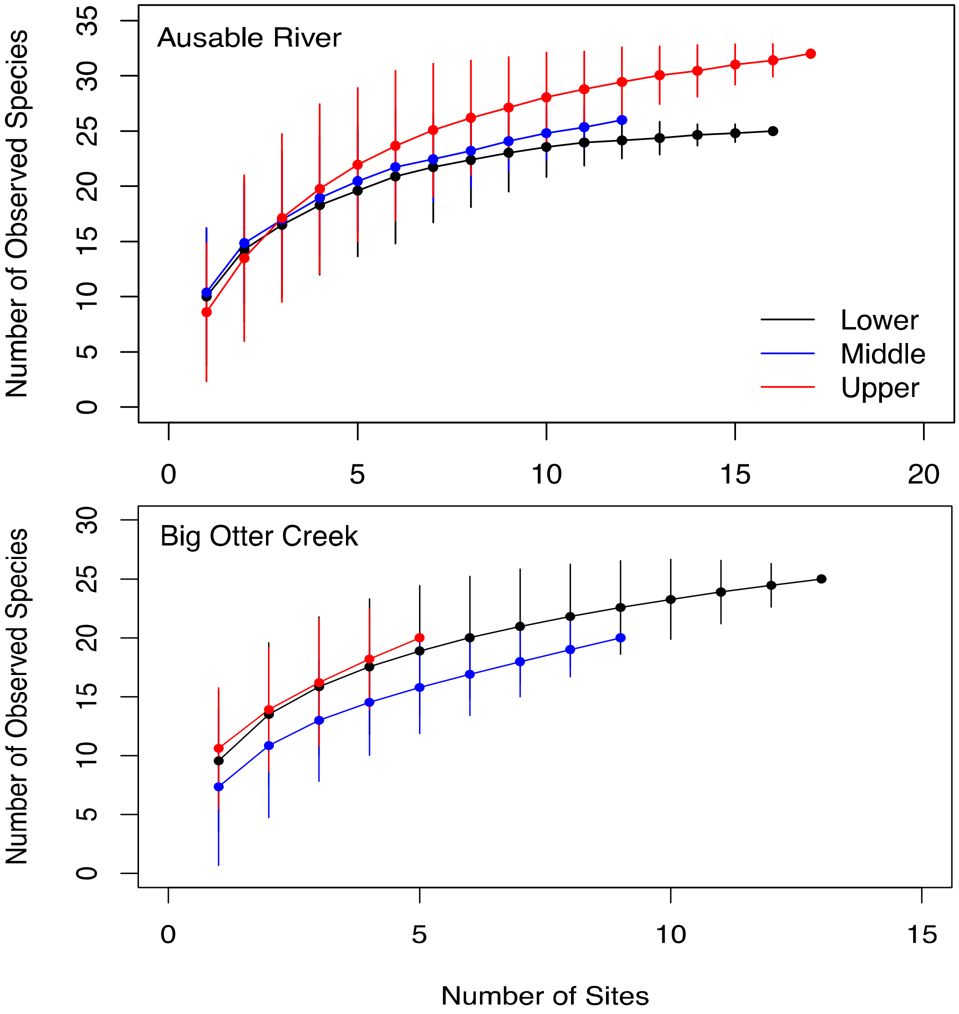

Figure 2.5 Species accumulation curves for Big Otter Creek (lower sites: 13.1 - 14.1 km from river mouth, n = 13; middle sites: 35.8 - 49.9 km from river mouth, n = 9; upper sites: 71.2 - 83.6 km from river mouth, n = 5) and the Ausable River (lower sites: 11.2 - 17.7 km from river mouth, n = 16; middle sites: 32.1 - 75.6 km from river mouth, n = 12 sites; upper sites: 91.6 - 126.4 km from river mouth, n = 17). Error bars are ± one standard deviation.

Table 2.1 Metrics used to compare fish communities within site groupings in each tributary where *s* defines the number of species, $p_i$ defines the proportion of individuals in the sample belonging to the *i*th species, $n_i$ defines the number of individuals of species *i* in the sample, $n_j$ defines the number of individuals of species *j* in sample, $x_i$ is the number of times species *i* is represented in the total *X* from one sample, and $y_i$ is the number of times species *i* is represented in the total *Y* from another sample.

| Metric | Formula |
|---|---|
| Shannon-Wiener index (*H*) | $H = -\sum_{i=1}^{s} p_i \ln p_i$ |
| Smith and Wilson's Index of Evenness ($E_{var}$) | $E_{var} = 1 - \left(\frac{2}{\pi}\right)\left[\arctan\left\{\frac{\sum_{i=1}^{s}\left(log_e(n_i) - \sum_{j=1}^{s} log_e(n_j)\,/s\right)^2}{s}\right\}\right]$ |
| Catch per unit area (*CPUA*) | $CPUA = \frac{Number\ of\ fish}{Seined\ area\ (m^2)}$ |
| Morisita-Horn Index of Similarity ($C_H$) | $C_H = \frac{2\sum_{i=1}^{s} x_i y_i}{\left(\frac{\sum_{i=1}^{s} {x_i}^2}{X^2} + \frac{\sum_{i=1}^{s} {y_i}^2}{Y^2}\right) XY}$ |

Table 2.2 Summary of linear regression correlation tests for CPUA of Round Goby and Percidae species in Big Otter Creek and the Ausable River, and the CPUA of the combined Percidae species in both tributaries. Sites where Round Goby CPUA = 0 were excluded.

| | Coefficients | Estimate | S.E. | t | p | Adj. $R^2$ | Spearman |
|---|---|---|---|---|---|---|---|
| Big Otter Creek | Intercept | 0.15 | 0.040 | 3.58 | 0.0011 | -0.032 | 0.016 |
| | Johnny Darter | 0.023 | 0.26 | 0.089 | 0.93 | | |
| | Intercept | 0.13 | 0.04 | 3.4 | 0.0017 | 0.055 | 0.29 |
| | Logperch | 15 | 9.1 | 1.7 | 0.1 | | |
| | Intercept | 0.17 | 0.049 | 3.6 | 0.0012 | -0.015 | -0.13 |
| | Blackside Darter | -1.1 | 1.4 | -0.73 | 0.47 | | |
| | Intercept | 0.15 | 0.043 | 3.49 | 0.0015 | -0.032 | 0.0010 |
| | All Percidae spp. | 0.0013 | 0.23 | 0.006 | 0.99 | | |

| | Coefficients | Estimate | S.E. | t | p | Adj. $R^2$ | Spearman |
|---|---|---|---|---|---|---|---|
| Ausable River | Intercept | 0.13 | 0.062 | 2.0 | 0.06 | -0.075 | 0.04 |
| | Johnny Darter | 0.14 | 0.9 | 0.16 | 0.88 | | |
| | Intercept | 0.11 | 0.059 | 1.8 | 0.09 | -0.042 | 0.18 |
| | Logperch | 3.1 | 4.7 | 0.66 | 0.52 | | |
| | Intercept | 0.15 | 0.055 | 2.8 | 0.016 | -0.054 | -0.15 |
| | Blackside Darter | -3.8 | 7.1 | -0.53 | 0.6 | | |
| | Intercept | 0.15 | 0.055 | 2.8 | 0.016 | -0.050 | -0.15 |
| | Greenside Darter | -0.27 | 0.48 | -0.56 | 0.58 | | |
| | Intercept | 0.15 | 0.065 | 2.3 | 0.039 | -0.068 | -0.093 |
| | All Percidae spp. | -0.12 | 0.37 | -0.34 | 0.74 | | |

Table 2.3 Summary of mean site habitat conditions (± standard error) for site groupings in Big Otter Creek and the Ausable River. Bold superscripts denote where mean habitat conditions differed significantly ($p < 0.05$) among site groupings within each tributary.

| | **Ausable River** | | | **Big Otter Creek** | | |
|---|---|---|---|---|---|---|
| | **Site Grouping** | | | **Site Grouping** | | |
| | **Lower** | **Middle** | **Upper** | **Lower** | **Middle** | **Upper** |
| **Stream Order** | 6 | 6 | 4 | 5 | 5 | 5 |
| **Water Temperature (°C)** | 22.7 (0.31) | 21.6 (0.56) | 22.7 (0.24) | 22.2 $^{\mathbf{A}}$ (0.63) | 19.6 $^{\mathbf{B}}$ (0.22) | 22.4 $^{\mathbf{A}}$ (0.61) |
| **Conductivity (μS)** | 474 $^{\mathbf{A}}$ (3.05) | 472 $^{\mathbf{A}}$ (8.03) | 589 $^{\mathbf{B}}$ (13.6) | 564 $^{\mathbf{A}}$ (2.51) | 599 $^{\mathbf{B}}$ (3.50) | 544 $^{\mathbf{C}}$ (7.46) |
| **Dissolved Oxygen (mg/L)** | 7.38 $^{\mathbf{A}}$ (0.07) | 7.36 $^{\mathbf{A}}$ (0.20) | 6.53 $^{\mathbf{B}}$ (0.35) | 9.10 $^{\mathbf{A,B}}$ (0.31) | 8.36 $^{\mathbf{A}}$ (0.14) | 10.2 $^{\mathbf{B}}$ (0.41) |
| **pH** | 8.28 $^{\mathbf{A}}$ (0.02) | 8.42 $^{\mathbf{B}}$ (0.02) | 8.29 $^{\mathbf{A}}$ (0.04) | 8.46 $^{\mathbf{A}}$ (0.03) | 8.37 $^{\mathbf{A}}$ (0.02) | 8.60 $^{\mathbf{B}}$ (0.05) |
| **Turbidity (NTU)** | 34.0 $^{\mathbf{A}}$ (5.57) | 31.9 $^{\mathbf{A}}$ (7.04) | 11.9 $^{\mathbf{B}}$ (1.91) | 26.8 (5.67) | 13.6 (1.53) | 9.35 (1.06) |
| **Stream Width (m)** | 17.3 $^{\mathbf{A}}$ (2.81) | 15.8 $^{\mathbf{A,B}}$ (1.24) | 10.4 $^{\mathbf{B}}$ (0.89) | 15.0 (0.56) | 15.0 (1.25) | 17.0 (4.98) |
| **Stream Depth (m)** | 1.1 $^{\mathbf{A}}$ (0.13) | 0.54 $^{\mathbf{B}}$ (0.07) | 0.46 $^{\mathbf{B}}$ (0.04) | 0.62 (0.04) | 0.60 (0.06) | 0.47 (0.12) |
| **Stream Velocity (m/s)** | 0.04 $^{\mathbf{A}}$ (0.01) | 0.11 $^{\mathbf{A,B}}$ (0.03) | 0.15 $^{\mathbf{B}}$ (0.03) | 0.28 (0.03) | 0.25 (0.03) | 0.24 (0.04) |
| **% Organic** | 0.00 (0.00) | 1.67 (0.94) | 2.65 (1.49) | 1.54 (1.54) | 0.00 (0.00) | 2.00 (2.00) |
| **% Clay** | 10.6 (4.78) | 0.00 (0.00) | 4.71 (2.41) | 0.00 (0.00) | 0.00 (0.00) | 0.00 (0.00) |
| **% Silt** | 10.6 (3.92) | 7.08 (2.57) | 10.6 (2.64) | 20.8 (2.39) | 17.8 (2.78) | 10.0 (5.48) |
| **% Sand** | 38.8 (6.82) | 27.1 (6.47) | 34.4 (7.93) | 68.5 $^{\mathbf{A}}$ (6.19) | 71.1 $^{\mathbf{A}}$ (7.72) | 34.0 $^{\mathbf{B}}$ (9.27) |
| **% Gravel** | 30.0 $^{\mathbf{A,B}}$ (6.12) | 41.7 $^{\mathbf{A}}$ (6.92) | 14.7 $^{\mathbf{B}}$ (6.25) | 5.38 $^{\mathbf{A}}$ (3.32) | 11.1 $^{\mathbf{A}}$ (6.55) | 42.0 $^{\mathbf{B}}$ (12.0) |
| **% Cobble** | 10.0 (3.65) | 8.75 (3.15) | 25.6 (8.26) | 3.10 (3.08) | 0.00 (0.00) | 12.0 (7.35) |
| **% Boulder** | 0.00 (0.00) | 13.8 (9.28) | 7.35 (4.31) | 0.77 (0.77) | 0.00 (0.00) | 0.00 (0.00) |
| **Dominant Substrate(s)** | Sand, Gravel | Sand, Gravel | Sand, Cobble | Sand | Sand | Sand, Gravel |

| | | | | | | |
|---|---|---|---|---|---|---|
| **Dominant Floodplain Use** | Shrubs/Woodland | Shrubs/Woodland,<br>Agricultural/Cropland | Shrubs/Woodland,<br>Agricultural/Cropland | Agricultural/Cropland | Agricultural/Cropland | Shrubs/Woodland |

Table 2.4 Summary of % CPUA (fish × $(m^2)^{-1}$) from site groupings in the Ausable River and Big Otter Creek.

| | | | Ausable River | | | Big Otter Creek | | |
|---|---|---|---|---|---|---|---|---|
| Family | Species | | Site Grouping | | | Site Grouping | | |
| | Common Name | Latin Name | Lower | Middle | Upper | Lower | Middle | Upper |
| **Atherinopsidae** | | | | | | | | |
| | Brook Silverside | *Labidesthes sicculus* | 1.97 | 0 | 0 | 0 | 0 | 0 |
| **Catostomidae** | | | | | | | | |
| | | *Catostomidae spp.* | 0 | 0 | 0 | 0.64 | 0.91 | 1.30 |
| | White Sucker | *Catostomus commersonii* | 0.29 | 0.18 | 0.28 | 17.5 | 7.83 | 12.1 |
| | Northern Hogsucker | *Hypentelium nigricans* | 0.04 | 0.45 | 1.85 | 0.79 | 0.21 | 2.49 |
| | Golden Redhorse | *Moxostoma erythrurum* | 0.98 | 0.03 | 0.28 | 0 | 0 | 0.08 |
| | Shorthead redhorse | *Moxostoma macrolepidotum* | 0 | 0.15 | 0 | 0 | 0 | 0.10 |
| | | *Moxostoma spp.* | 0.18 | 0 | 0 | 0.16 | 0 | 0 |
| | Greater Redhorse | *Moxostoma valenciennesi* | 0.46 | 0 | 0 | 0.16 | 0 | 0 |
| **Centrarchidae** | | | | | | | | |
| | Rock Bass | *Ambloplites rupestris* | 0 | 0.23 | 4.94 | 0 | 0 | 0 |
| | Pumpkinseed Sunfish | *Lepomis gibbosus* | 0 | 0 | 0.52 | 0.16 | 0 | 0 |
| | Bluegill Sunfish | *Lepomis macrochirus* | 0 | 0.07 | 0 | 0 | 0 | 0 |
| | Northern Sunfish | *Lepomis peltastes* | 0 | 0 | 0.57 | 0 | 0 | 0 |
| | | *Lepomis spp.* | 0 | 0 | 0.09 | 0 | 0 | 0 |
| | Smallmouth Bass | *Micropterus dolomieu* | 0.17 | 0.91 | 3.27 | 0 | 0.21 | 0 |
| | Black Crappie | *Pomoxis nigromaculatus* | 0 | 0 | 0.09 | 0 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Clupeidae** | | | | | | | | |
| | American Gizzard Shad | *Dorosoma cepedianum* | 0.20 | 0 | 0 | 0 | 0 | 0 |
| **Esocidae** | | | | | | | | |
| | Northern Pike | *Esox lucius* | 0 | 0 | 0.28 | 0 | 0 | 0 |
| **Gasterosteidae** | | | | | | | | |
| | Brook Stickleback | *Culaea inconstans* | 0 | 0 | 0 | 0 | 0 | 1.06 |
| **Gobiidae** | | | | | | | | |
| | Round Goby | *Neogobius melanostomus* | 7.36 | 0 | 0 | 8.59 | 2.43 | 0 |
| **Ictaluridae** | | | | | | | | |
| | Black Bullhead | *Ameiurus melas* | 0 | 0 | 0.07 | 0 | 0 | 0 |
| | Yellow Bullhead | *Ameiurus natalis* | 0 | 0 | 0.23 | 0 | 0 | 0 |
| | Channel Catfish | *Ictalurus punctatus* | 1.25 | 0.07 | 0 | 0 | 0 | 0 |
| | Stonecat | *Noturus flavus* | 0.14 | 0.20 | 0.08 | 0.16 | 0 | 0 |
| | Tadpole Madtom | *Noturus gyrinus* | 0 | 0 | 0 | 0 | 0.41 | 0 |
| **Leuciscidae** | | | | | | | | |
| | Central Stoneroller | *Campostoma anomalum* | 0 | 0.16 | 0.97 | 0 | 0 | 0.16 |
| | Northern Redbelly Dace | *Chrosomus eos* | 0 | 0 | 0 | 0 | 0 | 1.30 |
| | Emerald Shiner | *Notropis atherinoides* | 2.57 | 1.19 | 0.07 | 0 | 0 | 0 |
| | Ghost Shiner | *Notropis buchanani* | 65.7 | 1.99 | 0 | 0 | 0 | 0 |
| | Spottail Shiner | *Notropis hudsonius* | 0 | 0 | 0 | 0.16 | 0 | 0 |
| | Rosyface Shiner | *Notropis rubellus* | 0 | 28.0 | 17.3 | 1.75 | 1.03 | 0.49 |
| | | *Notropis spp.* | 0 | 0 | 0.36 | 0 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Mimic Shiner | *Notropis volucellus* | 6.04 | 25.0 | 1.78 | 2.86 | 0.82 | 0 |
| | Eastern Blacknose Dace | *Rhinichthys atratulus* | 0 | 0 | 0.07 | 0.48 | 0.21 | 5.75 |
| | Longnose Dace | *Rhinichthys cataractae* | 0 | 0 | 0 | 0 | 4.09 | 3.92 |
| | Spotfin Shiner | *Cyprinella spiloptera* | 0.91 | 5.75 | 8.10 | 8.43 | 1.85 | 0 |
| | | *Cyprinidae spp.* | 1.76 | 0.25 | 0.07 | 0.16 | 0 | 0.08 |
| | Striped Shiner | *Luxilus chrysocephalus* | 0.06 | 1.54 | 5.78 | 0 | 0 | 0 |
| | Common Shiner | *Luxilus cornutus* | 0.22 | 2.23 | 13.1 | 23.1 | 46.0 | 42.8 |
| | | *Luxilus spp.* | 0 | 0 | 6.46 | 9.54 | 0.41 | 2.28 |
| | Northern Pearl Dace | *Margariscus nachtriebi* | 0 | 0 | 0 | 0 | 0 | 0.08 |
| | Hornyhead Chub | *Nocomis biguttatus* | 0 | 0.08 | 8.39 | 0 | 0 | 0 |
| | River Chub | *Nocomis micropogon* | 0.24 | 1.47 | 1.08 | 8.90 | 1.64 | 0 |
| | | *Nocomis spp.* | 0 | 0 | 0 | 0.48 | 0 | 0 |
| | Golden Shiner | *Notemigonus crysoleucas* | 0 | 0 | 0 | 0.32 | 0 | 0 |
| | Bluntnose Minnow | *Pimephales notatus* | 1.85 | 12.7 | 8.84 | 0.95 | 1.03 | 1.63 |
| | Fathead Minnow | *Pimephales promelas* | 0 | 0 | 0 | 0 | 0.21 | 0.16 |
| | Creek Chub | *Semotilus atromaculatus* | 0 | 0.07 | 0 | 3.02 | 4.32 | 10.9 |
| **Percidae** | | | | | | | | |
| | Greenside Darter | *Etheostoma blennioides* | 3.80 | 9.83 | 3.88 | 0 | 0 | 0 |
| | Rainbow Darter | *Etheostoma caeruleum* | 0 | 0 | 1.31 | 0 | 0 | 0 |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | Johnny Darter | *Etheostoma nigrum* | 2.74 | 4.41 | 7.50 | 3.34 | 22.0 | 12.3 |
| | Yellow Perch | *Perca flavescens* | 0 | 0 | 0 | 1.59 | 0.21 | 0 |
| | Logperch | *Percina caprodes* | 0.50 | 0.23 | 0.61 | 0.16 | 0 | 0 |
| | Blackside Darter | *Percina maculata* | 0.26 | 2.84 | 1.80 | 6.68 | 4.21 | 0.98 |
| **Percopsidae** | | | | | | | | |
| | Trout-Perch | *Percopsis omiscomaycus* | 0.26 | 0 | 0 | 0 | 0 | 0 |

Table 2.5 Summary of Chao1 local richness estimates from each site grouping and the mean CPUA of Round Goby in sites within the site groupings in each tributary.

| **Tributary** | **Site Grouping** | **Number of Species** | **Number of Sites** | **Chao1 (+/- S.E.)** | **Mean Round Goby CPUA** |
|---|---|---|---|---|---|
| **Big Otter Creek** | Lower | 25 | 13 | 32.5 (7.68) | 0.04 |
| | Middle | 20 | 9 | 56 (43.7) | 0.01 |
| | Upper | 20 | 5 | 36.2 (16.2) | 0.0 |
| **Ausable River** | Lower | 25 | 16 | 26.4 (2.17) | 0.13 |
| | Middle | 26 | 12 | 48.5 (28.5) | 0.0 |
| | Upper | 32 | 17 | 39.5 (7.07) | 0.0 |

# Chapter 3: Ecological resource use and overlap of invasive Round Goby (*Neogobius melanostomus*) and native fishes in a Lake Erie tributary

## 3.1 Introduction

The ability to outcompete native species for resources (e.g., food and habitat) is a common attribute of aquatic invasive species (AIS) (Pimm et al. 1985; Volpe et al. 2001; Seiler and Keeley 2009). Species establishment is more likely when AIS invade ecosystems with low diversity, occupy vacant niches, and/or display a high plasticity in resource use (Peterson and Vieglais 2001; Stachowicz and Byrnes 2006; Hayden et al. 2013, 2014; Fridley and Sax 2014), all of which reduce interspecific competition with native species. Resource availability can also determine the success of an invader. Low resource availability within an ecosystem requires AIS to compete for limited resources with native species (Giller 1984; Chen et al. 2011) and can impede AIS establishment and/or negatively affect native species. However, once AIS become established, the trophic niches of native species can diverge and become partitioned due to shifts to different or previously underexploited resources (Syväranta and Jones 2008), thereby limiting competition, and facilitating the persistence of the invader within the community (Tran et al. 2015; Britton et al. 2019).

The Round Goby (*Neogobius melanostomus*), which is native to the Ponto-Caspian region, was first detected in the Great Lakes basin in 1990 in the St. Clair River (Jude et al. 1992). In the years following establishment, Round Goby reached high densities in nearshore lake habitats and has been associated with reduced abundances of Mottled Sculpin (*Cottus bairdii*), Logperch (*Percina caprodes*), Johnny Darter

(*Etheostoma nigrum*), Rainbow Darter (*Etheostoma caeruleum*), and Tessellated Darter (*Etheostoma olmstedi*) due to competition for food and habitat resources (French and Jude 2001; Lauer et al. 2004; Balshine et al. 2005; Krakowiak and Pennuto 2008; Bergstrom and Mensinger 2009; Morissette et al. 2018). Several studies have also provided experimental evidence for the ability of Round Goby to outcompete other species for resources due to their aggressive nature and propensity to achieve greater body sizes than similar benthic species (Charlebois et al. 2001; Balshine et al. 2005; van Kessel et al. 2011). Characteristic of successful invaders (Ruesink 2005), Round Goby have also been shown to display patterns of generalized feeding along the invasion front (Dashinov and Uzunova 2020) and adapt quickly to the biotic and abiotic conditions that determine trophic structure and energy flow (Herlevi et al. 2018).

Roughly a decade after their establishment in the Great Lakes, Round Goby underwent a secondary expansion by moving upstream into numerous Great Lakes tributaries, which has led to variable impacts on native fish communities. In Lake Michigan tributaries, no adverse impacts to native fish communities were detected after Round Goby establishment, although studies were conducted only shortly after invasion and over a limited spatial scale, i.e., < 20 km upstream from the lakes (Kornis et al. 2013; Malone 2016). In contrast, in several New York state tributaries of Lake Erie, the establishment of Round Goby was associated with the extirpation of Rainbow Darter and Johnny Darter, presumably due to competition for preferred benthic macroinvertebrate prey (Krakowiak and Pennuto 2008). Also supporting claims of competition between Round Goby and native species, gut content analyses indicated that six species (Brindled Madtom (*Noturus miurus*), Logperch, Eastern Sand Darter

(*Ammocrypta pellucida*), Blackside Darter (*Percina maculata*), Johnny Darter (*Etheostoma nigrum*), and Greenside Darter (*Etheostoma blennioidies*)) displayed significant dietary overlap with Round Goby after its establishment in the Sydenham River, a tributary of Lake St. Clair (Firth et al. 2020). While gut content analyses can provide direct evidence of competitive interactions, it reflects recent diets (typically within 48 hours of capture), tends to overestimate the importance of hard-shelled versus soft-bodied prey (Brush et al. 2012; Miano et al. 2021), and has previously misrepresented the diet of Round Goby by overestimating the proportion of dreissenids in its diet (Barton et al. 2005; Brush et al. 2012).

Compared to gut content analyses, stable isotope analysis (SIA) provides longer-term evidence of species' dietary patterns in an ecosystem (Grey 2006; Rybczynski et al. 2008). Thus, SIA can provide a longer-term view of the resource use of Round Goby and help to better predict their potential impacts on native stream fishes. In particular, carbon ($\delta^{13}C$) and nitrogen ($\delta^{15}N$) have been used to quantify the trophic relationships and resource use of invasive fish species (e.g., Vander Zanden et al. 1999; Cucherousset et al. 2012; Jacobs et al. 2017; Coulter et al. 2019; Dominguez Almela et al. 2021). SIA has also previously been used to characterize the resource use of Round Goby in the Great Lakes. For example, in the offshore habitats of Lake Ontario, Round Goby were shown to have the largest isotopic niche when compared to native forage fishes (indicative of a generalist feeding strategy), which has allowed them to partition their resource use to avoid competition with other native species (Mumby et al. 2018). In Lake Superior, Round Goby similarly displayed a generalist feeding strategy, with both a broad and plastic isotopic niche (Pettitt-Wade et al. 2015). While there is evidence for ontogenetic diet shifts toward preferential consumption of dreissenids once individuals reach a threshold size (100– 130 mm total length) in the Great Lakes (Miano et al. 2021), studies using SIA have also found that individuals of all sizes consume diverse diets composed of chironomids, cladocerans, copepods, and amphipods (Barton et al. 2005; McCallum et al. 2017).

Although resource use of Round Goby has been well studied with SIA within the Great Lakes (e.g., Barton et al. 2005; Pettitt-Wade et al. 2015; McCallum et al. 2017; Mumby et al. 2018; Miano et al. 2021), to our knowledge it has not been applied to assess the resource use effects of Round Goby on native fish communities in Great Lakes tributaries. Therefore, the trophic impacts of Round Goby on native fishes inhabiting tributary ecosystems remain uncertain. To gain a better understanding of the ecological impacts of an invasive species, such as Round Goby, it is critical to understand both the range of resources it can use and the nature of its trophic interactions with native species. Here, we first characterize the variability of resource use of Round Goby in Great Lakes populations and compare the resource use of Round Goby in a Lake Erie tributary (Big Otter Creek) to lake populations. We then determine how the presence of Round Goby has affected the isotopic niche, relative abundance, and diversity of the benthic and benthopelagic native fishes within Big Otter Creek by comparing sites where Round Goby is present and absent. Based on niche theory (e.g., Pianke 1974), we hypothesized that benthic and benthopelagic fishes would show altered isotopic niches (i.e., changes in position and size), decreased relative abundances, decreased condition, and decreased diversity in sites where Round Goby was present due to direct competition and resource overlap.

## 3.2 Methods

### 3.2.1 Sample Collection

Fishes were collected by Fisheries and Oceans Canada (DFO) between July 9 - 19th and September 24 - 26th, 2018 using a bag seine (3.0 mm bag mesh, 3.0 mm wing mesh, 9.144 m length) in Big Otter Creek (a tributary of Lake Erie, river mouth: 42°64′N, 80°81′W). Sites were chosen to ensure that a diverse set of native species were captured, and to ensure that there were some common species between sites where Round Goby was present and absent to facilitate comparisons. At each site, seining was completed in a downstream direction with three consecutive hauls. Where Round Goby was present, captured species included: Blackside Darter, White Sucker (*Catostomus commersonii*), Common Shiner (*Luxilus cornutus*), River Chub (*Nocomis micropogon*), and Spotfin Shiner (*Cyprinella spiloptera*). Where Round Goby was absent, captured species included: White Sucker, Longnose Dace (*Rhinichthys cataractae*), Johnny Darter, Northern Hogsucker (*Hypentelium nigricans*), Blacknose Dace (*Rhinichthys atratulus*), Common Shiner (*Luxilus cornutus*), Bluntnose Minnow (*Pimephales notatus*), Creek Chub (*Semotilus atromaculatus*), and Brook Stickleback (*Culaea inconstans*). Captured fishes were kept in bankside aquaria, identified to species, and enumerated for each haul. A subset of fishes was kept and preserved in a 10% formalin solution for confirmation of species identification and subsequently frozen for additional laboratory analyses (including measurements of total length (mm) and mass (g)). Complete sampling details can be found in Barnucz et al. (2020). Catch per unit area sampled (CPUA) was determined as the aggregate number of captured fish × seined area $(m^2)^{-1}$, as previously used in McAllister et al. 2022.

### 3.2.2 Stable Isotope Analysis

A minimum of 7 individuals of each captured species were selected for stable isotope analysis. Muscle samples (skin removed) were dissected from the dorsal area, dried for 48 hrs at 60ºC in a scientific drying oven (Yamato DX 600; Yamato Scientific Company, Tokyo, Japan), weighed, and analyzed for δ$^{13}$C and δ$^{15}$N at the University of Waterloo Environmental Isotope Laboratory (Waterloo, Ontario, Canada). SIA values were determined using a Delta Plus continuous-flow isotope ratio mass spectrometer (Therm Finnigan, Bremen, Germany) coupled to a Carlo Erba elemental analyzer (CHNS-O EA1108; Carlo Erba, Milan, Italy) with a reportable analytical precision of ±0.2‰ (δ$^{13}$C) and ±0.3‰ (δ$^{15}$N). Analytical precision was determined by analysis of laboratory working standards cross-calibrated to International Atomic Energy Agency standards $CH_6$ for δ$^{13}$C and N$^1$ and N$^2$ for δ$^{15}$N, with no less than 20% of the samples included in any run consisting of standards and reference materials. Results were expressed in standard delta notation (δ) as parts per thousand differences with respect to the international reference standards Vienna Pee Dee Belemnite (VPDB) and atmospheric nitrogen used respectively for δ$^{13}$C (Craig 1957) and δ$^{15}$N (Mariotti 1983). Measurement consistency was also established by running duplicates for every tenth sample. Because most of the C:N ratios in sampled muscle tissues were uniformly low (i.e., C:N < 4), tissues were not lipid-corrected (e.g., Jardine et al. 2013).

### 3.2.3 Round Goby Resource Use Comparison to Great Lakes

To assess the variation in resource use by Round Goby within and among studied populations in the Great Lakes basin, we collected literature-reported stable isotope measures from the following studies: Lake Ontario (Zhang et al. 2012; Brush et al. 2012; Rush et al. 2012; Fitzsimons et al. 2013, 2022; Paterson et al. 2014; Colborne et al. 2016; Jacobs et al. 2017; Mumby et al. 2018; Bruestle et al. 2019, Power, unpublished data), Lake Erie (Campbell et al. 2009; Guinan et al. 2015), Lake St. Clair (Pettitt-Wade et al. 2015) Lake Huron (Omara et al. 2015; Paterson et al. 2020), Lake Michigan (Feiner et al. 2018; Conard et al. 2021), and Lake Superior (Pettitt-Wade et al. 2015). Data for the Niagara River were assigned to Lake Erie or Lake Ontario depending on whether the data were collected from above or below Niagara Falls. Collected data consisted of means (fish length and $\delta^{13}C$), sample size, and standard deviation or mean error with conversion from standard error to standard deviation accomplished by multiplying the standard error by the square root of the sample size (Zar 2010). Given the minimal differences between bulk $\delta^{13}C$ and lipid extracted/corrected values reported in Mumby et al. (2018), no attempt was made to account for among-study differences in lipid extraction/correction. Resource ranges were estimated for each study as mean $\delta^{13}C$ standard deviation and coefficients of variation were estimated using mean and standard deviation for each study (Zar 2010). As baseline data were often missing or inconsistently reported, comparisons among studies with respect to $\delta^{15}N$ were not attempted. Patterns in the data related to differences in mean length-at-capture were examined and tested using standard ordinary least squares (OLS) regression methods (Zar 2010).

### 3.2.4 Statistical Analysis

For comparative purposes, estimated carbon ranges (mean ± SD) were plotted and compared to data obtained for Big Otter Creek. An among-study carbon range was similarly calculated from reported mean study data using standard parametric statistical methods (Zar 2010).

To determine whether the presence of Round Goby led to differences in the resource use of co-occurring stream fishes, sampled sites were classified as either Round Goby present (Round Goby catch per unit area (CPUA) > 0) or Round Goby absent (Round Goby CPUA = 0) (Fig. 3.1) based on McAllister et al. 2022. Species were further classified as either benthic or benthopelagic based on the Ontario Freshwater Fishes Life History Database (Eakins 2022), with such groupings reflecting among-species differences in habitat use and feeding. While Round Goby is a benthic species, Round Goby was excluded from the benthic species grouping for the purposes of between-group comparisons unless otherwise noted.

To compare between sites where Round Goby was present and absent, species diversity was determined using the widely applied Shannon-Wiener diversity index (Shannon and Weaver 1949; Spellerberg and Fedor 2003) and evenness was calculated using Smith and Wilson’s Index of Evenness as it is independent of species richness and sensitive to both rare and common species in the community (Smith and Wilson 1996). Benthic and benthopelagic species used in the calculations consisted of all species used for stable isotope analyses, which included (on a CPUA basis) 95.2% of all fishes captured, 98.8% of all benthic fishes captured, and 92.8% of all benthopelagic fishes captured. The relationship between CPUA and species richness of benthic and benthopelagic species at sites where Round Goby was present and absent was determined using linear and exponential regression.

The NicheRover package was used to calculate the 95% directional pairwise probabilities (%) that species A would occur within the niche region ($N_R$) of species B and vice versa (Swanson et al. 2015). Both $N_R$ and overlap are insensitive to variation in sample sizes (Swanson et al. 2015) and thus allow niche size estimates for species captured in unequal numbers. To determine whether the % overlap of benthic and benthopelagic species differed between sites where Round Goby is present or absent, one-way ANOVAs were used and assumptions of normality and homogeneity of variance were tested for each model using the Shapiro-Wilk *W* test and Levene's test. Changes to the proportion of significant overlaps (>60%) of benthic and benthopelagic groups in sites where Round Goby was present or absent were also tested using one-tailed or two-tailed two-proportion *Z*-tests, with the 60% threshold for ecological significance determined following (Zaret and Rand 1971; Wallace 1981).

The Stable Isotope Bayesian Ellipses in R (package 'SIBER') (Jackson et al. 2011) was used to calculate the Bayesian ellipse areas with small sample size correction (SEAc). Metrics proposed by Layman et al. (2007) were used to characterize resource use for each of the defined trophic groupings of species (i.e., benthic and benthopelagic). Metrics included: nitrogen range (NR; the distance between the two samples with the most enriched and most depleted $\delta^{15}N$ values), carbon range (CR; computed similarly to NR), nearest-neighbour distance (NND; the mean of the Euclidean distances to each sample's nearest neighbour in the SIA biplot and a measure of overall sample density), standard deviation of the nearest-neighbour distances (SDNND; a measure of evenness in the SIA biplot), distance to centroid (CD; average Euclidean distance of each sample to the $\delta^{13}C$-$\delta^{15}N$ centroid, i.e., the mean of the SIA data in a given sample grouping) and the area of the convex hull (TA; a measure of the total amount of niche space occupied). All analyses were conducted in R version 4.0.4 (R Core Team 2021). Differences in mean isotopic values and Layman metrics between life history groups (benthic and benthopelagic) where Round Goby was present and absent were evaluated using one-way ANOVAs. Finally, significant differences in condition as measured by Fulton's K were also assessed using one-way ANOVA.

## 3.3 Results

### 3.3.1 Round Goby Isotopic Niche in Waterbodies of the Great Lakes

Round Goby demonstrated high isotopic niche plasticity across the Great Lakes basin with a trend towards littoral resource use (i.e., more enriched $\delta^{13}C$) (Fig. 3.2). Most study values fell within the computed inter-population range (-24.3 to -17.7‰) with the exception of values reported for the Bay of Quinte (Power, unpublished data, Brush et al. 2012), the Lower Niagara River (Bruestle et al. 2019), and sites in Lake Superior (Pettit-Wade et al. 2015). Mean within-site range in resource use (2.1‰) was significantly smaller than the among-site range (6.6‰, t-test, $p < 0.001$). In Big Otter Creek, the range of resource use by Round Goby was significantly smaller (1.3‰) than within the Great Lakes generally (t-test, $p < 0.001$) and with the exception of sites in Lake Superior (Pettit-Wade et al. 2015), covered a lower range of $\delta^{13}C$ values.

### 3.3.2 Round Goby Impacts to Benthic and Benthopelagic Fish Communities

The isotopic niche occupied by benthic species (Fig. 3.3) was reduced by 51% in comparison to where Round Goby was present, whereas the isotopic niche occupied by benthopelagic species remained essentially the same when Round Goby was present (2.73‰$^2$) and absent (2.68‰$^2$) (Table 3.1). Benthopelagic species displayed a considerable shift in resource use in the presence of Round Goby, with the centroid of the trophic grouping shifting up in $\delta^{13}C$ (0.77‰) and down in $\delta^{15}N$ (-1.19‰) (Fig. 3.4). Benthic species displayed shifts in resource use and trophic position that were approximately half as large as those seen in the benthopelagic species, with the centroid for the benthic species shifting down in both $\delta^{13}C$ (-0.32‰) and $\delta^{15}N$ (-0.63‰) when Round Goby was present.

Coincident with shifts in trophic niche space, Fulton's K index of fish condition declined significantly for benthic fishes in the presence of Round Goby (ANOVA: $F_{1, 151} = 17.716$, $p < 0.001$) but not for benthopelagic fishes (ANOVA: $F_{1, 115} = 0.417$, $p = 0.520$). Relative to species richness, the CPUA of the combined benthic and benthopelagic species increased (Table 3.2, Fig. 3.5) at a higher and exponential rate (regression $p < 0.001$, $R^2 = 0.989$) when Round Goby was absent as compared to the linear rate when Round Goby was present (regression $p = 0.018$, $R^2 = 0.705$).

As for species found across both site classifications (i.e., Round Goby present and absent), the isotopic niche of the benthic White Sucker more than doubled at sites where Round Goby was absent as compared to where it was present (e.g., 2.94 vs. 1.24‰$^2$) while the isotopic niche of the benthopelagic Common Shiner did not differ (e.g., 1.97 vs. 1.92‰$^2$) (Appendix).

Round Goby had a high probability of overlapping the isotopic niche of both benthopelagic species (92.5%) and benthic species (85.3%) (Table 3.3), whereas the benthic species had a higher probability of overlapping with Round Goby (66.0%) than benthopelagic species (34.3%). Where Round Goby was present, benthopelagic species had a low probability of overlap with benthic species (27.8%), while benthic species had a 69.4% probability of overlap with benthopelagic species. Where Round Goby was absent, both benthic and benthopelagic species had a greater than 86% chance of overlap with each other.

Between sites where Round Goby was present or absent, no significant differences were detected in the % overlap between benthic species (ANOVA: $F_{1,20} = 0.731$, $p = 0.88$) or benthopelagic species (ANOVA: $F_{1,16} = 3.5$, $p = 0.12$) (Fig. 3.6). However, the % overlap between benthic and benthopelagic species is significantly higher in sites where Round Goby was absent (ANOVA: $F_{1,50} = 4.0$, $p = 0.002$). The proportion of significant overlaps (> 60%) between benthic species (one-tailed $Z = 0.135$, $p = 0.45$) and benthopelagic species (two-tailed $Z = 1.37$, $p = 0.17$) did not differ significantly between sites based on the presence or absence Round Goby, whereas significant overlaps between benthic and benthopelagic species were more common at sites where Round Goby was absent (0.55) than present (0.25) (one-tailed $Z = 1.824$, $p = 0.03$).

Blackside Darter, White Sucker, and River Chub had the highest probability (96.3%, 60.6%, and 56.8%, respectively) of overlapping the isotopic niche of Round Goby while Common Shiner and Spotfin Shiner had the lowest probabilities (32.0% and 10.7%, respectively) of overlap (Appendix). Round Goby had the highest probability of overlapping the isotopic niche of River Chub, White Sucker, and Common Shiner (91.5%, 77.9%, and 65.2%, respectively) and the lowest probabilities of overlapping with Blackside Darter and Spotfin Shiner (47.8% and 12.2%, respectively).

## 3.4 Discussion

Our study found that Round Goby displayed high niche plasticity across the Great Lakes basin. Where Round Goby was present in Big Otter Creek, benthic species underwent isotopic niche compression and occupied isotopic niches that overlapped significantly with Round Goby. While benthic species displayed some ability to shift the centroid of the isotopic niche, benthopelagic species were much more successful at shifting their isotopic niche and consequently experienced lower overlap with Round Goby. The effects of niche compression on benthic species were evident in the decline in mean fish condition, a fate which benthopelagic species appeared to have avoided due to the larger isotopic niche shift away from Round Goby.

High niche plasticity has been shown to lead to greater success for invasive fishes in novel environments (Layman and Allgeier 2012; Grabowska and Przybylski 2014; Nurkse et al. 2016; Tonella et al. 2018; Özdilek et al. 2019), by allowing invaders the flexibility to consume a wide variety of prey and grow rapidly (Hayden et al. 2014). Consequently, there are numerous examples of invasive fishes whose range expansions and successful introductions have been facilitated by high niche plasticity. For example, the Western Mosquitofish (*Gambusia affinis*) has successfully established in every continent but Antarctica (Pyke 2008) owing to its ability to consume a wide variety of prey typically found in degraded ecosystems (Lee et al. 2018). Establishment of other invasive fishes such as Largemouth Bass (*Micropterus salmoides*) in European rivers (Almeida et al. 2012) and Silver Carp (*Hypophthalmichthys molitrix*) in the Mississippi rivershed (Coulter et al. 2019) have also been attributed to high niche plasticity. Thus, the widespread establishment of Round Goby in the Great Lakes basin has likely also been facilitated by its high niche plasticity (Raby et al. 2010; Brush et al. 2012; Pettitt-Wade et al. 2015; Nurkse et al. 2016), with opportunistic feeding behaviours also suspected in the invasion success of Round Goby in the Baltic Sea (Nurkse et al. 2016).

Numerous diet studies of Round Goby in the Great Lakes basin and elsewhere have established Round Goby as a generalist feeder. In the Great Lakes, Round Goby consumes dreissenids, chironomids, cladocerans, copepods, crayfish, dragonflies, isopods, mayflies, fish eggs, and amphipods (Corkum et al. 2004; Barton et al. 2005; McCallum et al. 2017; Miano et al. 2021). After its range expansion from the Great Lakes upstream into tributaries, where dreissenids are less available, Round Goby has continued to demonstrate a generalist diet relying largely on chironomids, mayflies, amphipods, elmids, snails, caddisflies (Phillips et al.; Carman et al. 2006; Pennuto et al. 2018). Similarly in Europe, Round Goby preferred trichoptera, megaloptera, coleoptera, and gastropods in the Sava River (Piria et al. 2016), chironomids, amphipods, cladocerans, and bryozoans in the Danube River of Slovakia (Števove et al. 2013), and other organisms spanning 76 taxa including chironomids, trichopterans, and ephemeropterans in the Lower Danube River tributaries of Bulgaria (Dashinov and Uzunova 2020). Evidently, Round Goby can consume a wide variety of local prey sources regardless of geographical location and habitat, which has led to its proliferation and extensive competition with native species. This suggests, as has been noted here, that understanding the range of resource use in one environment, e.g., lakes, will not necessarily predict resource use in adjacent habitats such as tributary systems. For example, Round Goby resource use in Big Otter Creek tended to the extreme of the $\delta^{13}C$ values recorded for the other Great Lakes basin study sites.

The ability to outcompete native species for habitat and resources is a common trait among successful invasive species (Elton 1977; Vilà and Weiner 2004; Cucherousset and Olden 2011; Thomson et al. 2016). In addition to a highly plastic niche and generalist diet, a strong competitive ability has also facilitated the expansion and success of Round Goby in novel habitats (Kornis et al. 2012). Round Goby has caused the diminished abundance of prey in invaded lacustrine and riverine habitats (Lederer et al. 2008; Raby et al. 2010), and thus negatively affects the native fishes relying on those resources. Round Goby can also outcompete other species for habitat due to its aggressive behaviour and capacity to reach greater body sizes than species occupying similar habitats (Charlebois et al. 2001; Balshine et al. 2005), as has been shown in laboratory experiments (van Kessel et al. 2011). Thus, both a superior competitive ability and a generalist diet have likely contributed to the establishment of Round Goby in tributaries of the Great Lakes and the trophic alterations observed in this study.

The trophic shift displayed by benthopelagic species may be explained by competitive exclusion by Round Goby. Increased competitive interactions between species can result in niche compression and, therefore, shifts toward increased specialization (i.e., partitioning) by native species as a means of avoiding overlap in resource use (Van Valen 1965; Olsson et al. 2009; Thomson et al. 2016). For example, the isotopic niches of Gudgeon (*Gobio gobio*) and Roach (*Rutilus rutilus*) were reduced and individuals shifted to a more specialized diet after the introduction of Pumpkinseed Sunfish (*Lepomis gibbosus*) (Copp et al. 2017). Additionally, Lake Trout (*Salvelinus namaycush*) shifted their resource use from littoral prey fish to pelagic zooplankton after the introduction of invasive Rock Bass (*Ambloplites rupestris*) and Smallmouth Bass (*Micropterus dolomieu*) into several Canadian lakes (Vander Zanden et al. 1999). Lake Trout invasions in northwestern Montana in turn have caused trophic dispersion and subsequent trophic displacement via interference competition, as native Bull Trout (*Salvelinus confluentus*) and other fishes have increasingly come to depend on littoral resources (Wainright et al. 2021). The presence of Round Goby in Big Otter Creek appears to have caused similar trophic displacement and niche partitioning/specialization with benthic and benthopelagic fishes. Such shifts can result in the use of less preferred resources (Fausch and White 1981; Abbey-Lee et al. 2013; Sebastián et al. 2015), which may affect fish condition. Shifts detected here appear to have prevented reduction in condition among benthopelagic fishes but not among the benthic fishes likely to interact most with Round Goby due to competition (Henseler et al. 2020).

The substantial overlap in isotopic niche between several benthic species (Blackside Darter and White Sucker) and Round Goby in our study aligns with findings from a previous study of diet overlap between Round Goby and native benthic species in a Great Lakes tributary. Brindled Madtom (*Noturus miurus*), Logperch (*Percina caprodes*), Eastern Sand Darter (*Ammocrypta pellucida*), Blackside Darter, Johnny

Darter (*Etheostoma nigrum*), and Greenside Darter (*Etheostoma blennioides*) all shared significant overlap with Round Goby in the Sydenham River and increased their specialized feeding (Firth et al. 2020). While many invasive fishes are more likely to establish in ecosystems with low diversity and occupy vacant or underused niches upon introduction (Shea and Chesson 2002; Fridley and Sax 2014; Tran et al. 2015), Round Goby was still able to succeed in Great Lakes tributaries despite these ecosystems containing the most diverse freshwater fish communities in Canada (Staton and Mandrak 2005) and the evidence of its significant overlap in resource use with many native fishes (Firth et al. 2020). Given the renowned competitive ability of Round Goby (Charlebois et al. 2001; Balshine et al. 2005; Van Kessel et al. 2011) and the extensive overlap between Round Goby and benthic species observed in this study (especially Blackside Darter and White Sucker), native benthic species may experience further displacement and/or population declines if resources become limited (Pianka 1974; Ricciardi et al. 2013), or Round Goby abundances increase. Although niche overlap for extended periods of time is possible if resources remain abundant or if overlap is transient, prolonged competition can lead to eventual competitive exclusion or local extirpation (Elton 1927; Bolnick 2001).

Round Goby was associated with reduced relative abundance of native fishes in Big Otter Creek, which supports previous findings from the system (McAllister et al. 2022) and other Great Lakes tributaries (Krakowiak and Pennuto 2008). Generally, as the abundance of invasive species increases, populations of native species will undergo declines in abundance (Bradley et al. 2019). Despite the correlated reduction in relative abundance, species richness and other diversity metrics did not appear to be affected by the presence of Round Goby. Species richness is a stable ecosystem metric that often requires the removal or displacement of species to elicit change (Bradley et al. 2019). Nonetheless, the reduced relative abundance of fishes in sites where Round Goby were found may eventually lead to reduced community diversity in Big Otter Creek, especially given the evidence for reduced benthic fish condition at sites occupied by Round Goby.

Collectively, our study demonstrated the high niche plasticity and resource use among Round Goby in the Great Lakes and that even with restricted resource use in tributary ecosystems, Round Goby can trigger niche shifts in resident benthic and benthopelagic species. Smaller niche shifts among benthic species have been associated with reduced fish conditions, likely because of greater competitive interactions with Round Goby. Paired with the previous findings of Round Goby potentially causing population declines and/or displacement of native benthic fishes in Big Otter Creek (McAllister et al. 2022), this study adds to the literature that has documented the effects of Round Goby invasions on native fishes. The diet and resource use of Round Goby has been well studied in the Great Lakes with stable isotope analysis, but to our knowledge, this is the first study to use stable isotope analysis to determine the resource use and trophic impacts to fish communities by Round Goby in tributaries flowing directly into the Great Lakes. While findings here show how Round Goby has affected the native fish community in Big Otter Creek, future research should determine the resource use of Round Goby and its impacts on fish communities in other tributaries of the Great Lakes. Because the isotopic signature of fish muscle tissue only reflects the diet from the preceding few months (Hesslein et al.

1993; Maruyama et al. 2001), the resource use relationships found in this study are likely only reflective of the summer season. Thus, future research should also aim to determine whether Round Goby impacts on the resource use of native fishes vary intra- and interannually in Great Lake tributaries.

## 3.5 Figures and Tables

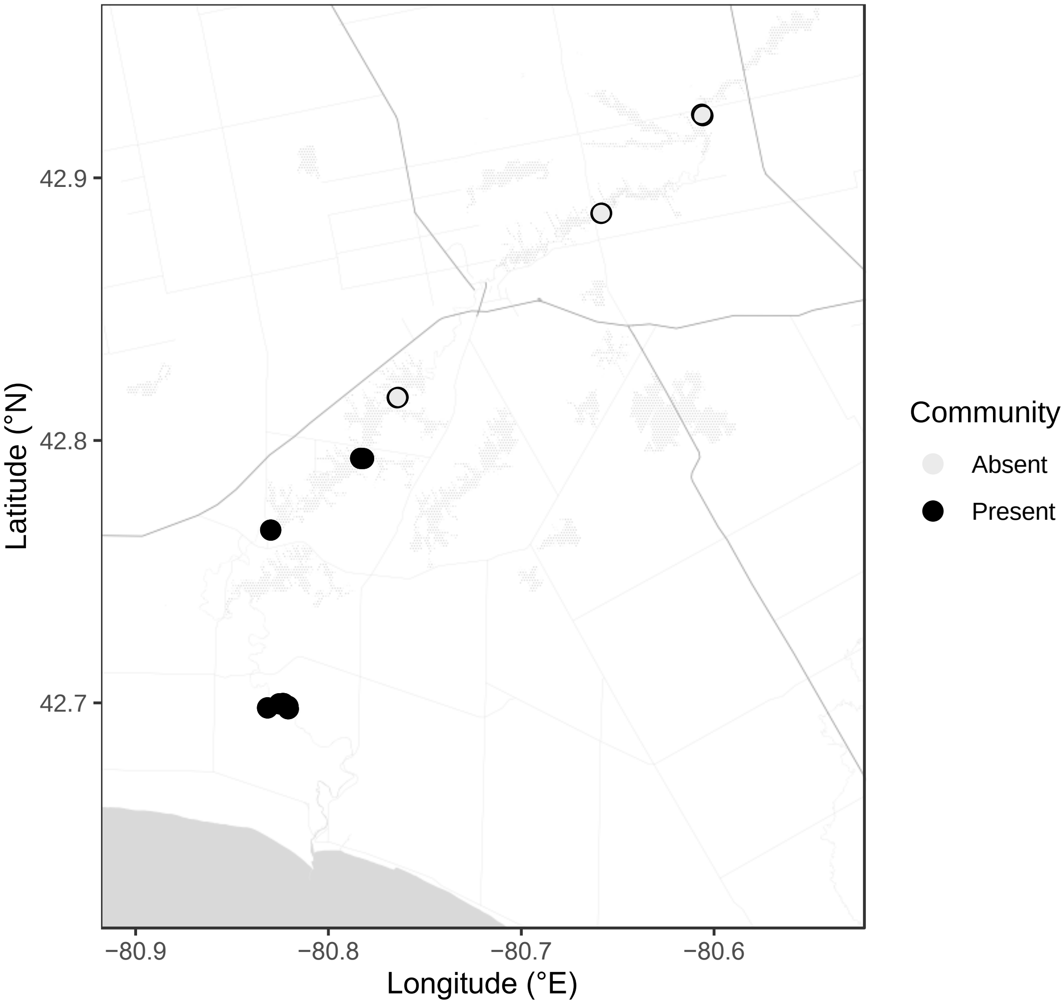

Figure 3.1 Map of sampling sites in Big Otter Creek where Round Goby was present (black) and absent (light grey) between July 9-19th and September 24-26th, 2018.

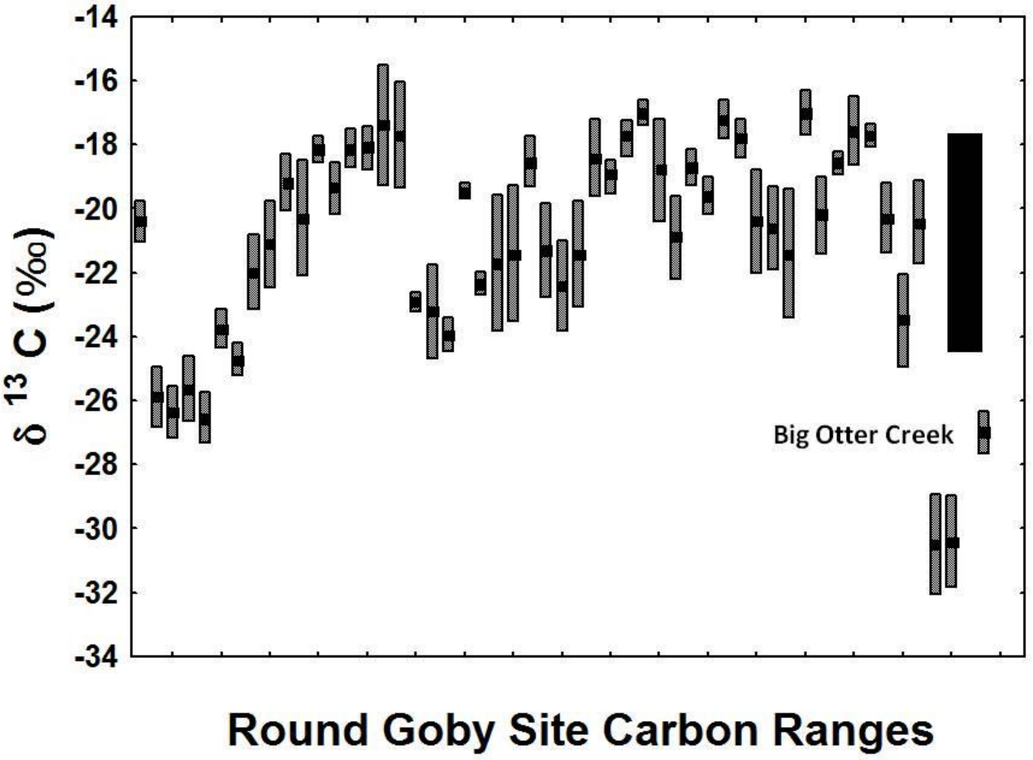

Figure 3.2 Plots of mean $\delta^{13}C$ values (± SD) of Round Goby from various waterbodies of the Great Lakes basin taken from past studies along with 2018 values from Big Otter Creek. The solid black bar plots the among-study range in $\delta^{13}C$ values (mean ± SD).

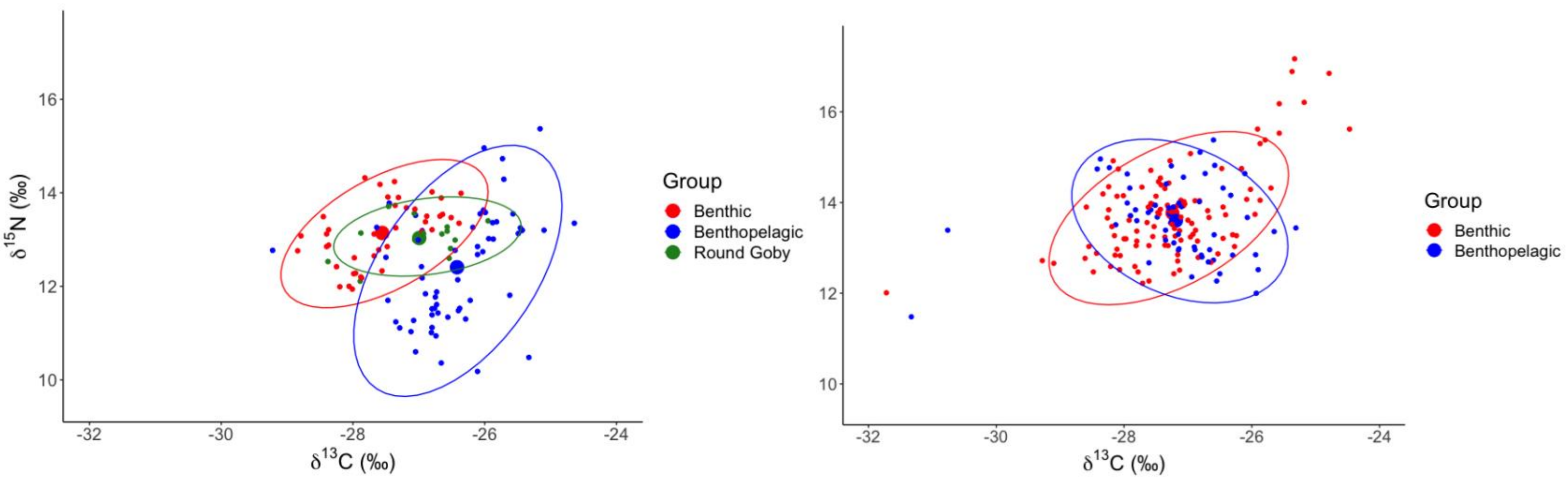

Figure 3.3 Isotopic niches of benthic (red) and benthopelagic (blue) fishes from sites where Round Goby (green) was present (left) and absent (right) in Big Otter Creek. Mean $\delta^{15}$N and $\delta^{13}$C are displayed as the enlarged dots.

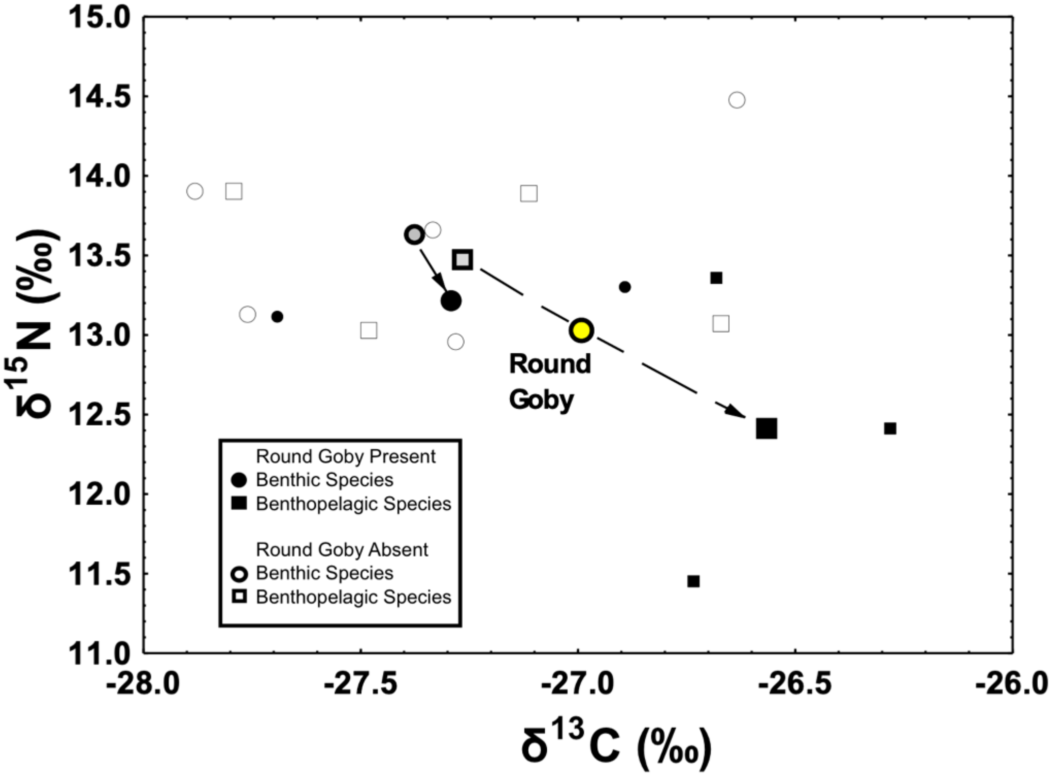

Figure 3.4 Bivariate plot of mean $\delta^{13}C$ and $\delta^{15}N$ of Round Goby (yellow circle) and benthic (enlarged circle) and benthopelagic (enlarged square) species where Round Goby was present (filled circles) and absent (grey and empty circles) in Big Otter Creek. Included are arrows displaying differences in isotopic niches of benthic and benthopelagic species between sites where Round Goby was absent and present, and mean $\delta^{13}C$ and $\delta^{15}N$ of individual species from both benthic and benthopelagic groupings (smaller shapes).

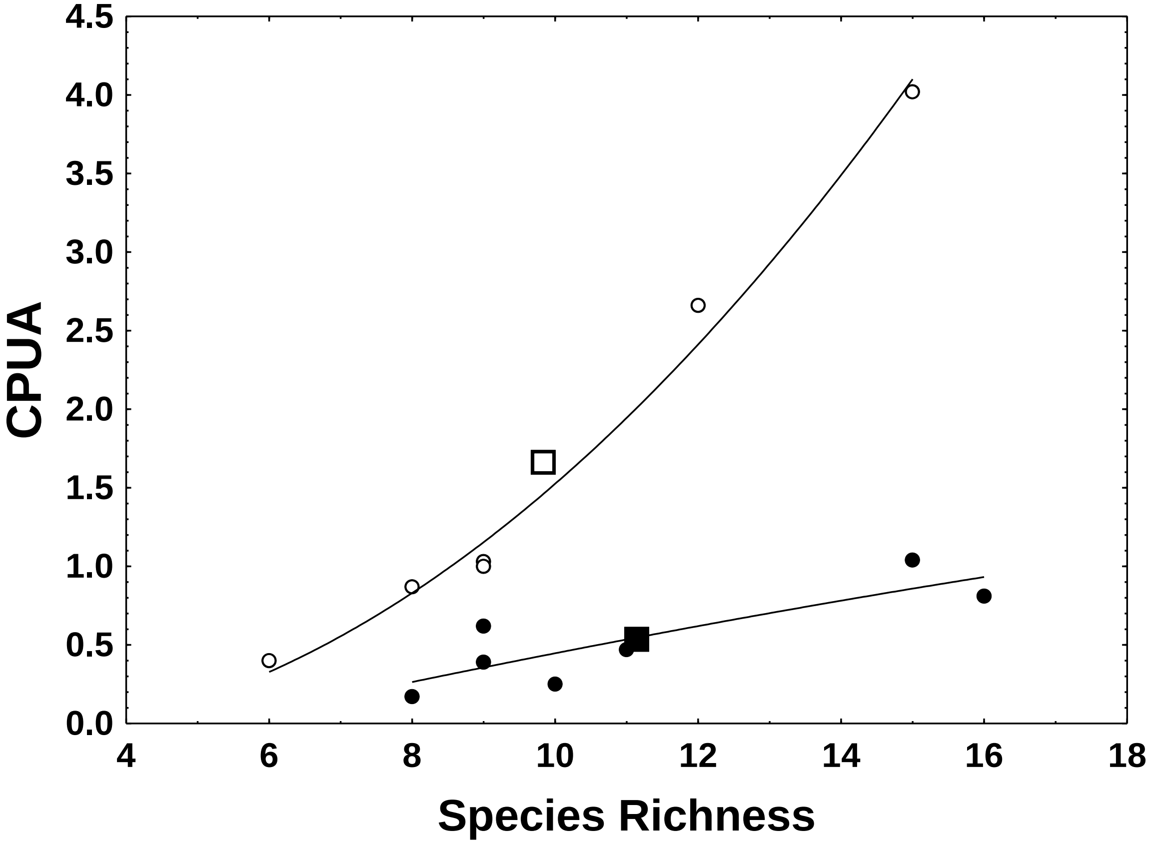

Figure 3.5 Linear and exponential regressions relating CPUA (catch per unit area sampled; number of captured fish × seined area $(m^2)^{-1}$) to species richness for fishes at sites where Round Goby was present (filled circles) and absent (open dots). Lines plot the significant ($p < 0.001$) regression lines. Large open and filled squares plot the mean CPUA and species richness for sites where Round Goby was present and absent.

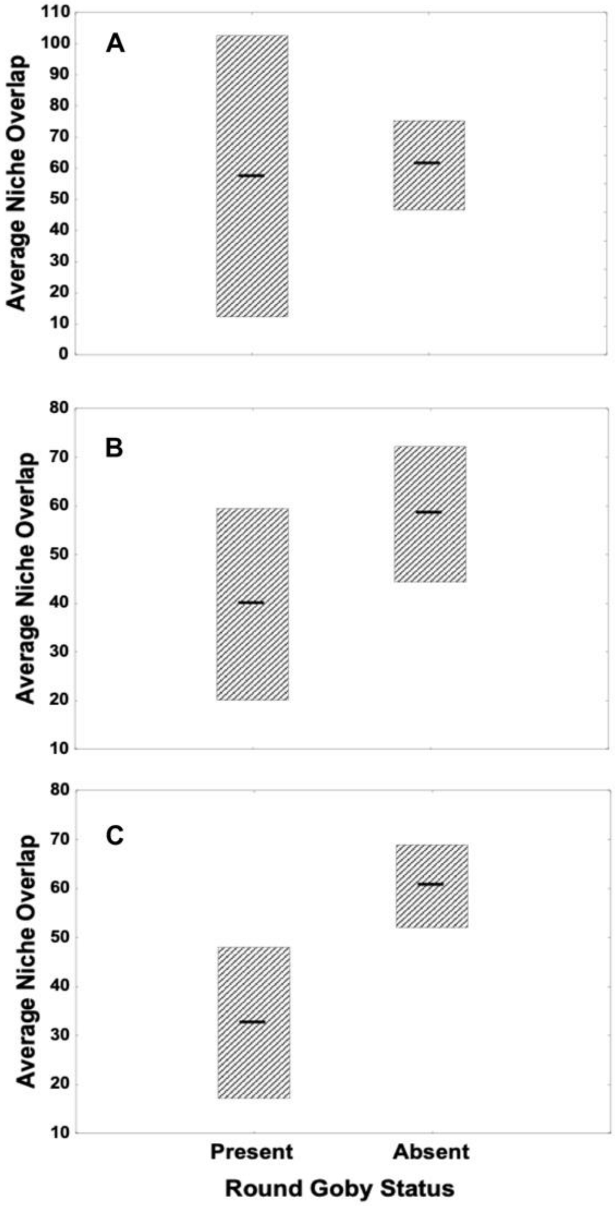

Figure 3.6 Boxplots of average niche overlap between (A) benthic species, (B) benthopelagic species, and (C) benthic and benthopelagic species in sites where Round Goby was present and absent.

Table 3.1 Summary of $\delta^{13}C$ and $\delta^{15}N$ values and Layman metrics for benthic and benthopelagic species captured from Big Otter Creek in sites where Round Goby was present and absent. Metrics include range of $\delta^{13}C$ (CR), range of $\delta^{15}N$ (NR), Bayesian ellipse size (SEAc), nearest neighbour distance (NND), standard deviation of nearest neighbour distance (SNND), and distance to centroid (CD).

| Community | Classification | Species | N | $\delta^{13}C$ ± std. | $\delta^{15}N$ ± std. | CR | NR | SEAc | TA | NND ± SDNND | CD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Round Goby Present | Benthic | All (Excluding Round Goby) | 42 | -27.56 ± 0.67 | 13.14 ± 0.67 | 2.48 | 2.38 | 1.22 | 3.53 | 0.16 ± 0.09 | 0.87 |
|  | Benthopelagic | All | 59 | -26.42 ± 0.80 | 12.41 ± 1.18 | 4.58 | 5.19 | 2.73 | 13.5 | 0.26 ± 0.27 | 1.26 |
| Round Goby Absent | Benthic | All | 110 | -27.24 ± 1.00 | 13.77 ± 1.00 | 7.25 | 5.16 | 2.51 | 15.31 | 0.19 ± 0.26 | 1.11 |
|  | Benthopelagic | All | 59 | -27.19 ± 1.00 | 13.60 ± 0.84 | 6.02 | 3.90 | 2.68 | 15.12 | 0.27 ± 0.35 | 1.05 |

Table 3.2 Summary of metrics comparing the benthic and benthopelagic species in communities where Round Goby was present and absent. Metrics include the mean CPUA of benthic and benthopelagic fishes and mean CPUA of Round Goby in sampled sites. Also given are the means of the site Shannon-Wiener indices, species richness, evenness, total length (mm), mass (g), and Fulton's K.

| Community | Classification | CPUA | Round Goby CPUA | Shannon-Wiener Index | Species Richness | Evenness | TL (mm) ± std. | Mass (g) ± std. | K ± std. |
|---|---|---|---|---|---|---|---|---|---|
| Round Goby Present | Benthic | 0.65 | 0.06 | 1.90 | 11.7 | 0.78 | 37.0 ± 6.45 | 0.46 ± 0.25 | 0.82 ± 0.16 |
| | Benthopelagic | 0.73 | 0.07 | 1.85 | 12.0 | 0.76 | 69.8 ± 28.4 | 9.52 ± 31.0 | 0.93 ± 0.12 |
| Round Goby Absent | Benthic | 1.92 | 0 | 1.68 | 10.6 | 0.71 | 52.5 ± 31.8 | 3.84 ± 9.99 | 0.93 ± 0.13 |
| | Benthopelagic | 2.36 | 0 | 1.76 | 11.0 | 0.75 | 60.5 ± 26.4 | 4.16 ± 10.2 | 0.96 ± 0.18 |

Table 3.3 Estimated overlap of isotopic niche among groups from sites where Round Goby was present and absent. Values are given as probabilities (%) of the isotopic niche of group A overlapping the isotopic niche of group B.

| **Community** | | | | | | | |
|---|---|---|---|---|---|---|---|
| **Round Goby Present** | | | | **Round Goby Absent** | | | |
| **Group B** | **Group A** | **Mean** | **95% Credible Interval** | **Group B** | **Group A** | **Mean** | **95% Credible Interval** |
| Benthic | Benthopelagic | 27.8 | (16-44) | Benthic | Benthopelagic | 86.2 | (76-94) |
| | Round Goby | 85.3 | (64-98) | | | | |
| Benthopelagic | Benthic | 69.4 | (45-90) | Benthopelagic | Benthic | 88.3 | (80-95) |
| | Round Goby | 92.5 | (76-100) | | | | |
| Round Goby | Benthic | 66.0 | (43-87) | | | | |
| | Benthopelagic | 34.3 | (20-53) | | | | |

# Chapter 4: General Conclusions

## 4.1 Summary

The overall objective of this research was to determine how Round Goby has affected native fishes in tributaries of the Great Lakes while addressing key knowledge gaps evident from previous research. The aim of Chapter 2 was to assess how Round Goby may have altered the populations and diversity of fish communities in Great Lakes tributaries at broader spatial scales than previous studies. Chapter 3 used stable isotope analysis to characterize the niche plasticity of Round Goby across the Great Lakes basin and determine how its resource use in Big Otter Creek has affected benthic and benthopelagic fish communities.

In Chapter 2, the catch per unit area of Round Goby and Percidae species from the Ausable River and Big Otter Creek were analyzed to determine whether Round Goby had impacted their relative abundances along longitudinal tributary gradients. Evidence suggested that Round Goby may have negatively affected the relative abundance of several darter species, but overwhelming evidence for negative associations with darter species was not detected. Additionally, Round Goby relative abundance in both tributaries was highest in downstream sections closest to the Great Lakes. The spatial differences suggest that communities in the lower reaches are most likely to be impacted by Round Goby invasion given that the relative abundance of several darter species was highest in upstream sections where Round Goby was either absent or at a low relative abundance. Diversity metrics varied across the lower, middle, and upper reaches of both tributaries, but did not correlate with patterns of Round Goby relative abundance. The evidence for the negative relationships between Round Goby and certain darter species reported here was found over similar time periods but across greater spatial scales than noted in earlier studies in Great Lakes tributaries. The findings from this chapter are important for understanding how Round Goby has impacted native fish communities throughout these and other tributaries of the Great Lakes. Ultimately, this chapter adds to the growing body of literature that provides evidence of the ecological impact of Round Goby in North American freshwater ecosystems.

Chapter 3 compared the stable isotope values of Round Goby in Big Otter Creek to literature-derived samples of Round Goby throughout the Great Lakes basin and found that Round Goby displayed high niche plasticity across the Great Lakes basin. The resource use ($\delta^{13}C$) of Round Goby in Big Otter Creek differed from lacustrine populations, being generally more depleted and less varied in the riverine ecosystem. The notably high niche plasticity of Round Goby has likely facilitated its expansion and successful establishment in numerous waterbodies (Pettitt-Wade et al. 2015). The resource use of benthic and benthopelagic species in Big Otter Creek was compared between sites where Round Goby was present and absent. Where Round Goby was present, benthopelagic species shifted their isotopic niche (presumably as a mechanism to limit trophic overlap with Round Goby); whereas benthic species were suspected of being less able to shift their isotopic niche and consequently shared significant overlap with Round Goby and, consequently, displayed reduced condition. These findings support previous studies that suggested Round Goby is more likely to negatively affect benthic fishes through competition for similar resources (French and Jude 2001; Raab et al. 2018; Firth et al. 2020).

Collectively, both chapters support one another in identifying that Round Goby has likely affected native fishes in tributaries of the Great Lakes through competition. Direct evidence of overlap in resource use between Round Goby and native fishes was required to support the hypothesized mechanism of competition and accounted for the observed patterns of benthic Percidae relative abundance along each tributary from Chapter 2. The finding of significant isotopic niche overlap between Round Goby and benthic species through the use of SIA in Chapter 3 reinforces that the displacement of several benthic Percidae species in the studied tributaries was likely due to Round Goby outcompeting those species for resources. As a consequence, many benthic species appear to be restricted to occupancy of upstream sites or to sites where Round Goby was either absent or present at low relative abundances.

## 4.2 Study Significance

Much of the past research on the ecological impacts of the Round Goby invasion in North America have focused on impacts in the Laurentian Great Lakes. After the secondary expansion of Round Goby upstream into tributaries of the Great Lakes, there has been limited focus on its subsequent impacts on native fishes in riverine habitats. While there have been studies of the ecological impacts of Round Goby in tributary ecosystems, several limitations from those studies exist, which this research sought to address. First, previous studies of Round Goby in tributaries were confined to less than 20 km from the Great Lakes (Krakowiak and Pennuto 2008; Kornis et al. 2013; Malone 2016) where Round Goby existed at high densities. The limited spatial scales of those studies may have misled important conclusions regarding ecological impacts. Several

European studies examining Round Goby populations and their impacts at broader spatial scales (248 km) in rivers observed considerable variation along the rivers with respect to condition, growth rate, abundance, and sex ratios (Brandner et al. 2018; Cerwenka et al. 2018), which supports the need to consider invasion impacts at greater spatial scales. Here, the spatial scale was expanded considerably (126 km in the Ausable River and 88 km in Big Otter Creek) relative to past studies in North America, and highlights how impacts are likely to differ along a tributary depending on the relative of abundance of Round Goby.

Secondly, stable isotope analyses have not been used to assess the resource use by Round Goby in Great Lakes tributaries. Many studies have used stable isotope analysis to quantify trophic impacts associated with species introductions (e.g., Vander Zanden et al. 1999; Britton et al. 2010; Cucherousset et al. 2012), including Round Goby in the Great Lakes (e.g., Paterson et al. 2014; Pettitt-Wade et al. 2015; Miano et al. 2021), confirming its use as a valuable tool when analyzing the impacts of invasive species on fish communities in Great Lakes tributaries. The finding of extensive resource overlap between Round Goby and benthic species supports a previous study that found diet overlap between Round Goby and several benthic species in another tributary of the Great Lakes (Firth et al. 2020) and corroborates earlier studies that predicted benthic fishes were likely to be the most susceptible to Round Goby invasion (French and Jude 2001; Raab et al. 2018). Indeed, benthic fishes in Big Otter Creek and other tributary ecosystems are likely experiencing competition for similar resources with Round Goby based on the high overlap of their isotopic niche.

Impacts associated with invasive species can occur shortly after establishment due to rapid population growth by the invader (Janssen and Jude 2001; Lauer et al. 2004; Britton et al. 2007; Connelly et al. 2007), but populations may eventually decline or collapse altogether (Simberloff and Gibbons 2004; Strayer and Malcom 2006), with impacts potentially decreasing over time (Kornis et al. 2014). Thus, analyzing the impacts by invasive species over longer temporal scales is necessary to understand how the invader will alter the native ecosystem. Results from previous studies of Round Goby impacts on fish communities in tributaries of Lake Michigan and Lake Erie (Krakowiak and Pennuto 2008; Kornis et al. 2013; Malone 2016) may have been dictated by the recency of Round Goby invasion in their study systems. The effects of Round Goby in the Ausable River and Big Otter Creek in this study were evaluated after longer post-invasion intervals (11-16 years) and may therefore better reflect the long-term impacts associated with Round Goby establishment.

The tributaries of the Great Lakes contain the highest diversity of freshwater fishes in Canada (Staton and Mandrak 2005), including several species listed under the *Species at Risk Act* (Poos et al. 2010). The reintroduction of the Threatened Eastern Sand Darter in Big Otter Creek and other tributaries is currently being planned by Fisheries and Oceans Canada (COSEWIC 2009; Fisheries and Oceans Canada 2012; Barnucz et al. 2020; Lamothe et al. 2021) as a key recovery measure for the species, yet hinges on the understanding of how Round Goby may affect Eastern Sand Darter in tributary ecosystems. Given that the findings from this research provide an improved understanding of how Round Goby may affect benthic fishes, including several darter species, this research can help to inform decisions regarding the reintroduction of

Eastern Sand Darter and potentially other management initiatives regarding SARA species in tributary ecosystems.

## 4.3 Future Research

While this research has provided a better understanding of how Round Goby has impacted native fishes in tributaries of the Great Lakes, several knowledge gaps remain. The data from Chapter 2 and 3 were collected over a limited time frame (two summers in the Ausable River, one summer in Big Otter Creek), and intra- and interannual variation of the relative abundance and resource use of both Round Goby and native fishes has not been assessed. Several studies have found that Round Goby populations in Great Lakes tributaries fluctuate seasonally with annual migration to lake habitat during the winter (Pennuto et al. 2010; Blair et al. 2019) that would lead to variable impacts being experienced by the native fish communities throughout the year. Additionally, the abundance of Round Goby can rapidly increase across years and upstream dispersal of populations in tributaries can occur typically between 0.5 to 5 km/year (Bergstrom et al. 2008; Bronnenhuber et al. 2011; Brownscombe and Fox 2012; Šlapanský et al. 2017), but can be as high as 17 km/year (Brandner et al. 2013), and so Round Goby populations in riverine ecosystems are spatially and temporally dynamic. The resource overlap of invasive and native species can also vary seasonally (Coulter et al. 2019), which would lead to variation in the competitive pressure experienced by native fishes. Therefore, future research into the relative abundance and resource use of Round Goby and native fishes in these ecosystems should be performed both intra- and interannually to highlight the potential variation of the ecological impacts of Round Goby.

Two tributaries were the focus of this research, but Round Goby has invaded numerous other tributaries of the Great Lakes, many of which contain species of conservation concern (Poos et al. 2010; Raab et al. 2018). The extent of Round Goby impacts on fish communities in many other tributaries of the Great Lakes is unknown. Monitoring impacts in additional tributaries would provide further insight regarding how and why Round Goby impacts vary spatially and temporally. For example, the habitat conditions (e.g., slow water velocity, watershed area, temperature) of certain tributaries facilitate establishment and promote more rapid upstream expansion of Round Goby populations (Kornis et al. 2013; Raab et al. 2018; Reid 2019), whereas the habitat conditions in other tributaries may constrain their population expansion and ecological impact (Madenjian et al. 2011; Burkett and Jude 2015). Evidently, the ecological impacts from Round Goby invasion are highly variable and depend on different abiotic and biotic conditions, so continued research on Round Goby impacts in additional tributary ecosystems is warranted to better understand the ecological impacts associated of this invasive species.

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## Appendix

Table 3.4 Estimated overlap of isotopic niche among species from sites where Round Goby was present and absent. Values are given as probabilities (%) of the isotopic niche of species A overlapping the isotopic niche of species B. Overlaps involving Round Goby are bolded.

| | | | Stable Isotope Overlap Probability | |
|---|---|---|---|---|
| **Community** | **Species B** | **Species A** | **Mean** | **95% Credible Interval** |
| Round Goby Present | Blackside Darter | Common Shiner | 8.74 | (2-22) |
| | | River Chub | 28.9 | (12-54) |
| | | **Round Goby** | **47.8** | **(24-77)** |
| | | Spotfin Shiner | 1.63 | (0-10) |
| | | White Sucker | 23.6 | (9-49) |
| | Common Shiner | Blackside Darter | 75.4 | (36-99) |
| | | River Chub | 52.7 | (30-75) |
| | | **Round Goby** | **65.2** | **(39-89)** |
| | | Spotfin Shiner | 98.1 | (88-100) |
| | | White Sucker | 18.5 | (5-41) |
| | River Chub | Blackside Darter | 99.2 | (92-100) |
| | | Common Shiner | 41.9 | (25-67) |
| | | **Round Goby** | **91.5** | **(69-100)** |
| | | Spotfin Shiner | 7.98 | (0-36) |
| | | White Sucker | 76.8 | (54-96) |
| | **Round Goby** | **Blackside Darter** | **96.3** | **(78-100)** |
| | | **Common Shiner** | **32.0** | **(16-52)** |
| | | **River Chub** | **56.8** | **(30-83)** |
| | | **Spotfin Shiner** | **10.7** | **(1-36)** |
| | | **White Sucker** | **60.6** | **(37-84)** |
| | Spotfin Shiner | Blackside Darter | 6.9 | (0-62) |
| | | Common Shiner | 34.3 | (19-56) |

|  |  |  |  |  |
|---|---|---|---|---|
|  |  | River Chub | 3.75 | (0-21) |
|  |  | **Round Goby** | **12.1** | **(0-50)** |
|  |  | White Sucker | 3.14 | (0-17) |
|  | White Sucker | Blackside Darter | 91.2 | (63-100) |
|  |  | Common Shiner | 12.3 | (3-30) |
|  |  | River Chub | 53.3 | (30-76) |
|  |  | **Round Goby** | **77.9** | **(53-96)** |
|  |  | Spotfin Shiner | 6.25 | (0-26) |
| Round Goby Absent | Blacknose Dace | Bluntnose Minnow | 30.4 | (9-57) |
|  |  | Brook Stickleback | 44.3 | (22-72) |
|  |  | Common Shiner | 43.3 | (24-70) |
|  |  | Creek Chub | 20.2 | (2-57) |
|  |  | Johnny Darter | 25.7 | (13-44) |
|  |  | Longnose Dace | 59.1 | (31-86) |
|  |  | Northern Hogsucker | 57.7 | (9-98) |
|  |  | White Sucker | 40.1 | (22-64) |
|  | Bluntnose Minnow | Blacknose Dace | 41.0 | (14-78) |
|  |  | Brook Stickleback | 37.8 | (9-77) |
|  |  | Common Shiner | 49.3 | (30-75) |
|  |  | Creek Chub | 63.4 | (36-91) |
|  |  | Johnny Darter | 34.2 | (17-60) |
|  |  | Longnose Dace | 90.7 | (72-100) |
|  |  | Northern Hogsucker | 86.0 | (58-100) |
|  |  | White Sucker | 54.9 | (33-81) |
|  | Brook Stickleback | Blacknose Dace | 90.2 | (66-100) |
|  |  | Bluntnose Minnow | 60.2 | (16-97) |
|  |  | Common Shiner | 76.0 | (53-97) |
|  |  | Creek Chub | 50.7 | (15-92) |
|  |  | Johnny Darter | 69.2 | (44-94) |
|  |  | Longnose Dace | 80.9 | (43-100) |
|  |  | Northern Hogsucker | 62.0 | (15-100) |

| | | | |
|---|---|---|---|
| | White Sucker | 74.9 | (49-96) |
| Common Shiner | Blacknose Dace | 93.4 | (74-100) |
| | Bluntnose Minnow | 59.4 | (35-83) |
| | Brook Stickleback | 68.4 | (41-91) |
| | Creek Chub | 85.2 | (59-99) |
| | Johnny Darter | 62.2 | (46-78) |
| | Longnose Dace | 72.2 | (50-92) |
| | Northern Hogsucker | 88.5 | (59-100) |
| | White Sucker | 73.8 | (55-90) |
| Creek Chub | Blacknose Dace | 38.2 | (4-92) |
| | Bluntnose Minnow | 49.0 | (25-77) |
| | Brook Stickleback | 35.7 | (10-69) |
| | Common Shiner | 64.1 | (39-90) |
| | Johnny Darter | 41.6 | (19-69) |
| | Longnose Dace | 52.0 | (24-87) |
| | Northern Hogsucker | 89.7 | (52-100) |
| | White Sucker | 49.8 | (26-78) |
| Johnny Darter | Blacknose Dace | 88.6 | (65-100) |
| | Bluntnose Minnow | 74.4 | (47-94) |
| | Brook Stickleback | 75.2 | (47-95) |
| | Common Shiner | 81.7 | (66-94) |
| | Creek Chub | 67.0 | (37-92) |
| | Longnose Dace | 92.9 | (77-100) |
| | Northern Hogsucker | 88.5 | (59-100) |
| | White Sucker | 80.8 | (62-94) |
| Longnose Dace | Blacknose Dace | 55.7 | (29-82) |
| | Bluntnose Minnow | 66.8 | (42-88) |
| | Brook Stickleback | 41.4 | (17-70) |
| | Common Shiner | 46.1 | (28-66) |
| | Creek Chub | 47.6 | (23-74) |
| | Johnny Darter | 32.5 | (19-50) |

|  |  |  |  |
|---|---|---|---|
|  | Northern Hogsucker | 83.4 | (55-99) |
|  | White Sucker | 51.1 | (32-71) |
| Northern Hogsucker | Blacknose Dace | 21.6 | (3-56) |
|  | Bluntnose Minnow | 25.4 | (12-45) |
|  | Brook Stickleback | 12.1 | (2-31) |
|  | Common Shiner | 21.1 | (8-42) |
|  | Creek Chub | 27.0 | (8-56) |
|  | Johnny Darter | 13.0 | (5-27) |
|  | Longnose Dace | 36.5 | (19-61) |
|  | White Sucker | 20.0 | (9-38) |
| White Sucker | Blacknose Dace | 95.9 | (80-100) |
|  | Bluntnose Minnow | 86.9 | (63-99) |
|  | Brook Stickleback | 87.2 | (61-99) |
|  | Common Shiner | 91.4 | (77-99) |
|  | Creek Chub | 85.6 | (58-99) |
|  | Johnny Darter | 80.3 | (61-96) |
|  | Longnose Dace | 98.4 | (91-100) |
|  | Northern Hogsucker | 96.9 | (81-100) |

Table 3.5 Summary of $\delta^{13}C$ and $\delta^{15}N$ values and Layman metrics for benthic and benthopelagic species captured from Big Otter Creek in sites where Round Goby was present and absent. Metrics include range of $\delta^{13}C$ (CR), range of $\delta^{15}N$ (NR), Bayesian ellipse size (SEAc), nearest neighbour distance (NND), standard deviation of nearest neighbour distance (SNND), and distance to centroid (CD).

| Community | Classification | Species | N | $\delta^{13}C$ ± std. | $\delta^{15}N$ ± std. | CR | NR | SEAc | TA | NND ± SDNND | CD |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Round Goby Present | Benthic | All (Excluding Round Goby) | 42 | -27.56 ± 0.67 | 13.14 ± 0.67 | 2.48 | 2.38 | 1.22 | 3.53 | 0.16 ± 0.09 | 0.87 |
| | | Round Goby | 15 | -26.99 ± 0.66 | 13.03 ± 0.41 | 2.43 | 1.60 | 0.85 | 2.15 | 0.31 ± 0.24 | 0.62 |
| | | Blackside Darter | 7 | -26.89 ± 0.38 | 13.30 ± 0.26 | 1.09 | 0.83 | 0.34 | 0.41 | 0.27 ± 0.18 | 0.36 |
| | | White Sucker | 35 | -27.69 ± 0.63 | 13.11 ± 0.72 | 2.48 | 2.38 | 1.24 | 3.21 | 0.17 ± 0.10 | 0.88 |
| | Benthopelagic | All | 59 | -26.42 ± 0.80 | 12.41 ± 1.18 | 4.58 | 5.19 | 2.73 | 13.5 | 0.26 ± 0.27 | 1.26 |
| | | Common Shiner | 39 | -26.28 ± 0.61 | 12.41 ± 1.23 | 2.31 | 5.19 | 1.97 | 7.87 | 0.23 ± 0.24 | 1.21 |
| | | River Chub | 10 | -26.68 ± 1.40 | 13.35 ± 0.48 | 4.58 | 1.67 | 2.12 | 3.78 | 0.63 ± 0.38 | 1.24 |
| | | Spotfin Shiner | 10 | -26.73 ± 0.28 | 11.45 ± 0.60 | 1.00 | 2.06 | 0.60 | 1.27 | 0.38 ± 0.18 | 0.53 |
| Round Goby Absent | Benthic | All | 110 | -27.24 ± 1.00 | 13.77 ± 1.00 | 7.25 | 5.16 | 2.51 | 15.31 | 0.19 ± 0.26 | 1.11 |
| | | White Sucker | 21 | -27.33 ± 1.09 | 13.66 ± 0.86 | 5.45 | 3.07 | 2.94 | 7.68 | 0.46 ± 0.83 | 1.00 |
| | | Longnose Dace | 31 | -27.76 ± 0.69 | 13.12 ± 0.47 | 2.75 | 1.58 | 0.91 | 2.69 | 0.19 ± 0.12 | 0.73 |
| | | Johnny Darter | 40 | -26.63 ± 1.02 | 14.47 ± 1.06 | 3.79 | 3.93 | 2.54 | 8.51 | 0.27 ± 0.16 | 1.29 |
| | | Northern Hogsucker | 8 | -27.28 ± 0.23 | 12.95 ± 0.51 | 0.77 | 1.48 | 0.42 | 0.59 | 0.25 ± 0.13 | 0.46 |
| | | Blacknose Dace | 10 | -27.88 ± 0.40 | 13.90 ± 0.64 | 1.17 | 2.33 | 0.89 | 1.56 | 0.37 ± 0.2 | 0.65 |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Benthopelagic | All | 59 | -27.19 ± 1.00 | 13.60 ± 0.84 | 6.02 | 3.90 | 2.68 | 15.12 | 0.27 ± 0.35 | 1.05 |
| | Common Shiner | 31 | -27.11 ± 0.82 | 13.88 ± 0.79 | 2.77 | 3.38 | 1.92 | 5.68 | 0.26 ± 0.15 | 1.00 |
| | Bluntnose Minnow | 10 | -27.48 ± 1.39 | 13.03 ± 0.63 | 5.03 | 2.42 | 1.93 | 3.30 | 0.63 ± 1.3 | 0.92 |
| | Creek Chub | 10 | -26.67 ± 0.54 | 13.07 ± 0.81 | 1.95 | 2.54 | 0.51 | 2.62 | 0.49 ± 0.44 | 0.77 |
| | Brook Stickleback | 8 | -27.79 ± 1.27 | 13.90 ± 0.64 | 4.18 | 2.15 | 2.81 | 3.76 | 0.76 ± 0.92 | 1.03 |