Understanding species life-history and ecology for improved conservation and recovery of the threatened Silver Shiner, *Notropis photogenis*

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: Urbanization correlates with altered growth and reduced survival of a small-bodied, imperilled freshwater fish. J. Burbank, D.A.R. Drake, M. Power

While the research was my own, this chapter was a collaborative effort involving all authors. JB conducted the field work, lab work, data analyses and wrote the paper. MP aided with the mortality analysis. JB, DARD and MP formulated the idea for the study and developed the study design. DARD and MP provided financial support and all authors provided editorial comments. This chapter was published as:

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Chapter 3: The influence of thermal cues on the reproductive phenology of Silver Shiner, Notropis photogenis. J. Burbank, D.A.R. Drake, M. Power

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Chapter 4: Silver Shiner, Notropis photogenis, fecundity and parasite infections during the reproductive period. J. Burbank, D.A.R. Drake, M. Power

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Chapter 5: Field-based oxygen isotope fractionation for the conservation of imperilled fishes: an application with the threatened silver shiner (Notropis photogenis). J. Burbank, D.A.R. Drake, M. Power

While the research was my own, this chapter was a collaborative effort involving all authors. JB conducted the field work, lab work, data analyses and wrote the paper. JB, DARD and MP formulated the idea for the study and developed the study design. DARD and MP provided financial support and all authors provided editorial comments. This chapter was published as:

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Chapter 6: Seasonal consumption of terrestrial prey by a threatened stream fish is influenced by riparian vegetation. J. Burbank, D.A.R. Drake, M. Power

While the research was my own, this chapter was a collaborative effort involving all authors. JB conducted the field work, lab work, data analyses and wrote the paper. JB, DARD and MP formulated the idea for the study and developed the study design. DARD and MP provided financial support and all authors provided editorial comments. This chapter was published as:

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Abstract

Conservation and recovery strategies are typically developed with the best available information. However, for many imperilled small-bodied freshwater fish species there is often a lack of basic information available on species life-history and ecology, which limits the ability to assess threats and develop comprehensive conservation actions. One species with a paucity of information pertaining to its life-history and ecology is Silver Shiner, *Notropis photogenis*, a small-bodied freshwater fish species listed as Threatened under Canada's *Species at Risk Act*. The goal of this thesis was to provide an in-depth examination of several life-history traits and ecological parameters of Silver Shiner, to better inform conservation and recovery efforts for the species. To achieve this goal, Silver Shiner were collected from Sixteen Mile Creek, Oakville, Ontario, Canada during 2017-2019 and studies were conducted on the growth, survival, fecundity, spawning phenology, thermal occupancy, and diet of the species. Chapter 2 determined that the probable maximum age of Silver Shiner is four years of age. Moreover, the species experienced altered growth and increased adult mortality when occupying urban (0.71 ± 0.05, average mortality ± standard error) compared to non-urban (0.61 ± 0.06) reaches of Sixteen Mile Creek. In chapter 3, logistic regression models were developed that predict spawning phenology in relation to a cumulative thermal cue and indicated a 50% probability that the population initiated and ceased spawning when cumulative growing degrees > 5^{o}C reached 68 °C•days and 368 °C•days, respectively. These models can be used to understand the impact of alterations in the thermal regime on spawn timing. In chapter 4, the first fecundity estimates for Silver Shiner in Canada were provided, ranging from 311-2768 eggs, and previously undocumented parasite infections were observed and quantified during the reproductive period. Chapter 5 developed a species-specific otolith thermometry equation that facilitates future examinations into the thermal occupancy of the species. In Chapter 6, the diet of Silver Shiner was quantified. The species was found to exhibit a generalist diet and consume a wide range of aquatic and terrestrial prey. Based on stomach contents Silver Shiner consumed more terrestrial prey in fall (41.53 ± 32.35 %, average ± standard deviation) compared to summer (20.45 ± 20.45) and exploited more terrestrial prey at reaches with intact riparian vegetation.

Overall, the project highlighted urbanization as an important threat, with knowledge of the extent and type of urbanization effects as necessary for better ecological understanding of Silver Shiner. The project also provided an assessment of the potential implications of alterations in the thermal regime on the reproductive dynamics of Silver Shiner. Moreover, results most notably point to the importance of intact riparian habitat for the species. Together, the thesis chapters provide valuable information on the life-history and ecology of Silver Shiner that will facilitate the development of more comprehensive, well-informed conservation and recovery action for the species in Canada.

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Dedication

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Chapter 1: Introduction

1.1 General context

Small-bodied freshwater fish species that lack commercial or recreational value often have a paucity of information available regarding their life-history and ecology, limiting the ability to develop comprehensive, well informed conservation and recovery strategies (Johnston, 1999; Kuehne & Olden, 2014; Saddlier *et al.*, 2013). Conservation and recovery strategies are typically developed with the best available information (Crouse *et al.*, 2002); however, a lack of basic information on species life-history and ecology restricts the ability to assess the impacts of threats on imperilled species. When comprehensive species-specific ecology and life-history information are lacking, managers are often forced to rely on analogs or anecdotal, rather than systematic appraisals of the evidence, limiting the effectiveness and relevance of conservation and recovery strategies developed for imperilled species.

Within Canada it is a requirement that recovery strategies are developed for imperilled species listed as Threatened or Endangered under Canada's *Species at Risk Act* (McDevitt-Irwin *et al.*, 2015). These listed species face a multitude of threats, and it is important to understand the ecology and life-history of species to appropriately assess threats and estimate responses to future habitat conditions, such as changes in the thermal regime, which may arise as a consequence of climate change and urbanization (Nelson *et al.*, 2009) . Throughout this thesis I highlight how in-depth life-history and ecological information can be used to advance conservation and recovery efforts for a species listed as Threatened under Canada's *Species at Risk Act*, Silver Shiner, *Notropis photogenis*.

1.2 Life-history and ecological information

1.2.1 Understanding life-history

Life-history information including but not limited to probable maximum age, growth, mortality, spawn timing and fecundity are necessary for evaluating population demographics (Beissinger & McCullough, 2002; Beissinger & Westphal, 1998; Morris *et al.*, 1999). Basic life-history information can provide valuable insight into a population's trajectory and resilience, thus such information is foundational for examining population dynamics (Winemiller, 2005) and is of particular importance for imperilled species. When species- or population-specific life-history information is unavailable, a common solution is to apply proxies or cross-species estimation techniques (e.g. Pauly survival equation (Pauly, 1980); Lorenzen age-0 survival equation (Lorenzen, 2000)) to inform population models. The use of proxies can be problematic as proxies may provide a poor representation of the species of interest, potentially leading to erroneous conclusions regarding requirements for species survival or recovery (Lindenmayer *et al.*, 2002; Wiens *et al.*, 2008). Because population models are sensitive to assumed life-history parameters (Gross *et al.*, 2006; Morris *et al.*, 2002; Tear *et al.*, 1995), poor correspondence between the proxy and the species of interest can have significant implications when models are used to inform decision-making.

Probable maximum age is an important life-history trait to obtain for imperilled species. Short-lived, small-bodied fish species display vastly different population dynamics and general population sizes in contrast to longer-lived species. Short-lived, small-bodied species often require high population abundances to compensate for their vulnerability to predation, low survival among age classes, and short reproductive lifespans. Demographic models that underpin recovery targets can be sensitive to probable maximum age (Morris *et al.*, 2002), thus the inclusion of an incorrect probable maximum age could have repercussions, leading to underestimates or overestimates of the population abundance needed for effective conservation or recovery.

Growth and mortality are also important life-history parameters to quantify when developing comprehensive conservation and recovery plans. Both are crucial inputs into population demographic models such as population viability analysis, which is commonly used to develop recovery targets for imperilled species (Beissinger & McCullough, 2002; Beissinger & Westphal, 1998; Morris *et al.*, 2002). Additionally, reductions in growth and increases in mortality in response to differing habitat conditions can provide clear indications of how a species is responding to various threats. For instance, reduced growth and survival in urban areas caused by alterations in food supply (Eitzmann & Paukert, 2010) or changes in flow and thermal regime (Nelson *et al.*, 2009) can be indicators of the negative impacts of urbanization on fish populations.

Spawn timing and fecundity are critical and sensitive components of a species life-history. Understanding and developing approaches to predict spawning phenology can allow managers to provide adequate protection to species during the reproductive period. Many lotic species initiate and terminate spawning activity in response to environmental factors such as cumulative temperature and or discharge (LovellFord *et al.*, 2020). Developing the ability to predict spawning phenology in relation to environmental factors can help elucidate potential responses to changing thermal regimes or discharge that may arise as a result of common threats such as climate change, urbanization or installation of dams.

1.2.2 Thermal and Feeding Ecology

A paucity of information on the general ecology of a species, including the temperatures occupied and feeding habits, can restrict the ability to identify and protect critical habitat, including important food resources. Within lotic systems, it is essential to identify and describe the habitats where a species is found, where dietary items are sourced from, and what dietary items are consumed.

Understanding the temperature occupied by a fish species is essential for evaluating its potential vulnerability to shifting thermal regimes that may arise as a consequence of urbanization or climate change (Nelson & Palmer, 2007). Knowledge of the temperatures occupied by fish species in the wild can help identify important thermal refugia that may be valuable for the conservation and development of science-based recovery strategies for threatened freshwater fish. However, evaluating temperature use can be difficult for small-bodied fishes due to a lack of suitable field methods. One opportunity to estimate the range of occupied temperatures is the retrospective estimation of the average relative temperature occupied by fish through measures of the oxygen isotope ($\delta^{18}O$) values of otoliths (Godiksen *et al.*, 2010a; Willmes *et al.*, 2019) . Fish otoliths are commonly archived for ageing during fish sampling programs and retain a geochemical chronological history of an individual's environment (Campana, 1999). Empirical temperature-dependent fractionation equations can be developed that describe the relationship between occupied temperature and oxygen isotope values, and thus can be employed to evaluate previous temperature used by individuals (e.g. Godiksen *et al.*, 2010b; Minke-Martin *et al.*, 2015). In this thesis I construct a species-specific temperature dependent fractionation equation for a threatened small-bodied fish that can be applied to evaluate temperature use.

In addition to thermal ecology, knowledge of feeding ecology is crucial for the effective conservation of fish species. Understanding what prey a species consumes, and the origin of prey, is a cornerstone of defining and protecting critical habitat. Information on feeding ecology aids in identifying important prey items (Amundsen *et al.*, 1996), potential competitive interactions with co-occurring members of the fish community (Burbank *et al.*, 2019), and habitats that contribute to prey production and are used for forging (Naman *et al.*, 2017). Some small-bodied stream dwelling fishes exploit prey items sourced from the adjacent riparian habitat (Sullivan *et al.*, 2012), including imperilled species such as Redside Dace, *Clinostomus elongatus* (Daniels & Wisniewski, 1994) and Silver Shiner (Burress *et al.*, 2016; McKee & Parker, 1982), highlighting the importance of habitat outside the confines of the stream itself for providing energetically valuable prey items. Therefore, a paucity of knowledge pertaining to a species' feeding ecology can limit conservation efforts by neglecting potentially important habitat not directly occupied by the species of interest.

1.3 Focal species – Silver Shiner *Notropis photogenis*

Silver Shiner is a small-bodied freshwater fish (approximate maximum length: 144 mm (Bouvier et al., 2013)) within the superfamily Cyprinioidea currently listed as Threatened under Canada's *Species at Risk Act*. Silver Shiner exists in Canada at the northern edge of its geographic range and occupies only five drainages within the country (Bronte Creek, Grand River, Thames River, Saugeen River, and Sixteen Mile Creek), all of which are located in Southern Ontario (Bouvier *et al.*, 2013; Gáspárdy *et al.*, 2021; Glass *et al.*, 2016) at latitudes between approximately 42.5-43.5 °N. Identifying Silver Shiner in the field can be difficult as the species closely resembles both Emerald Shiner, *Notropis atherinoides* and Rosyface Shiner, *Notropis rubellus* (Glass *et al.*, 2016). Silver Shiner can be distinguished from Emerald Shiner and Rosyface Shiner by subtle characteristics including the origin of the dorsal fin that falls within the base of the pelvic fin, and two dark crescent shaped melanophores that lie between the nostrils that can be seen when viewing specimens from above (Glass *et al.*, 2016; Gruchy *et al.*, 1973).

Currently little information exists on the life-history and ecology of Silver Shiner, resulting in the application of anecdotal information and analog information from surrogate species such as Emerald Shiner when evaluating the recovery potential of the species (DFO, 2013). The life-history and ecological information that is available for Silver Shiner is sparse, based on limited sample numbers, and generally outdated, which has restricted the ability to develop well informed recovery action. Sparse information based on small sample sizes leads to increased bias and uncertainty when developing population models (Doak *et al.*, 2005). Limited information on Silver Shiner feeding ecology is available from examination of n=5 (Burress *et al.*, 2016) individuals captured in the New River, North Carolina and n=35 (McKee & Parker, 1982) individuals captured in the late 1970's and early 1980's in southern Ontario. While useful such information did not capture either size or seasonal variation in diet. Further, current and future conservation and management of the species would be better informed by feeding studies based on current environmental conditions in Canada, especially in ecosystems that have undergone extensive change as a result of urbanization. Knowledge of growth incorporated in recovery potential modelling is based on n=50 individuals, and information on fecundity is non-existent, resulting in the need to rely on fecundity information from a surrogate species, Emerald Shiner (Young & Koops, 2013b). Available estimations of spawn timing are relatively outdated, coarse and highly variable (Baldwin, 1983; McKee & Parker, 1982). Furthermore, prior to this thesis uncertainty remained regarding the probable maximum age of Silver Shiner, which resulted in large discrepancies in minimum viable population (MVP) sizes estimated when conducting the recovery potential assessment for the species. Short-lived (maximum age of 3+) and long-lived age interpretations (maximum age of 10+) yielded MVP size estimates of ~780,000 adults compared to ~700 adults, respectively, resulting in drastically different recovery targets (DFO, 2013). Therefore, it is necessary to resolve the maximum age of Silver Shiner to refine and develop relevant recovery targets for the species. For this thesis I captured Silver Shiner in Sixteen Mile Creek, Oakville, Ontario from 2017-2019 to better understand the age, growth, mortality, fecundity, spawning phenology, thermal ecology and feeding ecology of the species within Canada to improve the information base available for current and future conservation and recovery efforts.

1.4 Study location

This study focuses on the Sixteen Mile Creek population of Silver Shiner. The Sixteen Mile Creek watershed drains an area of approximately 372 km^2 and the creek flows through a mix of forested, agricultural, and urbanized land, including two large urban centers (Milton and Oakville, Ontario, Canada), prior to entering Lake Ontario (Dunn, 2006; Conservation Halton, 2009). Six reaches were selected to include both non-urbanized and urbanized landscape along Sixteen Mile Creek (See Fig. 2.1 below), with reaches located from just north of Hwy 407 (Lower Base Line Rd W.), downstream to Upper Middle Rd in Oakville, Ontario, Canada. Urban reaches were defined as reaches where the surrounding land use (3 km radius) was predominately urban or undergoing urban development and consisted of predominantly impervious surfaces. Alternatively, the non-urban reaches were defined as reaches with a surrounding landscape that was not urbanized. The three most upstream reaches were considered non-urban, with surrounding landscapes that primarily consisted of forested and agricultural land, and the three most downstream sites were considered urbanized, with the surrounding landscape consisting of urban development dominated by impervious surfaces and man-made structures. Within each reach, three unique in-stream habitats were targeted; a run, a riffle and a pool. Therefore, a total of 18 sites were sampled. The study location was chosen because Silver Shiner is known to be more abundant in Sixteen Mile Creek relative to the other systems inhabited within Canada (Burbank *et al.*, 2021b; Glass *et al.*, 2016).

1.5 Research objectives

1.5.1 Chapter 2: Urbanization correlates with altered growth and reduced survival of a small-bodied, imperilled freshwater fish

Given the paucity of life-history data available for Silver Shiner in Canada, the goal of this study was to examine the age, growth, and mortality of the species using three years (2017-2019) of field sampling. Furthermore, as urbanization is known to have negative impacts on freshwater fish (Paul & Meyer, 2001), this chapter investigates the influence of urbanization on the growth and mortality of Silver Shiner by comparing the vital rates between urban and non-urban reaches of Sixteen Mile Creek, Oakville, Ontario, Canada. Specifically, the hypothesis that urbanization would correspond with reduced growth and increased mortality at the local reach scale was tested.

1.5.2 Chapter 3: The influence of thermal cues on the reproductive phenology of Silver Shiner, *Notropis photogenis*

Small-bodied stream-dwelling fishes often initiate and cease spawning in response to environmental cues such as discharge and cumulative temperature (Archdeacon *et al.*, 2020; Heins, 2020). To better understand the reproductive phenology of Silver Shiner in Canada, this study evaluated the spawn timing of Silver Shiner in relation to environmental conditions in 2018 and 2019. The overall objectives of this chapter were to describe the reproductive phenology of a Silver Shiner population at the northern edge of its range in Canada, evaluate if spawning occurrence could be predicted by thermal cues, such as cumulative growing degree days, and develop models that would predict Silver Shiner reproductive phenology based on the thermal regime of a given year.

1.5.3 Chapter 4: Silver Shiner, *Notropis photogenis*, fecundity and reproductive period parasite infections

As little to no information exists on the fecundity of Silver Shiner within Canada, this chapter aimed to quantify the fecundity of a Canadian population of Silver Shiner to improve the ability to develop well informed conservation and recovery strategies for the species. Specifically, Silver Shiner collected from Sixteen Mile Creek, Oakville, Ontario, Canada in spring 2018 and 2019 were examined to estimate fecundity (egg count), egg diameter and the length-fecundity relationship of the species in Canada.

1.5.4 Chapter 5: Field-based oxygen isotope fractionation for the conservation of imperilled fishes: an application with the threatened silver shiner (*Notropis photogenis*)

Currently it is difficult to estimate the temperature use of small-bodied fishes such as Silver Shiner in nature due to a lack of appropriate field methods. Accordingly, the overall objectives of this study were to: i) develop an oxygen isotope fractionation equation from archived otoliths to better understand variation in individual thermal habitat use of Silver Shiner, ii) quantify the accuracy and predictive error associated with using alternative species-specific fraction equations to evaluate the importance of deriving species-specific fractionation equations, and iii) illustrate the application of oxygen isotope techniques for evaluating the realized thermal use of small-bodied freshwater fishes in the hope of motivating similar research for small-bodied species lacking a detailed understanding of thermal ecology.

1.5.5 Chapter 6: Seasonal consumption of terrestrial prey by a threatened stream fish is influenced by riparian vegetation

Minimal and outdated information currently exists on the feeding habits of Silver Shiner in Canada. In light of the value of understanding feeding ecology for effective species conservation, this study aimed to provide a general description of the diet of Silver Shiner in summer and fall. Furthermore, given the suspected importance of terrestrial prey to Silver Shiner diet, this chapter tested the hypotheses that: i) Silver Shiner has a broad feeding niche typical of generalist drift feeding fishes, ii) within the feeding niche, Silver Shiner demonstrate a reliance on terrestrial prey, iii) terrestrial prey consumption varies seasonally, increasing in the fall, iv) relative bankside terrestrial invertebrate abundance is positively correlated with upstream riparian vegetation cover, v) terrestrial prey consumption varies positively with upstream riparian vegetation cover, and vi) terrestrial consumption is higher in adults (ages 1 and greater) than in juveniles (age 0).

Chapter 2: Urbanization correlates with altered growth and reduced survival of a small-bodied, imperilled freshwater fish

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2.1 Introduction

Life-history and vital rate information is necessary for understanding population demographics. Age, growth, and mortality, in particular, provide insight into the resilience and trajectory of a population (Winemiller, 2005), and thus provide the necessary foundation for evaluating the population dynamics of imperilled species. Although recovery strategies for imperilled species are typically developed with the best available information (Crouse *et al.*, 2002), for many small-bodied, non-commercial, or non-recreational species, life-history information is often lacking due to limited research (Kuehne & Olden, 2014; Saddlier *et al.*, 2013). The paucity of such information limits the ability to understand basic population dynamics, forcing managers to rely on anecdotal rather than systematic appraisals of the evidence (Sutherland *et al.*, 2004). The lack of age, growth and mortality information also hinders an understanding of how threats to imperilled species may be leading to population declines, or whether recovery targets are attainable (Morris *et al.*, 2002). Thus, the collection, archiving, and analysis of species-specific life-history and vital rate information is necessary to ensure that effective conservation approaches are developed for small-bodied species.

When life-history and vital rate information has been obtained, understanding if and how vital rates change in response to threats (e.g. urbanization, climate change, and agriculture, among others for freshwater fishes) provides an opportunity to evaluate the significance of the threat for the population. Urbanization has demonstrated negative impacts on aquatic ecosystems such as increased flow variability, elevated nutrient and contaminant loading, and reduced species diversity (Paul & Meyer, 2001), and is considered a major threat for many imperilled aquatic species (Venter *et al.*, 2006). Urbanization can lead to declines in the growth and survival of fish populations through habitat stressors such as changes in flow, temperature, siltation, and turbidity (Nelson *et al.*, 2009; Nelson & Palmer, 2007), contaminants (Collier *et al.*, 1998), food supply (Eitzmann & Paukert, 2010; Yoder & Rankini, 1997), and loss of habitat complexity (Paukert & Makinster, 2009), all of which may act independently or interactively (Rosso & Quiros, 2009; Walton *et al.*, 2007). For example, studies of Endangered Redside Dace (*Clinostomus elongatus*) in relation to impervious land use highlighted the chronic adverse impacts of urban development on population viability, with strong negative associations between population abundance and urbanization at multiple spatial scales (e.g. pool and sub-catchment (Poos *et al.*, 2012)). Given that future human population growth will occur rapidly and in areas that are already urbanized (Cohen, 2003; Meyer *et al.*, 2005), it is imperative to examine the potential impacts of urbanization on the growth and survival of freshwater fishes.

Silver Shiner (*Notropis photogenis*) is a small-bodied (maximum total length: 144mm (DFO, 2013)) freshwater fish listed as Threatened under Canada's *Species at Risk Act* (SARA) that occupies areas with increasing urban pressure. Like many SARA-listed species, Silver Shiner lacks current, accurate, and validated life-history and vital rate information, including age, growth, and mortality estimates (COSEWIC, 2011). Silver Shiner exists in Canada at the northern edge of its range and is located in only five drainages (Bronte Creek, Grand River, Thames River, Sixteen Mile Creek and Saugeen River) in southern Ontario between the latitudes of approximately 42.5-43.5 °N (Burbank *et al.*, 2020). Species-specific age, growth, mortality and other vital rate information, where it exists, has typically been derived from a relatively low number of individuals (e.g. McKee and Parker, 1982; Baldwin, 1983, 1988). These small sample-based estimates have been used for population viability analyses and to make extinction risk and recovery target predictions. For instance, a recent recovery potential modelling exercise for Silver Shiner used growth estimates based on 50 individuals collected between 2011 and 2013 to assess vital rate elasticity and determine population-based recovery targets for the species (short-lived model; Young and Koops, 2013). Small sample sizes can be problematic when evaluating population demographics, leading to an increased likelihood of uncertainty and bias from demographic models (Doak *et al.*, 2005). Estimating life-history and vital rates with a larger sample will, therefore, help to increase confidence in modelled recovery targets. Furthermore, as Silver Shiner is expected to continue to face increased urban pressure, more robust estimates of vital rates will also help determine the potential severity of urbanization as a threat to the species.

Given the paucity of population-specific biological data of Silver Shiner, this study uses data from three years of field collections at the northern edge of its geographic distribution to describe key demographic characteristics such as age-structure, growth, and mortality. We then compare estimated vital rates between urban and non-urban environments to better understand the possible implications of watershed disturbance for the species. Specifically, we test the hypothesis that urbanization will be associated with reduced growth and increased mortality at the local reach scale.

2.2 Methods

2.2.1 Sample collection and processing

Silver Shiner (n=1403) was captured at six reaches along Sixteen Mile Creek, Oakville, Ontario, Canada, in Summer 2017 (July), Fall 2017 (September), Winter 2018 (March), Spring 2018 (April-June), Summer 2018 (August) and Spring 2019 (April-June). In light of the Threatened status of Silver Shiner, sampling centered on Sixteen Mile Creek as a precautionary measure, as previous sampling efforts (Glass et al., 2016) captured a larger number of individuals in Sixteen Mile Creek compared to other systems in which the species occurs. Sample reaches were chosen based on historical capture locations of the species within Sixteen Mile Creek (Glass et al., 2016) and reaches were chosen to encompass both urban and non-urban affected areas. The three most upstream and downstream reaches, respectively, were classified as non-urban (surrounding catchment largely forest and agriculture) and urban (surrounding catchment largely urban landcover; Figure 2.1). Urban and non-urban reaches were separated by a minimum distance of approximately 7 km and the study took place over approximately 13 km. The separation between the closest urban and non-urban reaches was presumed to preclude fish movement between the two reach classes based on the limited linear home ranges that have been estimated for Yellowfin Shiner, *Notropis lutipinnis* (Goforth & Foltz, 1998) and small-bodied fishes in general (Minns, 1995; Woolnough *et al.*, 2009). At sample reaches fishes were captured in each of a run, riffle and pool habitat with three repeated hauls of a 9.14 m bag seine (3 mm mesh). Captured fishes were identified to species, counted, and a random subset of Silver Shiner was euthanized with clove oil, placed on ice, brought to the lab and stored in a freezer (-20 °C) prior to further processing. All fishes were collected and retained under an animal use permit (AUP 1846) approved by the Canadian Council on Animal Care. At each sample location measures of stream width (m), depth (m), water velocity (m/s), conductivity (µS), dissolved oxygen (mg/L) and pH were taken following fish collections. Average stream width (m), depth (m), water velocity (m/s), conductivity (µS), dissolved oxygen (mg/L) and pH were compared between urban and non-urban reaches using two-sample t-tests. Additionally, stream temperatures at urban and non-urban reaches were measured every 30 minutes using instream temperature loggers (Onset HOBO Pro V2). Average temperatures from May-November 2017 were compared using a two-sample t-test to evaluate if average temperatures were significantly different at urban and non-urban reaches.

Individual Silver Shiner were thawed in the lab and measured for length (mm) and weight (g) (n=1403). The opercula and the largest (lapilli) otoliths were removed for ageing purposes. Opercula were cleaned with warm deionized water by gently rubbing between the thumb and forefinger to remove any flesh (Gallucci *et al.*, 1996). The opercula were then dried and stored in a wax-paper envelope prior to ageing. Otoliths were cleaned with deionized water in a similar manner, dried, and placed in snap-cap vials prior to ageing.

A subset of Silver Shiner (n=262) from across the size range was selected for ageing using a stratified random sampling design such that ~15 individuals were aged per 5 mm length interval across the length range from < 35 mm to ≥ 125 mm. Storage and handling damaged some structures and only individuals with both otoliths and opercula structures (n=254) were used (see Table 2.2). Opercula structures were placed on a clear slide, viewed, and photographed with transmitted light using a stereo-microscope and camera (SMZ 1000 and DS-Fi1; Nikon Instruments Europe B.V., England) at magnifications ranging from 4-16x. Otoliths were placed on a small black wooden stick, fixed sacculus side down with cyanoacrylate and polished using high grit silicon carbide waterproof sandpaper ranging from 1500-7000 grit (Starcke GmbH &

Co., Germany) until the center was reached, and growth rings became evident. Otoliths were checked under the microscope often (i.e. approximately every 3-5 seconds) during the polishing process to avoid over-polishing or cracking. Following polishing, otoliths were soaked in a 50:50 solution of water and glycerin, viewed on a black background under reflected light at ~45x magnification and photographed using a dissecting microscope (SZN-2; Optika, Italy). Opercula and otoliths were aged twice to evaluate the consistency of age estimates within and among structures, with the second assessment being blind to the initial results. When age estimates for a given structure differed between readings, the structure was read a third time to resolve differences.

2.2.2 Age, growth and mortality analyses

Age estimates were assessed for consistency within and among structures following Horwitz et al., (2018) by evaluating the percent agreement between paired structure readings, such that:

$$\text{Percent Agreement} = \left(\frac{A}{T}\right) \times 100$$

where A is the number of structures read (opercular or otolith) with identical age estimates, and T is the total number of structures (opercular or otolith) aged. For assessment of among-structure percent agreement, the final age estimates from the separate opercular and otolith readings were used (i.e. if initial readings disagreed the third reading was considered).

Length-at-age von Bertalanffy growth models (Beverton & Holt, 1957) were fit to otolith and opercular age estimates using year and month time-steps as follows:

$$L_t = L_\infty\left(1 - e^{-K(t-t_0)}\right)$$

where L_t is total length at time-step t (mm), L_∞ is the asymptotic length (mm), K is a growth coefficient defining the rate at which the asymptote is approached, and t_0 is the theoretical time at which length is zero. Month-based estimates assumed individuals hatched on June 1st of the birth year (i.e. individuals captured in July and estimated to be 0+ and 1+ were considered 2 and 14 months old respectively). Silver Shiner is a spring spawner and is typically captured in Ontario with ripe gonads from May through early June, after which the prevalence of spent individuals increases rapidly (Baldwin, 1988; Burbank, unpublished data). Accordingly, June 1st was selected as a realistic hatch date for modelling purposes. The assumptions of the von Bertalanffy growth models, including homoscedasticity and normality of residuals, were examined using plots of residuals versus fitted values and histograms of residuals, respectively. Models developed using opercular and otolith age data were compared using analysis of the residual sum of squares (ARSS) following (Chen *et al.*, 1992; Haddon, 2001) to determine if the structure-specific growth curves differed significantly. Additionally, growth models from urban and non-urban sites (see Figure 2.1) were compared to evaluate differences in growth between environments using ARSS (Chen *et al.*, 1992; Haddon, 2001). The von Bertalanffy model parameters were further compared statistically using hierarchal ratio tests (Kimura, 1980).

Age estimates (year) derived from otolith structures were used to create a probabilistic age-length key for Silver Shiner following Isermann & Knight (2005) (Table S-2.1). The age of all non-structure aged Silver Shiner with length measurements collected during the study were assigned using the age-length key. Actual and estimated age assignments of individuals were then used to determine the total number of Silver Shiner captured in each age class required for the population mortality estimates. Furthermore, differences in the proportion of age-0 and adult (age-1 and older) fish in urban and non-urban reaches were tested using Fisher's exact test (Zar, 2010). Age and growth analyses were conducted using the FishR package (Ogle, 2018) in R version 3.6.1 (R Development Core Team, 2013).

Given the importance of mortality for defining population dynamics and the difficulty associated with estimating it because of the need for both an extensive aging program and limited selection bias in sampling (Kenchington, 2014), a suite of mortality estimators suited for information-limited fisheries were applied here. Estimates were derived using summer 2017 and 2018 data only for which grouped sample sizes were sufficiently large (n > 200). Mortality approaches were broadly grouped into those based on life-history theory (Charnov *et al.*, 2013; Cubillos *et al.*, 1999; Taylor, 1958), those based on length- or weight-at-age data (Beverton & Holt, 1956, 1957; Hoenig, 1983; Huynh *et al.*, 2018; Peterson & Wroblewski, 1984; Robson & Chapman, 1961), those based on abundance or density (CPUE used as a surrogate) data (as described in Power 2007 and Skalski *et al.*, 2005) and those based directly on age data (Robson & Chapman, 1961; Sekharan, 1975; Skalski *et al.*, 2005). Mean population estimates of mortality across pooled adult age-classes (considered here as ages 1 to 3+) were computed for each class of estimator following the methods described in the references above. The resulting urban and non-urban estimates were grouped and compared for differences using a two-sample t-test to determine if mean estimated mortality in urban affected reaches exceeded that of the non-urban reaches. Because the null hypothesis was directional, a one-tailed test was used (Zar, 2010).

2.3 Results

Silver Shiner captured in Sixteen Mile Creek (n=1403) over the 2017-2019 period varied in length from 23-135mm (mean ± standard deviation; 73.75 ± 19.59 mm). The average length of individuals captured was 68.54 mm in urban reaches and 75.57 mm in non-urban reaches. Overall, the CPUE for Silver Shiner was higher in non-urban (13.12 fish/seine haul) reaches compared to the urban reaches (9.163 fish/seine haul). The majority of Silver Shiner was captured in runs (82.74%), followed by pools (11.79%) and riffles (5.47%). Habitat characteristics (shown in Table 2.1) were not significantly different between urban and non-urban reaches (average stream width: t-test, $t_{3.8}=0.793$, $P = 0.474$; average depth: t-test, $t_{2.6}=0.211$, $P = 0.848$; average water velocity: t-test, $t_{4.0}=-1.32$, $P = 0.257$; average pH: t-test, $t_{3.6}=-0.298$, $P = 0.782$; average conductivity: t-test, $t_{3.6}=-1.43$, $P = 0.234$; average DO: t-test, $t_{3.6}=0.332$, $P = 0.758$). Average stream temperature at urban (15.71 ± 7.2 °C) and non-urban (15.75 ± 6.6 °C) reaches did not significantly differ between March-November 2017 (t-test, $t_{23217} = 0.452$, $P = 0.651$).

Age estimates of calcified structures from a subset of individuals (n=254) resulted in between-reading agreement of 85.4 % and 88.9% for otolith and opercular structures respectively. Between-structure agreement was 88.9% (Pearson’s r = 0.92), indicating both structures yielded similar age estimates (Figure 2.2). The age estimates of 28 individuals differed between the two structures. For 6 individuals the age estimated from the otolith was one year older than that of the opercular and for 22 individuals the age estimated with the opercular was one year older compared to the otolith. Disagreement among structures was most prominent for individuals between 100-119 mm (Table 2.2). The oldest age estimated for Silver Shiner was 3+ for both otolith and opercular structures.

Seasonal length-frequency plots indicated two distinctive modes for Silver Shiner that shifted through time (Figure 2.3). The von Bertalanffy growth models describing the relationship between Silver Shiner age and length based on otolith and opercular structures did not differ significantly when modelled on either a yearly or monthly basis (year: ARSS, $F_{3,502} = 1.07$, $P = 0.361$; month: ARSS, $F_{3,502} = 0.99$, $P = 0.398$; Table 2.3, Figure 2.4). Growth models for Silver Shiner captured at urban and non-urban reaches differed significantly, whether estimated using annual or monthly data (otolith-year: ARSS, $F_{3,248} = 5.29$, $P < 0.01$; opercular-year: ARSS, $F_{3,248} = 3.05$, $P < 0.05$; otolith-month: ARSS, $F_{3,248} = 11.36$, $P < 0.00001$; opercular-month: ARSS, $F_{3,248} = 13.10$, $P < 0.00001$) with urban growth models showing significantly and consistently lower $L\infty$ and higher K parameter estimates (Table 2.4, Figure 2.5).

The proportion of age-0 fish captured in urban and non-urban affected reaches differed significantly (Fisher's Exact Test, $P < 0.001$), with the proportion of age-0 fish in non-urban reaches (76%) being significantly greater than in urban reaches (67%). The mean of all mortality estimates for urban reaches (70.8%, range: 30.1-96.2%) exceeded that of non-urban reaches (61.0%, range: 25.1-90.6%), with the difference being significant (t-test, $t_{12} = 2.91$, $P = 0.006$; Table 2.5). Within the classes of estimators considered, mean estimates of mortality for urban reaches equaled or exceeded estimates for non-urban reaches (Table 2.5). Density and age-based mortality estimates did not differ significantly (density-based: t-test, $t_1 = 0.881$, $P = 0.270$; age-based: t-test, $t_2 = -0.121$, $P = 0.457$) between urban and non-urban reaches, whereas life-history and length-based estimates of mortality differed significantly (life-history-based: t-test, $t_2 = 13.30$, $P = 0.003$; length-based: t-test, $t_4 = 2.97$, $P = 0.021$), with mortality in urban reaches always exceeding that in non-urban reaches.

2.4 Discussion

Accurate life-history and vital rate information is critical for the conservation of imperilled fishes, including determining whether threats lead to changing demographics. Here we evaluated the age-structure, growth, and mortality of Silver Shiner and found it to be a short-lived, quick growing species with low annual survival. Furthermore, we found that growth was altered, and survival and the proportion of age-0 fish within the sample were significantly reduced within urban sites, which suggests a negative effect from urbanization and a probable link between urbanization and the viability of the species in Canada.

Our study indicates Silver Shiner reaches a probable maximum age of 4, similar to previous longevity estimates for the species in Canada (Baldwin, 1983; Gruchy *et al.*, 1973; McKee & Parker, 1982). The maximum age identified for Silver Shiner is identical to that reported for Emerald Shiner, *Notropis atherinoides* and River Shiner, *Notropis blennius* (Froese & Pauly, 2019). Longevity in Silver Shiner, however, exceeds that of Rosyface Shiner, *Notropis rubellus* and Pugnose Shiner, *Notropis anogenus* (Froese & Pauly, 2019). Rosyface Shiner is more ecologically similar to Silver Shiner, typically occupying stream habitats, whereas Emerald Shiner and Pugnose Shiner typically occupy lakes and wetlands respectively (Holm *et al.*, 2009). While the ages of Silver Shiner reported here have not been validated *sensu* Campana (2001), it is important to note that maximum age estimates fall within the range (age 2-5) of those reported for *Notropis* spp. in the Great Lakes basin (e.g. Holm *et al.*, 2009; Froese and Pauly, 2019).

The von Bertalanffy growth curves for Silver Shiner were similar to what has been previously described for the species based on limited aging (n=50) and an assumption of size at hatch based on Emerald Shiner (Young & Koops, 2013b). Here, however, different growth trajectories were evident for individuals captured in urban and non-urban environments. The urban population exhibited suppressed asymptotic length (L_∞) and approached L_∞ at a faster rate (higher K value) than the non-urban population, suggesting growth may be impacted by urbanization. Increases in K may be related to reduced age-0 abundances in urban reaches, as observed here with age-0 CPUE being substantially lower (average 43%) at each seasonal sampling. Differences in growth at urban compared to non-urban reaches were not the result of early maturation as Silver Shiner begin to reproduce at age 1+ (Baldwin, 1983). Similar reductions in juvenile abundances have been noted in studies of urban and wastewater affected streams (e.g. Adams *et al.*, 1992; Yeom *et al.*, 2007). Decreased age-0 abundances may reduce overall densities and competition, thereby increasing resource availability. With increased food and habitat availability more energy could lead to faster juvenile growth (Adams *et al.*, 1992), embodied here as a higher K for urban fishes. Regardless, growth models and average fish length suggest a lower final size is achieved in urban reaches and occurs in conjunction with elevated mortality. When density dependent effects are prominent, density and mortality are positively correlated as adjustments in mortality alter abundance to adapt it to habitat carrying capacity, with high densities typically being associated with increased mortality (Rose *et al.*, 2001). In contrast we found low CPUE reflective of lower densities to be associated with higher mortalities in urban environments, a combination that would rule out differences in mortality between urban and non-urban reaches as being due to density-dependent effects.

Overall Silver Shiner experienced high mortality (61-71%) within Sixteen Mile Creek. Mortality estimates were comparable with the modelling based estimate (75%) reported by Young and Koops (2013) and are within the ranges of adult mortality estimated for other *Notropis* spp.: Sand Shiner, *N. stramineus* (77.9%) in Iowa (Smith *et al.*, 2010), Carmine Shiner *N. percobromus* (78%) in Canada (Young & Koops, 2013a) and Pugnose Shiner (85.9%) in Canada

(Venturelli *et al.*, 2010). Mortality, however, varied significantly among urban and non-urban reaches, with the higher rates observed in urban reaches, likely attributable to the effects of urbanization at the reach scale.

Altered growth and elevated mortality in the urban compared to non-urban reaches was evident based on the von Bertalanffy growth curves and estimated mortality using the life-history and length-based estimators (e.g. Kenchington, 2014). Collectively the weight of evidence provided by the mortality estimates suggests significant declines in survival associated with occupancy of urban reaches. Further, the reduced proportion of age-0 Silver Shiner may be linked to reduced recruitment from larval stages, with urban stream channels known to have a variety of effects on juvenile fish recruitment (Weber & Wolter, 2017). For example, urbanization may impact stream resident fishes by introducing pollutants, increasing storm water runoff, and/or altering channel substrate and structure (Klein, 1979). Lack of suitable riparian vegetation may also reduce areas with suitable cover, exposing fish to changes in temperature that can have varying lethal and sub-lethal impacts on the smallest fishes, depending on the intensity of temperature changes (Regetz, 2003). In severe cases, urbanization impacts may limit recruitment among a wide variety of species to less impaired headwater reaches (Siligato & Böhmer, 2002).

Urbanization is a major stressor in stream systems (Paul & Meyer, 2001). Because the physiological consequences of dealing with stress are energetically expensive (Barton & Iwama, 1991; Van Weerd & Komen, 1998), urban development might be expected to alter the growth and reduce survival of fish, as observed here. For example, Spanjer *et al.* (2018) observed reduced growth in juvenile Coho Salmon, *Oncorhynchus kisutch* across an urbanization gradient, while Monteiro Pierce *et al.* (2020) identified reduced length, growth rate, and condition factor in Alewife, *Alosa pseudoharengus* along coastal watersheds of New England. Altogether, urbanization has been associated with negative implications for the growth and survival of a wide range of fish species in lakes (Francis & Schindler, 2009), streams (Nelson *et al.*, 2009), and coastal environments (Monteiro Pierce *et al.*, 2020).

Significant negative community impacts of urbanization have been identified in small-bodied stream fishes including altered species composition, increases in tolerant species, and homogenization of the fish community (Morgan & Cushman, 2005; Wang *et al.*, 2000); however, the impacts of urbanization on the vital rates of small-bodied fishes are not commonly examined. Changes in land use associated with urbanization such as increases in impervious surfaces have been linked to reduced population abundance for Redside Dace in the Greater Toronto Area (Poos *et al.*, 2012), and reduced occupancy of species such as Speckled Madtom (*Noturus leptacanthus*) and Etowah Darter (*Etheostoma etowahae*) in Georgia (Wenger *et al.*, 2008). The observed reduced asymptotic length (L_∞) and higher mortality in urban reaches (Table 2.4 and 2.5) indicates that urbanization may have negative fitness implications for Silver Shiner given the importance of size and survival for determining overall abundance (Power, 2002). Therefore, urbanization should be considered an important threat for the species and considered when developing conservation approaches.

Urbanization impacts on stream fishes have often been related to increased stream channel temperatures owing to landcover changes (i.e. Wang *et al.*, 2003; Wheeler *et al.*, 2005; Nelson and Palmer, 2007). However, in the current study, stream temperatures at urban and non-urban sites did not significantly differ, suggesting observed differences in growth and mortality cannot be attributed to thermal effects. It is important to acknowledge that urban impacts were examined within only one stream along a longitudinal gradient, a sampling design necessitated by the Threatened status of the species and its limited occurrence within Canada. While other longitudinal upstream-downstream effects in addition to urbanization may be present, habitat characteristics and water quality (Table 2.1) did not vary among urban and non-urban reaches as might be expected if longitudinal effects prevailed. Nevertheless, alternate factors such as food quality and availability should be considered. Silver Shiner actively forages at surface and mid-levels of the water column (Bouvier *et al.*, 2013) and consumes terrestrial drift material within the river. The loss of intact high-quality riparian habitats, therefore, is potentially important for the species. Urban development in areas surrounding occupied river reaches may reduce the quality, quantity and diversity of terrestrial drift material entering the system (Gimenez *et al.*, 2015; Jones & Leather, 2012), thereby reducing the amount of food available and leading to reduced growth and survival. Riparian habitats are also essential components of the critical habitat for fishes (Richardson *et al.*, 2010) and suitable riparian vegetation has been identified as important for the persistence of species such as Redside Dace (Reid *et al.*, 2019). Terrestrial habitats both immediately adjacent to stream banks and further inland are particularly important for some fishes (Correa & Winemiller, 2018), including drift feeding fishes such as Silver Shiner due to their direct reliance on terrestrial resources. Silver Shiner and other small-bodied fishes in Ontario streams are expected to continue to face changing conditions and increased pressures from urbanization due to projected increases in human populations within Southern Ontario (Ontario Ministry of Finance, 2019). Given the observed alteration in growth, reduction in the relative proportion of age-0 individuals and the elevated mortality of Silver Shiner in urban reaches, it is important to fully understand the collective impacts of urban development on Silver Shiner and other imperilled species.

The study has assessed the relationship between species-specific vital rates and a dominant threat, and as such, highlights the importance of obtaining suitable life-history information for the conservation of small-bodied freshwater fishes. We have illustrated the potential negative impacts of urbanization for the growth and survival of Silver Shiner, a species that exists within increasingly urbanized watersheds in Canada. The results of the study indicate the need to understand, consider and mitigate the impacts of urban development on this and other small-bodied imperilled fishes. We recommend that future studies evaluate the mechanisms behind reduced vital rates in urban regions to further understand how the threat impacts freshwater fishes.

Table 2.1. Measures of average stream width (m), average depth (m), average water velocity (m/s), average pH, average conductivity (µS), and average dissolved oxygen (mg/L) collected at each sample reach following fish collections.

Reach	Urban Status	Avg Stream Width (m)	Avg Depth (m)	Avg Water Velocity (m/s)	Avg pH	Avg Conductivity (µS)	Avg DO (mg/L)
1	Non-Urban	16.04	0.65	0.37	8.58	677.74	10.02
2	Non-Urban	14.24	0.31	0.41	8.46	642.50	9.10
3	Non-Urban	11.83	0.35	0.46	8.88	586.57	11.71
4	Urban	12.33	0.49	0.51	8.55	735.11	8.96
5	Urban	9.91	0.40	0.50	8.65	744.97	10.05
6	Urban	15.21	0.35	0.41	8.85	626.02	10.87

Table 2.2. The number of fish aged in each length interval, the number of individuals with agreement between otolith and opercular age estimates, and the percent agreement between otolith (OTO) and opercular (OP).

Length Bin (mm)	**N**	**Agreed**	**Agreement (%)**	**Notes**
<35	1	1	100.00	
35-39	14	14	100.00	
40-44	14	14	100.00	
45-49	15	15	100.00	
50-54	15	15	100.00	
55-59	15	15	100.00	
60-64	15	14	93.33	OTO<OP
65-69	15	15	100.00	
70-74	14	14	100.00	
75-79	14	12	85.71	OTO>OP, OTO<OP
80-84	13	13	100.00	
85-89	15	15	100.00	
90-94	15	15	100.00	
95-99	15	13	86.67	2OTO<OP
100-104	14	8	57.14	6OTO<OP
105-109	15	10	66.67	OTO>OP,4OTO<OP
110-114	14	8	57.14	2OTO>OP,4OTO<OP
115-119	14	9	64.29	OTO>OP,4OTO<OP
120-124	4	3	75.00	OTO>OP
≥ 125	3	3	100.00	

Table 2.3. Estimated von Bertalanffy growth curves for Silver Shiner captured in Sixteen Mile Creek during 2017-2019, using age estimates from otolith and opercular structures.

Age Type	**Structure**	**Model**
Year	Otolith	L_{age}=128.70 (1- $e^{(-0.758(ageyear+0.764)}$)
	Opercular	L_{age}=128.73 (1- $e^{(-0.690(ageyear+0.838)}$)
Month	Otolith	L_{age}=120.46 (1- $e^{(-0.0923(agemonth+3.80)}$)
	Opercular	L_{age}=112.81 (1- $e^{(-0.0818(agemonth+3.73)}$)

Table 2.4. Estimated von Bertalanffy growth curves for Silver Shiner captured at urban and non-urban reaches in Sixteen Mile Creek during 2017-2019 using age estimates from otolith and opercular structures. Differing superscripts in the location column indicate where growth curves are significantly different.

Age Type	**Structure**	**n**	**Location**	**Model**
Year	Otolith	136	Non-urban^{A}	L_{age}=144.73 (1-e$^{(-0.491(\text{ageyear}+1.079)}$)
		118	Urban^{B}	L_{age}=117.52 (1-e$^{(-1.238(\text{ageyear}+0.501)}$)
	Opercular	136	Non-urban^{A}	L_{age}=139.34 (1-e$^{(-0.507(\text{ageyear}+1.091)}$)
		118	Urban^{B}	L_{age}=117.44 (1-e$^{(-1.081(\text{ageyear}+0.577)}$)
Month	Otolith	136	Non-urban^{C}	L_{age}=131.59 (1-e$^{(-0.0634(\text{agemonth}+6.220)}$)
		118	Urban^{D}	L_{age}=106.34 (1-e$^{(-0.208(\text{agemonth}+0.655)}$)
	Opercular	136	Non-urban^{C}	L_{age}=126.70 (1-e$^{(-0.0672(\text{agemonth}+6.232)}$)
		118	Urban^{D}	L_{age}=106.13 (1-e$^{(-0.213(\text{agemonth}+0.599)}$)

Table 2.5. Annualized mortality (proportion) estimates for adult (pooled age 1 – 3+) Silver Shiner computed using age, length, abundance, and life-history based methods reported in the literature for urban and non-urban sampling environments. Mean ± standard error of the mean for each group of estimation methods. Differences given as urban minus non-urban estimates. Estimates derived using summer 2017 and 2018 data only for which sample sizes where sufficiently large (n>200). *P* is the one-tailed t-test *P*-value for the t-test testing for significantly higher mortality in urban than non-urban environments (i.e., that the difference between the environments is positive).

Estimation Method	**Urban**	**Non-Urban**	**Difference**	***P***
Life-history-based	0.71 ± 0.04	0.42 ± 0.04	0.29	0.003
Length-based	0.68 ± 0.12	0.62 ± 0.10	0.06	0.021
Density-based	0.59 ± 0.12	0.54 ± 0.18	0.05	0.270
Age-based	0.82 ± 0.08	0.82 ± 0.05	0.00	0.457
Average of all methods	0.71 ± 0.05	0.61 ± 0.06	0.10	0.006

Figure 2.1. Capture locations of Silver Shiner at non-urban (green; upstream) and urban (red; downstream) locations in Sixteen Mile Creek, Ontario, Canada, sampled from 2017-2019.

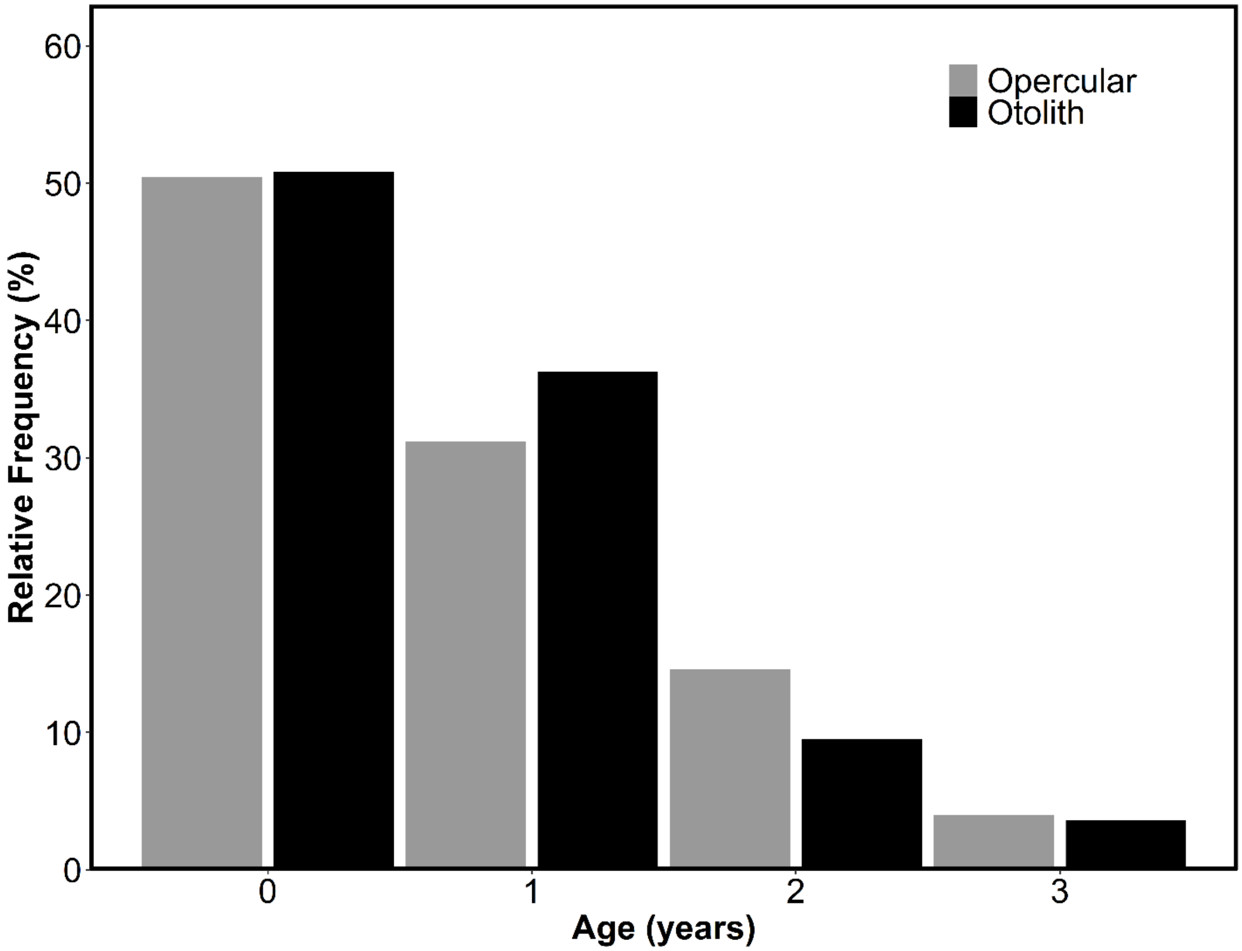

Figure 2.2. The relative frequency of individuals in each age class based on age estimates using opercular (grey) and otolith (black) structures.

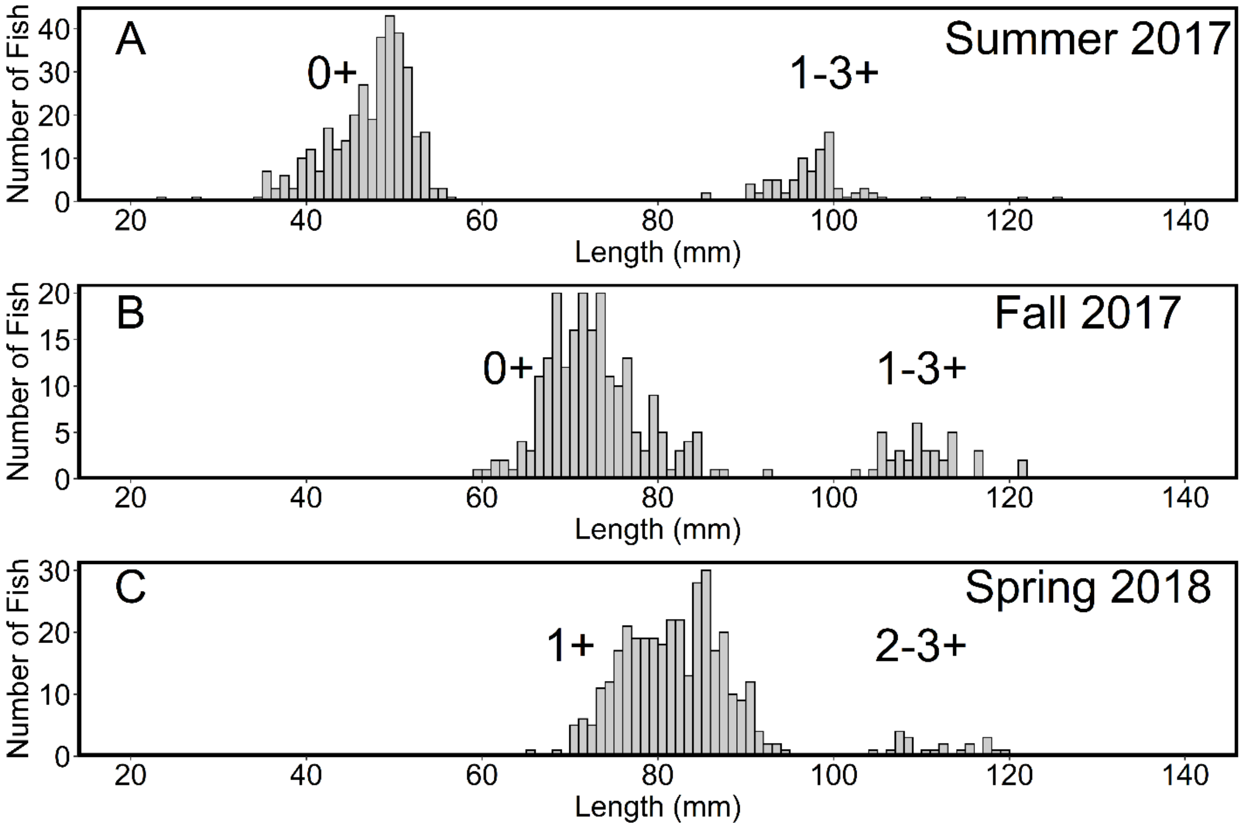

Figure 2.3. Length-frequency (total length, mm) histograms for Silver Shiner captured in Sixteen Mile Creek, Oakville, Ontario in A) Summer 2017 (n = 435), B) Fall 2017 (n = 252) and C) Spring 2018 (n = 367).

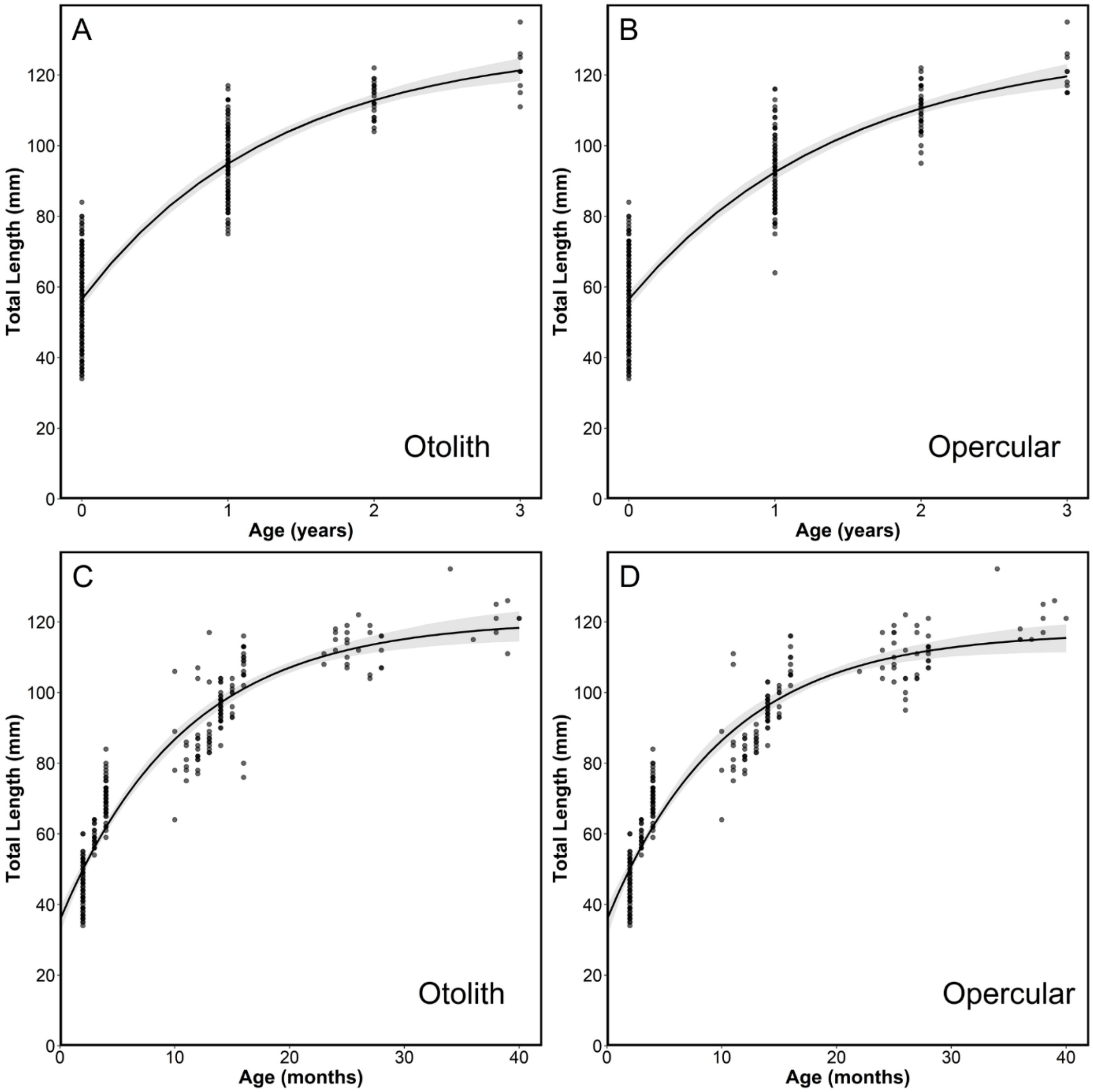

Figure 2.4. Estimated von Bertalanffy growth models for Silver Shiner captured in Sixteen Mile Creek, Ontario, Canada between 2017-2019. Individual length at age is plotted. Age is expressed as year and estimated with A) Otolith and B) Opercular structures. Age was also expressed as month for ages estimated with C) Otolith and D) Opercular.

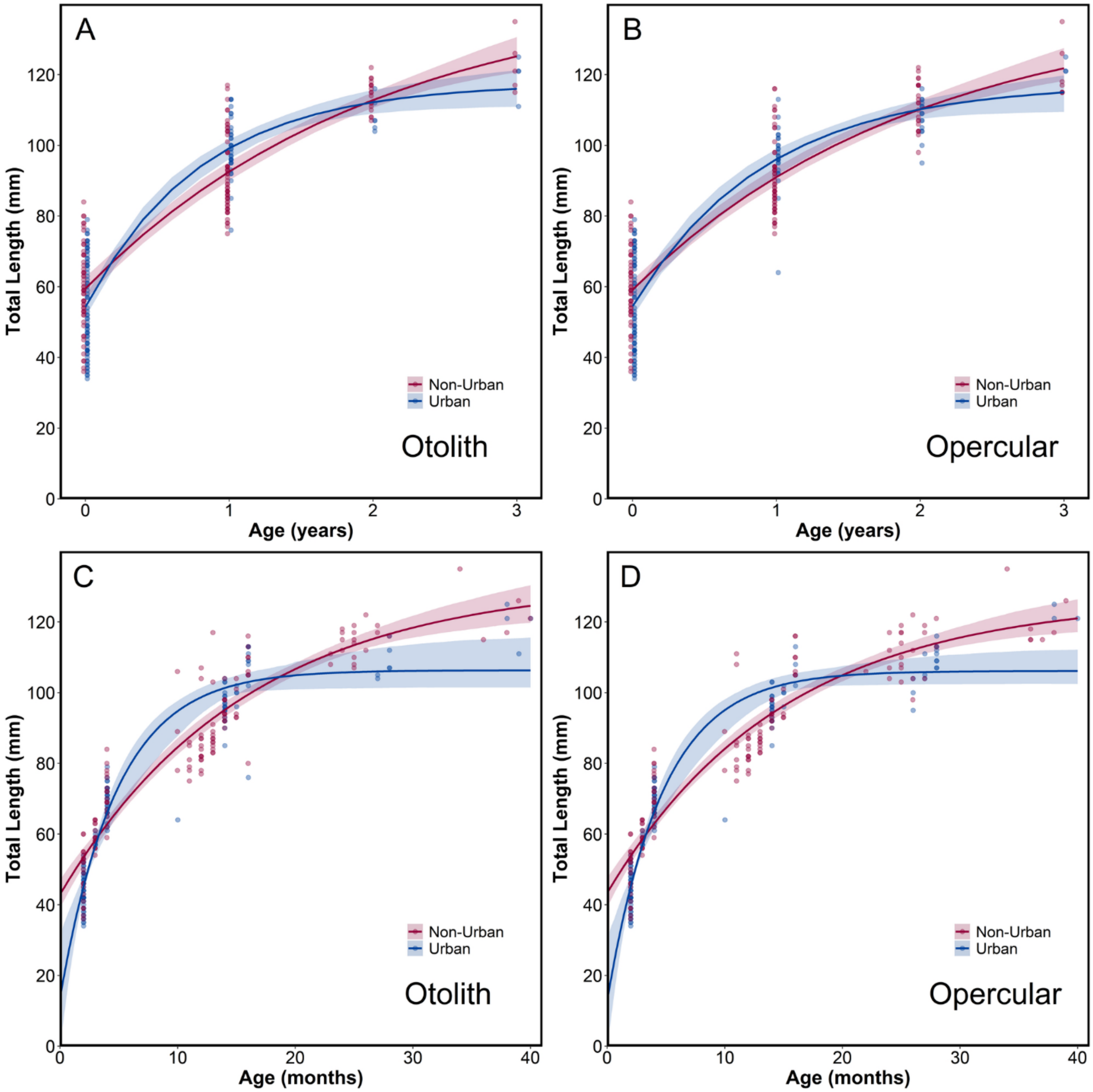

Figure 2.5. Estimated von Bertalanffy growth models for Silver Shiner captured at urban and non-urban reaches sampled in Sixteen Mile Creek, Ontario, Canada between 2017-2019. Individual length at age is plotted. Age is expressed as year and estimated with A) Otolith and B) Opercular structures. Age was also expressed as month for ages estimated with C) Otolith and D) Opercular.

Table S-2.1 Probabilistic age-length key developed from sampled Silver Shiner used in this study.

Length Bin (mm)	**Age 0+**	**Age 1+**	**Age 2+**	**Age 3+**
<35	1	0	0	0
35-39	1	0	0	0
40-44	1	0	0	0
45-49	1	0	0	0
50-54	1	0	0	0
55-59	1	0	0	0
60-64	1	0	0	0
65-69	1	0	0	0
70-74	1	0	0	0
75-79	0.57	0.43	0	0
80-84	0.23	0.77	0	0
85-89	0	1	0	0
90-94	0	1	0	0
95-99	0	1	0	0
100-104	0	0.93	0.07	0
105-109	0	0.6	0.4	0
110-114	0	0.5	0.43	0.07
115-119	0	0.14	0.71	0.14
120-124	0	0	0.25	0.75
≥125	0	0	0	1

Chapter 3: The influence of thermal cues on the reproductive phenology of Silver Shiner, *Notropis photogenis*

The definitive publisher authenticated version of this paper is: Burbank, J., Drake, D.A.R. and Power, M. (2021) The influence of thermal cues on the reproductive phenology of Silver Shiner, *Notropis photogenis*. Journal of Fish Biology 100(2): 416-424.

3.1 Introduction

Reproductive ecology is an important component of a species' life-history (Winemiller & Rose, 1992). Reproductive phenology (i.e., the timing of the initiation and cessation of spawning) and the length of the growing season have implications for population growth and overwinter survival of freshwater fishes (Cargnelli & Gross, 1996). Fish must balance their allocation of energy to ensure sufficient gonadal development prior to spawning while leaving adequate time for the growth of offspring to maximize young of year (YOY) overwinter survival (Shuter *et al.*, 2012, 1980). Therefore, the timing of spawning is often reflective of favorable environmental conditions that optimize larval development and fitness (Munro, 1990) and is expected to vary in response to interannual variation in environmental conditions (Heins, 2020). Understanding the reproductive phenology of species, particularly imperilled species, in relation to environmental conditions is needed to better evaluate their potential responses to changing environmental conditions, estimate future population dynamics, and develop comprehensive recovery strategies.

Reproductive phenology can vary in response to environmental conditions (i.e. cues) such as, but not limited to, the accumulation of thermal energy (Coulter *et al.*, 2016; Heins, 2020), flow pulses (Archdeacon *et al.*, 2020), or a combination of both (Falke *et al.*, 2010; Munz & Higgins, 2013). Research indicates that the reproductive phenology of many small-bodied freshwater fishes is temperature dependent, including species such as Roundtail Chub, *Gila robusta* (Fraser *et al.*, 2019) and Oregon Chub, *Oregonichthys crameri* (Scheerer & McDonald, 2003). The accumulation of thermal energy throughout spring has been identified as one important way in which temperature affects reproductive phenology, having been shown to influence reproductive readiness and spawn timing of Longnose Shiner, *Notropis longirostris* that shift to earlier initiation of spawning with faster warming (Heins, 2020). Thermal energy is also responsible for the timing and duration of spawning activity in Blackbanded Darter, *Percina nigrofasciata*, with different populations exhibiting unique reproductive life-history traits linked to the dominant thermal regime (Hughey *et al.*, 2012). Furthermore, spawning is temperature dependent and adapted to the seasonal thermal cycle for other percids including: Yellow Perch, *Perca flavescens*, European Perch, *Perca fluviatilis* and Pikeperch, *Sander lucioperca* (Hokanson, 1977). Although temperature is a prevalent determinant of spawning, the among-year variation of the thermal regime and its effect on reproductive phenology is still poorly understood for many small-bodied freshwater fishes (Krabbenhoft *et al.*, 2014; Woods *et al.*, 2021), particularly imperilled fishes.

The accumulation of thermal energy, measured as cumulative growing degree days (GDD) (Chezik *et al.*, 2014), can dictate the rate of gonadal development in adults (Akhoundian *et al.*, 2020; Liu *et al.*, 2021; De Vlaming, 1975) and result in year-to-year variation in spawn timing (Lappalainen *et al.*, 2003). Variation in spawn date, in turn, can affect incubation period, hatch date, daily growth of YOY, the probability of overwinter survival, and resulting year-class strength (Shuter *et al.*, 1980). Therefore, among-year variation in the reproductive phenology of a species arising from differences in the thermal regime can have important implications for species conservation (Woods *et al.*, 2021). A first step for describing the influence of thermal energy is to understand the relationship between GDD and the initiation and cessation of spawning. Flexible models that predict spawn timing in relation to the environmental conditions in a given year (i.e. GDD) have the potential to improve the understanding of spawning phenology in general (Coulter *et al.*, 2016; Embke *et al.*, 2016), and for imperilled fish species, could help evaluate shifts in the reproductive period in response to thermal changes driven by threats such as, but not limited to, climate change (Hovel *et al.*, 2017) and urbanization (Firkus *et al.*, 2018).

One small-bodied fish species for which there is a limited understanding of reproductive phenology is Silver Shiner, *Notropis photogenis*, which is listed as Threatened under Canada's *Species at Risk Act* (Burbank *et al.*, 2020, 2021b). In particular, the reproductive ecology of this species at the northern edge of its range where it is threatened is largely unknown (DFO, 2013). The limited examinations of spawn timing in Canada are coarse and outdated (Baldwin, 1983; McKee & Parker, 1982), which has restricted the relevance and utility of the information in the development of species recovery strategies. Conversely, in Alabama, at the southern edge of the species' range, a recent study has indicated that Silver Shiner can undergo multiple synchronized spawning events between February and April in response to stream discharge and temperature, with large spawning events occurring following substantial increases in spring discharge (Hodgskins *et al.*, 2016). In Canada, reproduction is expected to be seasonally delayed by winter, implying latitudinal gradient effects (Blanck & Lamouroux, 2007; Leggett & Carscadden, 1978; Mills, 1988). Given the paucity of information regarding the reproductive phenology of Silver Shiner in Canada we: 1) describe the reproductive phenology of Silver Shiner for a population at the northern edge of the species' range; and, 2) determine if spawning occurrence can be predicted on the basis of thermal cues such as GDD.

3.2 Methods

Silver Shiner was captured during the spring (April-June) of 2018 and 2019 in Sixteen Mile Creek, Oakville, Ontario, Canada (43.49908^{o}N, 79.77673^{o}W). The Sixteen Mile Creek watershed drains an area of approximately 372 km^2 and the creek flows through a mix of forested, agricultural, and urbanized land, including two large urban centers (Milton and Oakville), prior to entering Lake Ontario (Conservation Halton, 2009). Sampling effort was focused on a rural reach of Sixteen Mile Creek (see Reach 1 in Burbank *et al.*, (2021)), with a channel width ranging from approximately 10-30 m. The study location was chosen because Silver Shiner is known to be more abundant in Sixteen Mile Creek relative to the other systems inhabited within Canada (Burbank *et al.*, 2021b; Glass *et al.*, 2016). Fish were captured in run and pool habitats using repeat hauls of a 9.14 m bag seine with a 3mm mesh bag (Table S-3.1). Sampling began each spring as soon as spring flow conditions allowed safe operation of the seine net. All fishes were collected and retained under an animal use permit (AUP 1846) approved by the Canadian Council on Animal Care. Upon capture fish were identified to species and a random subset of Silver Shiner were selected for further examination. Individuals were labelled and frozen for subsequent detailed gonad examinations completed in the laboratory. Furthermore, in 2018 a subset of larvae that were incidentally captured during seine netting were retained (May 25th, n=56; June 1st, n=41; June 8th, n=13; June 14th, n=45) and subsequent barcoding analysis (e.g. Ivanova *et al.*, 2007) was used to confirm species identification (DFO, unpublished data).

Water temperature at the site of capture was measured every half hour between March 29th, 2017 and June 29th, 2019 using two instream temperature loggers (Onset HOBO Pro V2) placed near the bottom of the river adjacent to the sampling site. Average daily water temperature throughout spring 2018 and 2019 was estimated using the instream temperature loggers. Daily discharge (m^3/s) data were obtained from an Environment Canada gauging station (station 02HB004) located approximately 100m upstream of the capture location.

In the lab, Silver Shiner were thawed and measured for total length (mm) and mass (g). Individuals were dissected and their gonads (Figure 3.1) were examined under a dissecting microscope (SZN- 2; Optika, Italy). Sex was determined from gonad examinations and only females were used during the study. The ratio of mature females to males of retained Silver Shiner was 0.78 females per male and 0.84 females per male in 2018 and 2019, respectively. The gonads of n=160 and n=81 adult females captured in 2018 and 2019, respectively, were examined in detail. We used a combination of field observation (i.e. if eggs were visibly shedding (Figure 3.1) or not upon handling in the field), the Gonadosomatic Index (GSI) value, and the visual appearance of ovaries when examined under the microscope (Hodgskins *et al.*, 2016) to determine the stage of reproduction (not spawned, spawning, or spawned) for each captured individual. The GSI was computed following Durham & Wilde (2014) as:

$$\mathrm{GSI} = \left(\frac{\text{Gonadal Mass (g)}}{(\text{Body Mass (g)} - \text{Gonadal Mass (g)})}\right) * 100$$

An individual with a GSI ≤ 4.0 was considered as either: i) not spawned, if ovaries appeared opaque, undeveloped and contained latent eggs, or ii) already spawned, if ovaries were ruptured, largely void of eggs and visibly spent. An individual with a GSI > 4.0 and GSI ≤ 11.0 was considered as either: i) not yet spawned with ripening gonads, if ovaries filled a substantial portion of the abdominal cavity and contained white- and cream-coloured eggs, but showed no evidence of gonad rupture and egg extrusion, or ii) spawning with partially spent gonads, if ovaries showed evidence of rupture and egg extrusion and contained eggs that were orange and yellow in colour. An individual with a GSI > 11.0 was considered as either: i) not spawned with ripe gonads, if ovaries were filled with orange and yellow eggs, but showed no evidence of rupture and egg extrusion, or ii) spawning, if ovaries showed evidence of rupture and egg extrusion and eggs were readily shed when handling in the field.

We evaluated the relationship between spawn initiation, or cessation, and cumulative growing degree days with a base of 5 ^{o}C (GDD_5) using logistic regression following Coulter *et al.*, (2016). GDD_5 was computed following Trudel *et al.*, (2010) as:

$$\mathrm{GDD}_5 = \sum_{i=1}^{n} T_i - T_0$$

Where T_i is the average daily water temperature, T_0 is the base temperature of 5 °C and only non-negative values (days with an average daily temperature greater than 5 °C) are summed. A base temperature of 5 °C was selected as some stream-dwelling small-bodied fishes such as Blacknose Dace *Rhinichthys atratulus* have been observed to resume open-water activity following winter when water temperatures exceed 4 °C (Cunjak & Power, 1986a). A base temperature of 5 °C was used in favour of 4 °C following the recommendation to standardize base temperatures when computing GDD (Chezik *et al.*, 2014). We used the 2018 data to construct the spawn initiation and cessation models and evaluated models using the pseudo R^2 values and the likelihood ratio test (McCullagh & Nelder, 1983). The ‘logistf’ package in R was used to apply Firth's penalized likelihood method for logistic regression (Firth, 1993) to model spawn initiation (spawning and spawned = 1, not yet spawned = 0) and cessation (spawning = 1, no longer spawning = 0). Further, we applied the 2018 models to the 2019 data to validate the models and evaluate their predictive ability. The use of data splitting for model testing and evaluation whereby new data are used to test the predictive powers of a model is a robust procedure for evaluating the predictive performance of statistical models in ecology (Montgomery & Peck, 1982; Power, 1993). Finally, the predictive performance of the 2018 models were evaluated using a confusion matrix to determine if model predictions of spawning state were correct using the measures of sensitivity, the conditional probability that a given individual spawning state was correctly predicted, and specificity, the inverse of sensitivity (see Fielding & Bell (1997)). All analyses were conducted in R version 3.6.1 (R Development Core Team, 2013).

3.3 Results

Based on weekly gonad examinations, GSI values and expressed ripe eggs visible at time of capture, spawning in the population was initiated on May 5th in 2018 and May 6th in 2019 and continued until May 25th in 2018 and June 12th in 2019, after which the majority of examined gonads were spent. Spawning occurred between GDD_5 of 97 – 340 °C•days in 2018 and 107 – 486 °C•days in 2019 (Figure 3.2) and at average daily temperatures ranging from 15 - 22 °C in 2018 and 12 - 20 °C in 2019. Larval Silver Shiner were observed and collected on June 8th, 2018 (n=4; 585 °C•days) and June 14th, 2018 (n=25; 671 °C•days). On June 8th and June 14th, larval Silver Shiner total lengths ranged from 14-23 mm (18.5 ± 3.7 mm; mean ± sd) and 24-29 mm (26.0 ± 1.2 mm) respectively.

Spawning did not coincide with high discharge (spate) events or predictable changes in spring discharge in either 2018 or 2019 but was initiated on the descending limb of the spring hydrograph and was generally completed before the onset of the extended summer low flow period (Figure 3.3). Overall mean discharge was 2.3 ± 4.3 m^3/s and 3.5 ± 4.8 m^3/s in 2018 and

2019 respectively, with low, stable flows established earlier in 2018 compared to 2019 (Figure 3.3). The initiation and cessation of spawning were described by logistic regression models as follows (Figure 3.4):

$$\text{Spawn Initiation: } \log\left(\frac{\pi_i}{1-\pi_i}\right) = -3.44 + 0.050(\text{GDD}_5)$$

(Likelihood Ratio Test=94.29, $p<0.0001$, Pseudo $R^2=0.84$)

$$\text{Spawn Cessation: } \log\left(\frac{\pi_i}{1-\pi_i}\right) = 13.63 - 0.037(\text{GDD}_5)$$

(Likelihood Ratio Test=124.70, $p<0.0001$, Pseudo $R^2=0.84$)

Based on the spawn initiation model, there was a 50% probability the population initiated spawning when GDD_5 reached 68 °C•days (May 3rd in 2018 and April 28th in 2019) and a 95% probability the population initiated spawning by 127 °C•days (May 8th in 2018 and May 9th, 2019). Based on the spawn cessation model, there was a 50% probability the population completed spawning by 368 °C•days (May 27th in 2018 and June 4th in 2019) and 95% probability the population completed spawning by 448 °C•days (May 31st in 2018 and June 10th in 2019; Figure 3.2). Within the 2018 model estimation data set, the spawn initiation model had a misclassification rate of 0.02 and the sensitivity and specificity were 0.99 and 0.91, respectively. The spawn cessation model had a misclassification rate of 0.04 and the sensitivity and specificity were 1 and 0.92 respectively.

The spawn timing models built using 2018 data effectively predicted spawn initiation and cessation in 2019 with misclassification rates of 0.05 and 0.26 for spawn initiation and cessation, respectively. The sensitivity and specificity of the initiation model when applied to 2019 data was 1 and 0.56 respectively and 0.67 and 1 respectively for the spawn cessation model.

3.4 Discussion

Reproduction is an essential component of life-history and interannual variation in reproductive phenology can have important implications for fitness (Munro, 1990). We used two years of field observations and laboratory data to evaluate the reproductive phenology of a Threatened freshwater fish, Silver Shiner, and determined that the cumulative temperature experienced by Silver Shiner is an important determinant of the initiation and cessation of spawning in a Canadian population at the northern edge of its geographic range. Logistic regression models incorporating GDD_5 effectively predicted spawning initiation and cessation, providing useful models for examining the anthropogenic impacts of alterations to the thermal regime on spawn timing and improving the ability to evaluate changes in the larval growth period.

Interannual variation in reproductive phenology linked to the available thermal regime of the occupied ecosystem was similar to what has been observed elsewhere for Silver Shiner (Baldwin, 1983) and is consistent with findings for a range of small-bodied stream-dwelling species such as Longnose Shiner, *Notropis longirostris* (Heins, 2020), Golden Shiner, *Notemigonus crysoleucas* (De Vlaming, 1975), Roundtail Chub, *Gila robusta* (Fraser *et al.*, 2019), and Pikeperch (Hokanson, 1977). Estimates of Silver Shiner reproduction in Canada are highly variable. Previous studies simply used the presence of ripe gonads followed by spent gonads to estimate spawning windows of 17-22 °C (McKee & Parker, 1982), 10-19 °C (Baldwin, 1983) and when temperatures approach 24 °C (Bunt, 2016). Previous research on Silver Shiner spawning in Alabama indicated that spawning is associated with increases in daily mean discharge, with initiation beginning during moderate flows and the largest synchronized spawning events occurring following peak flow events. Modelled relationships with temperature further suggested the majority of spawning in Alabama occurred between average daily temperatures of 12-17 °C, similar to the range reported by Baldwin (1983). Examination of GSI also indicated multiple synchronized spawning events can occur depending on the year and thermal regime (Hodgskins *et al.*, 2016). While the observed range of spawning temperatures in our study was larger than that observed in Alabama (10 versus 5 ^{o}C), spawning in Canada occurred over a shorter period of time (approximately 2-5 weeks compared to 2-4 months), likely as a result of differences in the rate of spring warming in the two localities (Arguez *et al.*, 2012; Lapen & Hayhoe, 2003). The shorter reproductive period may explain why we did not find evidence of multiple synchronized spawning events by Silver Shiner in either year of sampling.

The presence of multiple synchronized spawning events over several months within the southern US (Hodgskins *et al.*, 2016) and the relationship between reproductive phenology and cumulative temperature suggests Silver Shiner populations in Canada may have the potential to spawn multiple cohorts in years with protracted spring warming, which could become more prevalent in the face of climate change. The presence of multiple cohorts resulting from climate change might enhance larval production but also increase intraspecific competition among YOY (Rose *et al.*, 2001), thereby impacting YOY growth and survival. For instance Hovel *et al.*, (2017) found Three-Spined Stickleback, *Gasterosteus aculeatus* spawned multiple cohorts in response to earlier ice break up dates resulting from climate change. The associated increase in intraspecific competition among YOY Three-Spined Stickleback contributed to the decrease in the average length of YOY by the end of the growing season (Hovel *et al.*, 2017), which for many species has implications for over-winter survival (e.g. Meyer & Griffith, 1997; Post & Evans, 1989; Shoup & Wahl, 2011). Consequently, additional research is warranted to evaluate the potential impacts of multiple cohorts arising from protracted spring warming on Silver Shiner YOY growth and year-class strength.

We did not observe an association between spawning and large discharge events. Rather spawning generally occurred on the descending limb of the spring hydrograph and was completed before the onset of the extended period of low summer flow. Extended periods of high spring flows and flood events have the potential to delay the onset of spawning in Silver Shiner as has been seen in other spring spawning riverine fishes such as Redhorse, *Moxostoma* spp. (Cooke & Bunt, 1999; Reid, 2006). In contrast, the earlier establishment of natural low, stable flows characteristic of the summer hydrograph may accelerate the initiation and cessation of spawning and lead to increased reproductive success (Cull Peterson *et al.*, 2013). Silver Shiner is a drift-feeding fish, actively capitalizing on both aquatic and terrestrial prey in the surface drift (Burbank *et al.*, 2022). Therefore, delaying reproduction until high spring flows have passed, and initiating spawning on the descending portion of the hydrograph, may be an adaptive strategy critical for obtaining sufficient prey prior to spawning. Following winter, individuals must acquire adequate energy for gonad development and reproduction (Mcbride *et al.*, 2015), particularly when winters are harsh and there is significant overwinter depletion of metabolic reserves even for cold-adapted salmonid fishes (e.g. Cunjak, 1988). The importance of discharge and water velocity for increased invertebrate drift (Brittain & Eikeland, 1988) suggests that high spring flows may deliver considerable amounts of aquatic and terrestrial prey to Silver Shiner via the surface drift and provide critical resources for post-winter reconditioning prior to spawning. Substantial pre-spawning (i.e. post-winter for spring spawners) prey consumption can improve gonad development (Bunnell *et al.*, 2007) and increase fecundity (Lambert & Dutil, 2000) whereas restricted prey consumption prior to spawning can have negative implications such as reduced egg production (Mcbride *et al.*, 2015; Wootton, 1977). Thus, for small-bodied drift-feeding fishes such as Silver Shiner, reduced prey supply within the surface drift resulting from disrupted flow regimes with dampened spring melts may negatively impact spawning success, highlighting the importance of maintaining natural flow regimes (e.g. Poff & Zimmerman, 2010). Furthermore, spawning during the descending limb of the hydrograph following the spring melt compared to during the spring melt ensures deposited eggs stay closer to the point of release and provides larval fish with shallow, faster warming water that may help promote rapid growth (Haworth & Bestgen, 2016). For example, the average length of larval Silver Shiner increased by 7.5 mm (18.5 to 26.0 mm; ~ 5% of the species maximum length) in 6 days over 86 °C•days during June 2018, suggesting individuals undergo relatively fast larval growth as the water warms. Nevertheless, discharge events or particular discharge thresholds alone do not have the capacity to initiate spawning of Silver Shiner in Canada. Rather, it appears the species has timed its reproduction to optimize post-winter prey consumption, maximizing gonadal development and subsequent egg and larval survival.

We observed GDD_5 as a key determinant of Silver Shiner spawning activity and developed flexible statistical models that can be used to explore variation in the species' spawning phenology as a result of interannual variation in the spring thermal regime. The models can facilitate the development of real-time estimates of spawning activity when daily average water temperatures are available, and therefore ensure that disturbance to the species is minimized during the sensitive reproductive period. It is important to note that our results indicate GDD_5 is a better predictor of spawning initiation than spawning cessation. Heins (2020) similarly noted cumulative temperature was a better predictor of reproductive readiness in Longnose Shiner, with photoperiod playing a more important role in spawning termination. Thus, it is possible that other unconsidered factors, such as photoperiod, may similarly influence the termination of spawning activity in Silver Shiner.

Silver Shiner continue to face urbanization pressures within Canada (Burbank *et al.*, 2021b), with increases in impervious surfaces and reductions in riparian vegetation having the potential to contribute to more rapid spring warming and increases in stream flashiness (Wallace *et al.*, 2013), which could negatively impact spawning success as a result of decreased egg survival (e.g. Rutherford *et al.*, 2016). Our models suggest a contracted spring with faster than average warming would result in the earlier onset and termination of spawning activity and subsequent extension of the larval growing season. Although an extended growing season is often beneficial for YOY growth and overwinter survival (Post *et al.*, 1998; Shuter *et al.*, 1980; Teal *et al.*, 2008), faster spring warming may shorten the pre-spawning reconditioning period, thereby reducing overall reproductive potential of the population. Alternatively, if spring warming is initially rapid and then plateaus, the pattern of repeat spawning following discharge peaks extended over several months as seen in Alabama may become established in Canadian populations provided that critical (and currently unknown) thermal tolerance boundaries for larval fishes are not surpassed. However, advancement of the spawning period may also result in a mismatch in the timing of resource availability (Durant *et al.*, 2007), which can have negative impacts on YOY growth and survival as has been observed for Atlantic Cod *Gadus morhua* (Gotceitas *et al.*, 1996) and hypothesized for Three-Spine Stickleback (Hovel *et al.*, 2017). Alterations to reproductive phenology can also result in changes in the fish species that recently hatched individuals encounter, potentially increasing interspecific competitive interactions through reductions in resource partitioning (Krabbenhoft *et al.*, 2014). Accordingly, we suggest that for imperilled species such as Silver Shiner there remains a need for further research on quantifying the population-level impacts of potential mismatches between reproduction and resource availability and alterations in intra- and interspecific competition associated with altered reproductive phenology.

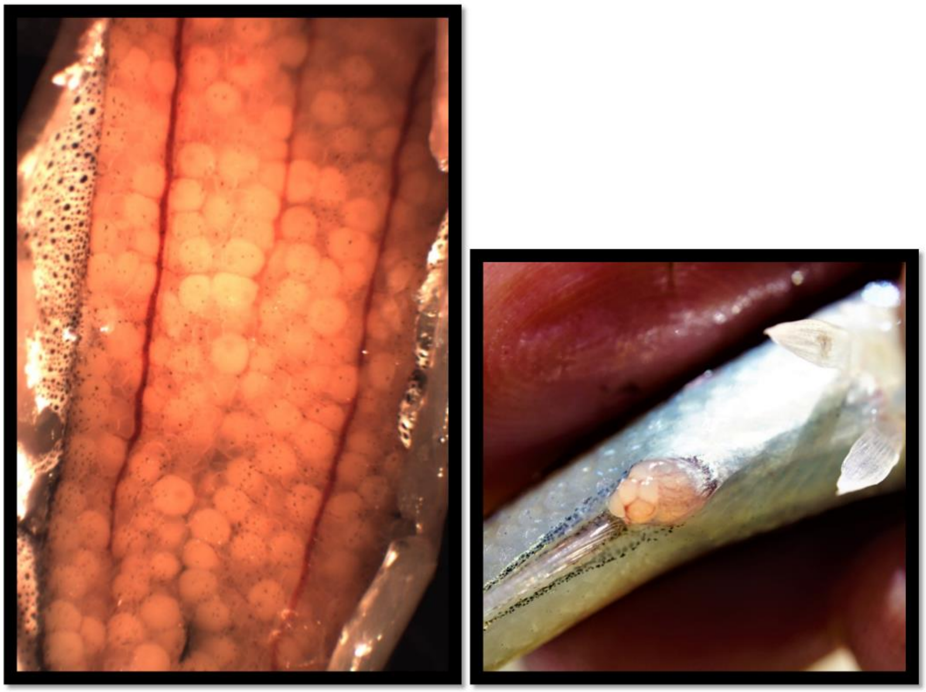

Figure 3.1. Ripe Silver Shiner gonads displayed under the microscope (left) and eggs being visibly shed upon handling in the field (right).

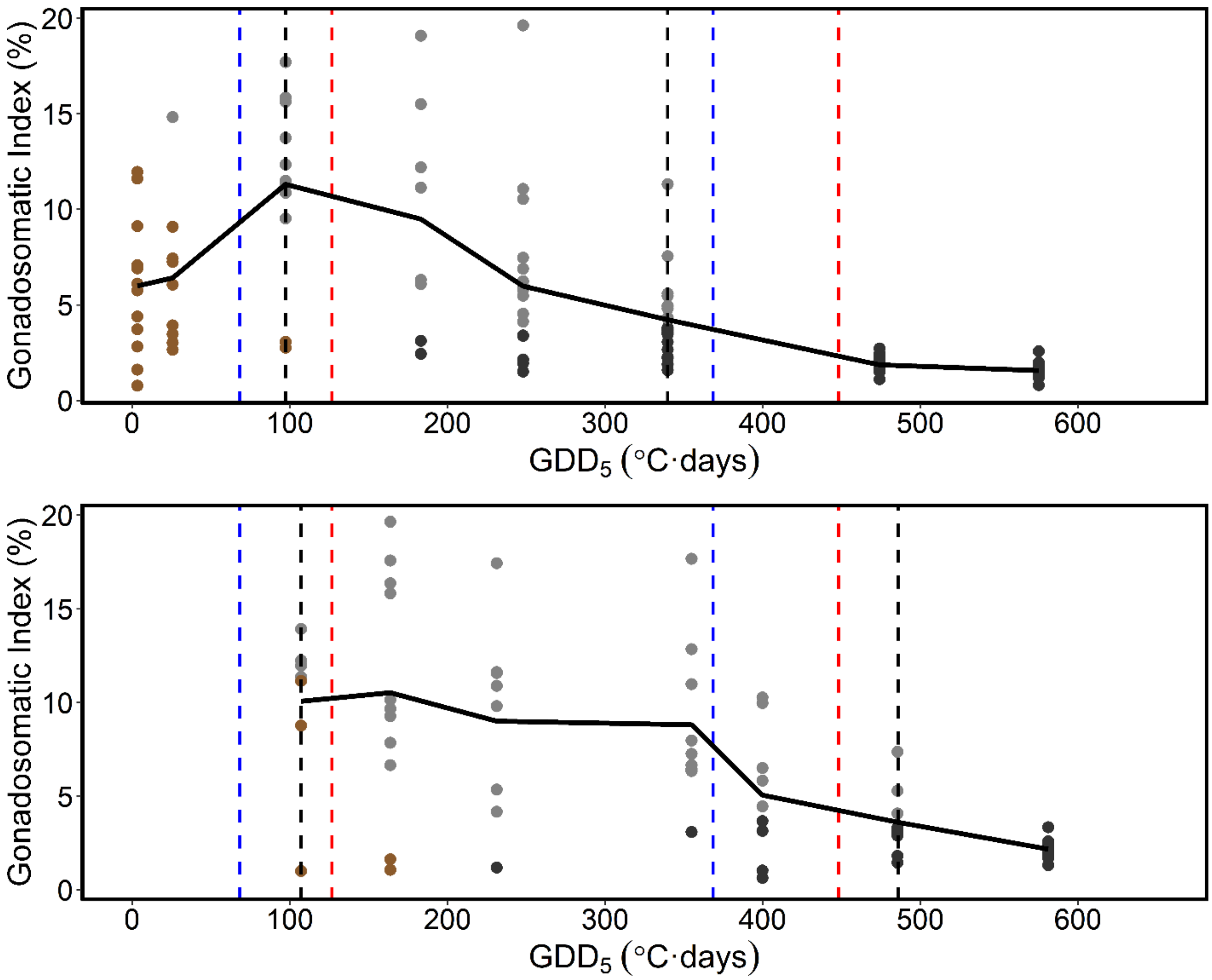

Figure 3.2. The individual (points) and average (black line) gonadosomatic index (GSI) for Silver Shiner captured in Sixteen Mile Creek in spring 2018 (top) and 2019 (bottom) by cumulative growing degree days computed using a base of 5 °C. The colour of the points indicates if an individual was classified as not spawned (brown), spawning (light grey) or spawned (dark grey). The observed spawn window estimated from visual observation of gonads is depicted by the black dotted lines. The blue and red dotted lines indicate the point when there is a 50% and a 95% probability respectively that the population had initiated and ceased spawning based on the spawn initiation and cessation models.

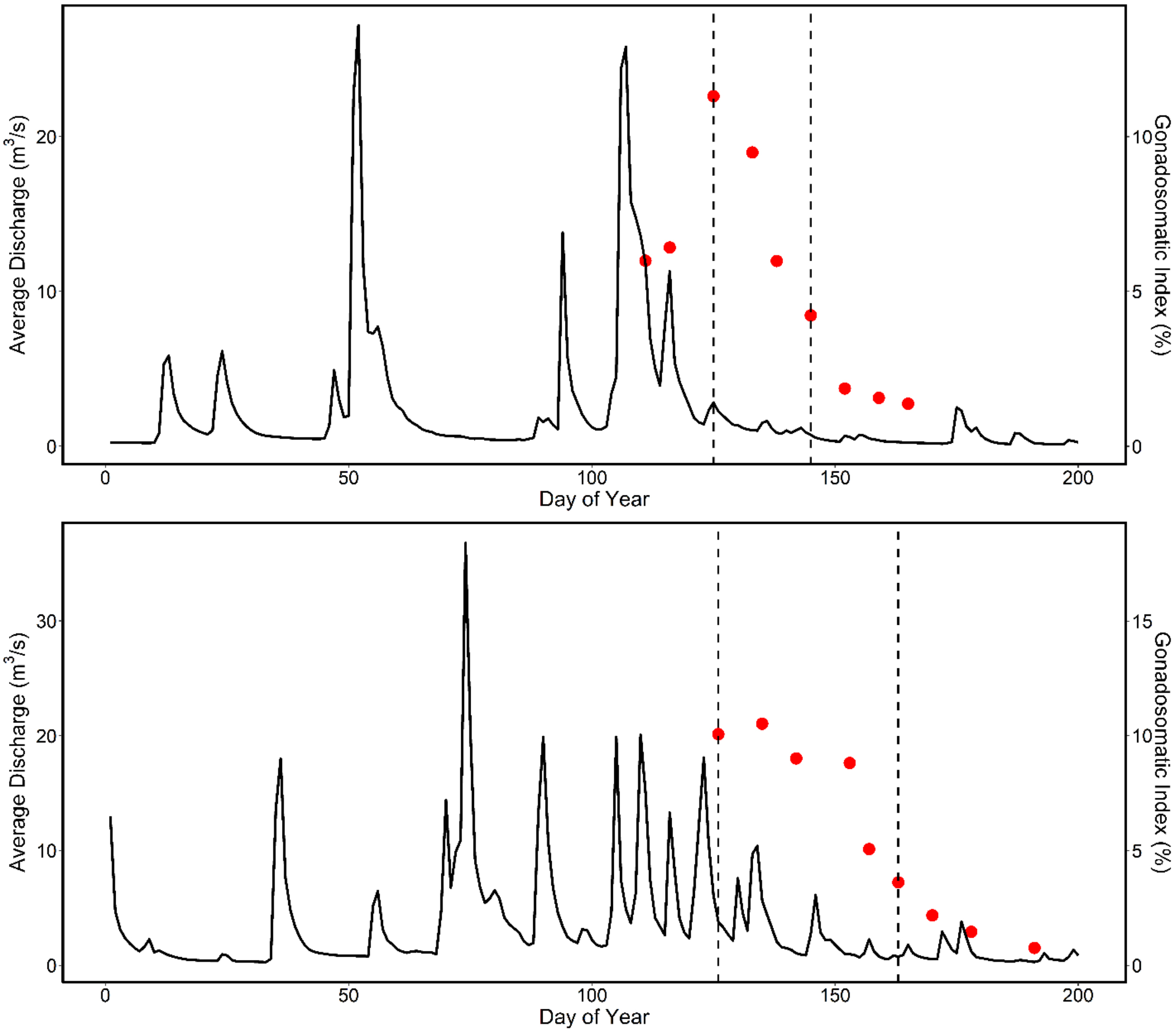

Figure 3.3. The average daily discharge (m^3/s) (black line) and average GSI (red dots) during 2018 (top) and 2019 (bottom) in Sixteen Mile Creek, Oakville, Ontario, Canada. The observed spawn window estimated from visual observation of gonads is depicted by the black dotted lines.

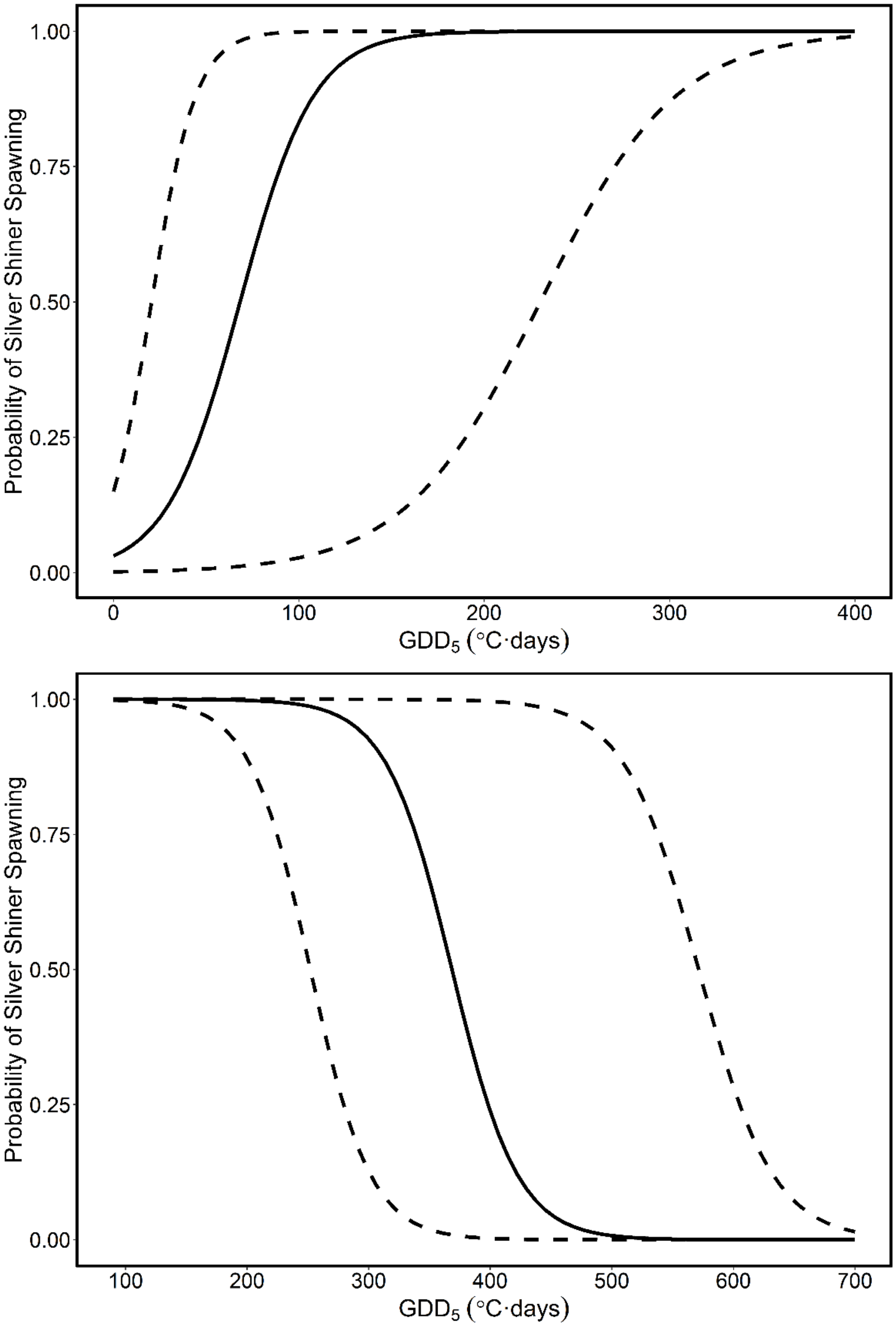

Figure 3.4. The logistic regression model (solid line) and associated 95 % confidence interval (dotted lines) describing the probability Silver Shiner initate (top) and cease (bottom) spawning in relation to GDD$_5$.

Table S-3.1. The number of hauls, number of fish captured, and number of adult Silver Shiner captured at each sampling event during spring 2018 and 2019 in Sixteen Mile Creek, Oakville, Ontario.

Sample Date	Number of Hauls	Number of Fish Captured	Number of Adult Silver Shiner Captured
21-Apr-18	10	69	55
26-Apr-18	16	77	34
05-May-18	3	73	38
13-May-18	50	157	34
18-May-18	4	123	65
25-May-18	9	105	79
01-Jun-18	3	111	109
08-Jun-18	2	72	66
14-Jun-18	1	86	82
29-Jun-18	2	166	70
06-May-19	8	114	30
15-May-19	22	153	20
22-May-19	3	78	27
02-Jun-19	5	42	27
06-Jun-19	10	150	22
12-Jun-19	2	94	40
18-Jun-19	7	39	27
27-Jun-19	4	66	29
07-Jul-19	2	129	59

Chapter 4: Silver Shiner, Notropis photogenis, fecundity and parasite infections during the reproductive period

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4.1 Introduction

Reproduction is a critical component of life history (Winemiller & Rose, 1992). Fecundity, in particular, has implications for population dynamics, species persistence, and estimates of minimum viable population size (MVP) (Power, 2007; Wang *et al.*, 2019). Fecundity can be influenced by a multitude of factors, including climate (Mills, 1988; Power *et al.*, 2005), fish size (Lauer *et al.*, 2005; Schemske, 1974), and environmental stressors (Miller & Ankley, 2004; Power, 1997). Therefore, obtaining contemporary, population-specific estimates of fecundity can improve the predictive ability of demographic models aimed at estimating MVPs and assessing the recovery potential of imperilled species. Failure to utilize suitable fecundity estimates can lead to erroneous conclusions regarding the recovery potential of a species and may lead to unrealistic recovery targets due to the importance of fecundity as an input in population models (Beissinger & McCullough, 2002; Beissinger & Westphal, 1998).

Silver Shiner, *Notropis photogenis* (Cope,1865) is a small-bodied freshwater fish listed as Threatened under Canada's *Species at Risk Act*. Currently no fecundity estimates exist for Silver Shiner within Canada and current estimates of recovery potential incorporate fecundity from a surrogate species, Emerald Shiner *Notropis atherinoides* (Rafinesque, 1818) (DFO, 2013; Schapp, 1989; Young & Koops, 2013b). The use of surrogate species for conservation can be problematic as surrogates may provide a poor representation of the species of interest, possibly leading to erroneous conclusions regarding species survival and recovery potential (Lindenmayer *et al.*, 2002; Wiens *et al.*, 2008). Furthermore, previous fecundity studies for Silver Shiner in Alabama, USA, (Hodgskins *et al.*, 2016) did not directly relate fecundity to fish size and may not reflect fecundity of the species in Canada owing to different thermal conditions, growth, spawn timing, and the number of spawning events (Burbank *et al.*, 2021a). Thus, estimation of contemporary, population-specific fecundity information for Silver Shiner can improve estimates of recovery potential by avoiding the use of surrogate species (Caro & O'Doherty, 1999; Cooke *et al.*, 2017) and fecundity estimated from individuals occupying different climatic conditions.

Furthermore, the presence and impact of parasites during the reproductive season is poorly understood for many small-bodied freshwater fishes, including Silver Shiner. The infestation of parasites during the reproductive period can have population-level impacts for the host species (Krkošek *et al.*, 2013; Marcogliese & Pietrock, 2011; Thomas *et al.*, 1996). Parasite infestation can also alter the behavior of the host, resulting in increased susceptibility to predation (Barber *et al.*, 2000; Latham & Poulin, 2002). Therefore, it is important to examine and identify the presence of parasites during spawning, particularly for imperilled species.

To address the absence of relevant Canadian data, we examined Silver Shiner from an Ontario population to examine: i) egg diameter, ii) the fish length-egg diameter relationship, iii) fecundity (egg count), and iv) fish age-, length- and weight-fecundity relationships to improve the ability to inform recovery strategies for Silver Shiner in Canada. Additionally, we identified an acanthocephalan parasite (thorny-headed worm) *Leptorhychoides thecatus* (Linton, 1891) within the gastrointestinal tract of Silver Shiner and examined v) the timing of parasite infection, vi) the relationship between parasite load and length, and vii) the impact of parasite load on fish condition.

4.2 Methods

Silver Shiner were captured during the spring (April-June) of 2018 and 2019 in Sixteen Mile Creek, Oakville, Ontario, Canada (43.49908, -79.77673). Fishes were captured using repeat hauls of a 9.14 m bag seine (see Burbank et al. (2021a) for details regarding fish collections), with a sub-sample of Silver Shiner (*n*=367 and *n*=178 in spring 2018 and 2019) retained for study under an animal use permit (AUP 1846) approved by the Canadian Council on Animal Care. Retained Silver Shiner were euthanized and frozen following approved Fisheries and Oceans Canada protocols.

In the lab Silver Shiner were thawed and measured for total length (mm) and mass (g). Individuals were dissected and their gonads and gastrointestinal tract were thoroughly examined under a dissection microscope (SZN- 2; Optika, Italy). Sex was determined based on gonad examinations. Of the Silver Shiner kept, *n*=160 (2018) and *n*=81 (2019) were identified as adult females.

The gonadosomatic index (GSI), which is an indication of reproductive status, was calculated as gonad mass expressed as the percentage of somatic mass for all females following Hodgskins et al. (2016) and Burbank et al. (2021a). Body condition (Fulton's *K*) and body condition calculated net of gonad weight were also estimated (Ricker, 1975). Body condition calculated net of gonad weight was estimated in addition to Fulton's *K* to control for gonad weight when estimating body condition. For a random subset of mature reproductive females captured in spring 2018 (n=40) the gonads were placed in Gilson's fluid (Bagenal & Braum, 1978) prior to being separated in a petri dish split into 9 equal-area sections. All eggs from mature females were included in the analysis, with egg stages that ranged from early maturing to ripe (Hodgskins *et al.*, 2016). The eggs were photographed, and egg diameter and fecundity were assessed using a Nikon DS-Fi3 camera mounted on a Nikon

SMZ800 stereo-microscope. To estimate the average egg diameter per fish, the diameters of the longest axis of n=20 randomly selected eggs from each female were measured (mm) using the measurement function on the Nikon DS-L4 tablet interface and the mean was calculated. The relationship between fish length and average egg diameter was evaluated using simple linear regression. To estimate fecundity, the total number of eggs in each fish were counted three times and the average of the three estimates was taken. We evaluated the relationship between fish length (mm) and fecundity (measured as the number of eggs) using a power function model following Bagenal and Braum (1978) and Power et al. (2005) as:

$$F=aL^b$$

Where F is fecundity (number of eggs), L is the total fish length (mm), a is the estimated constant, and b is the estimated exponential coefficient. The relationship between fish weight (g) and fecundity was estimated using the same approach. Furthermore, we evaluated if older individuals (ages 2-3+; generally 2nd or 3rd time spawners) had significantly higher average fecundity than younger individuals (age 1+; generally 1st time spawners), using a two-sample one-tailed t-test. Age was estimated using the age-length key and length-frequency histogram for spring-captured fish provided in Burbank et al. (2021b).

Parasites within the gastrointestinal tract of Silver Shiner were discovered during fish dissections. Subsequently, each tract was thoroughly examined for the presence of parasites. The parasitic species *L. thecatus* was identified (Figure 4.1), distinguished by the number of hooks per longitudinal row of the proboscis following Steinauer and Nickol (2014). Taxonomic confirmation of the parasite was made by parasitology experts. The number of *L. thecatus* in each gastrointestinal tract was counted to estimate the parasite load (the total number of parasites found in an individual fish) for each fish. Additionally, the frequency of occurrence of parasites in individual fish retained at each sampling event was calculated and the correlation between frequency of occurrence of parasites and GSI of females was examined in each year using Pearson's correlation coefficient. The relationship between fish length and the parasite load was investigated with non-linear regression using a power function similar to the length-weight relationship. The correlations between the parasite load of females and body condition and body condition calculated net of gonad weight were examined using Pearsons's correlation coefficient. Grubbs' test (Sokal & Rohlf, 2012) was used to examine the ordered data sets for outliers, both high and low. We also used a two-sample two-tailed *t*-test to determine if the parasite load differed significantly between all younger (age 1+; generally, 1st time spawners) and older (ages 2-3+; generally 2nd or 3rd time spawners) fish. For the *t*-tests conducted, assumptions of normality and homogeneity of variance were assessed using the Shapiro-Wilk *W*-test and Bartlett's homogeneity of variance test respectively (Zar, 2010). If assumptions were not met, the non-parametric Mann-Whitney *U*-test was used instead. Assumptions of linear regression including normality and homoscedasticity were evaluated for each regression with the Shapiro-Wilk *W*-test and graphs of residual versus fitted values respectively (Zar, 2010). For all non-linear regressions residuals were examined for statistical adequacy following Bates and Watts (1988). All analyses were conducted in R version 4.0.2 (R Development Core Team, 2013).

4.3 Results

The diameter of eggs averaged (± standard deviation) 0.61 ± 0.15 mm and ranged from 0.13 – 0.92 mm (Table S-4.1). The fish with the smallest egg diameter was an outlier (Grubb's test p = 0.03) and was excluded from further analysis. For the remaining fish, there was a significant positive linear relationship between fish length (mm; L) and average egg diameter (mm; ED) as follows:

$$\mathrm{ED} = 0.00387L + 0.281$$

(Linear Regression, $F_{1,37} = 12.14$, $p = 0.001$, $R^2=0.23$)

Fecundity estimates for Silver Shiner ranged from 311-2768 eggs (Table S-4.1). The fish with the smallest egg diameter also had the highest fecundity and within the fecundity data similarly tested as an outlier (Grubbs' test $p = 0.03$). Fecundity was positively related to fish total length (Figure 4.2) as follows:

$$F = 0.0168L^{2.39}$$

(Non-linear Regression, $p<0.001$, n=39, $R^2 = 0.52$)

Fecundity was also positively related to fish weight (g; W) as follows:

$$F = 217.3W^{5.38}$$

(Non-linear Regression, $p<0.001$, $n=39$, $R^2 = 0.52$)

The average fecundity of ages 2-3+ individuals (1475 ± 570 eggs) was significantly higher than the average fecundity of age 1+ individuals (576 ± 270 eggs; *t*-test, $t_{11.8}= 5.01$, $p<0.001$).

L. thecatus was found within the gastrointestinal tracts of Silver Shiner in 2018 and 2019 but no other parasitic species were discovered. There was a significant positive correlation between the frequency of occurrence of parasites and GSI (Figure 4.3) in 2018 (Pearson's $r = 0.83$, $p = 0.001$) and 2019 (Pearson's $r = 0.60$, $p = 0.03$). The relationship between GSI and parasite frequency of occurrence (PF) was described by the equation:

$$\mathrm{PF} = 0.0474\mathrm{GSI} + 0.355$$

(Linear Regression, $F_{1,16} = 13.66$, $p = 0.002$, $R^2=0.43$)

The frequency of occurrence of parasites followed the temporal pattern of GSI in both years (Figure 4.3), indicating parasite infections were highest during the spawning period and declined in the weeks following spawning. Parasite load averaged ~6 parasites per fish (range 0-56 per fish) during the spawning period. When considering individuals infected with parasites, there was a significant non-linear relationship between fish length (mm) and parasite load (number of parasites in gastrointestinal tract; PL) described by the equation:

$$\mathrm{PL} = 9.02\text{x } 10^{-9}\, L^{4.44}$$

(Non-linear Regression, $p<0.001$, n=296, $R^2 = 0.22$)

The average parasite load was significantly higher in age 2-3+ individuals (8.31 ± 11.62) compared to age 1+ individuals (2.24 ± 3.81; Mann-Whitney *U* Test, $W=15100$, $p<0.001$). Furthermore, for females, parasite load was significantly negatively correlated with body condition (Pearson’s $r = -0.15$, $p = 0.02$) and body condition calculated net of gonad weight (Pearson's $r = -0.31$, $p<0.001$).

4.4 Discussion

Here we provide fecundity estimates for the threatened Silver Shiner at the northern edge of its geographic range and identify a high frequency of occurrence of *L. thecatus* during the reproductive period. To our knowledge there are no previous published records of acanthocephalan parasites including *L. thecatus* in Silver Shiner. Our results indicate parasite load is negatively correlated with body condition; therefore, further research is required to understand the ecological implications of parasite infection for Silver Shiner.

On the basis of our data, previous recovery potential modelling for Silver Shiner over-estimates the reproductive potential for the species as a result of using higher than actual fecundity estimates from surrogte Emerald Shiner. Feundity estimates from Emerald Shiner ranged from 2452-2630 eggs (Young & Koops, 2013b), whereas we found Silver Shiner fecundity was generally below 1000 eggs, with some older individuals (ages 2-3+) exhibiting fecundity of up to a maximum of 2768 eggs. Previously studied Silver Shiner captured in Alabama, USA, were estimated to have average fecundity of 5115 eggs, with a range of 2499-9115 (Hodgskins *et al.*, 2016), suggesting southern populations display substaitially higher fecundity, possibly as a result of the thermal factors on growth and reproduction (Kirk *et al.*, 2022) or differences in productivity (Power *et al.*, 2005) in northern habitats. Comparatively, Silver Shiner within Canada display fecundity that is generally less than half of that estimated for southern USA populations. Latitudinal variation in life-hitstory traits, including reproductive traits, is common in fish species (Blanck & Lamouroux, 2007; Winemiller & Rose, 1992), and has been demonstrated for Walleye, *Sander vitreus* (Mitchill, 1818) (Johnston & Leggett, 2002), Arctic Charr, *Salvelinus alpinus* (Linnaeus, 1758) (Power *et al.*, 2005), American Shad, *Alosa sapidissima* (A. Wilson, 1811) (Leggett & Carscadden, 1978), and Roach, *Rutilus rutilus* (Linnaeus, 1758) (Lappalainen *et al.*, 2008). Such findings suggest it would be misleading to utilize fecundity estimates from popuations of the species in other climates, as they would lead to positively biased estimates of individual fecundity, population-wide reproductive potential and estimated MVP sizes. The lower estimates for reproductive output based on our fecundity estimates have significant conservation implications. Populations with lower fecundity will (*ceteris paribus*) have lower intrinsic rates of population increase and with lower abundance, will have consequent increased risk of extripation (e.g. Pimm et al. 1988). Together such effects may suggest poorer prospects for the recovery of this imperilled species within Canada than previously assumed.

The discovery of parasites during the reproductive season raises several questions about the potential ecological consequences of infestation for Silver Shiner. In fishes, parasitic infection has been repeatedly shown to markedly increase susceptability to predation (e.g. Lafferty and Morris (1996), Seppälä et al. (2004), Lafferty (2008), and Krause et al. (2010)). Acanthocephalan parasites, in particular, commonly alter the behaviour of their host in a manner that increases susceptiblity to predation, thereby facilitating parasite transmission to the final host (Moore, 1984). For instance, acanthocephalan parasites have been observed to reduce the burrowing and hiding behaviour of three species of shore crabs in New Zealand, with consequent increases in their susceptability to predation (Latham & Poulin, 2002). Although the occurrence and impacts of acanthocephalan parasites on the population ecology of fishes in Canada has often been overlooked, the parasite is known in some Canadian fish and bird species (MacLulich, 1943; Sanford, 1978; Song & Proctor, 2020). For example, *L. thecatus* are common in Smallmouth Bass, *Micropterus salmoides* (Richardson & Richardson, 2009), where damage to the intestinal wall by the parasite can cause extensive tissue damage and death (Nickol & Crompton, 1985). Smallmouth Bass are known to prey upon Silver Shiner (Bunt, 2016; McKee & Parker, 1982), thus predation of infected Silver Shiner may facilitate transmission of the parasite to Smallmouth Bass, which is a preferred definitive host for the parasite (Muzzall & Gillilland, 2004).

Previous studies have identified parasites within Silver Shiner including small numbers of black-spot trematode, *Uvulifer cysts* and infestations of the trematode, *Neodactulogyrus* (Baldwin, 1987, 1988; Hoffman, 1999). The identification of *L. thecatus* within Silver Shiner is a novel finding but not surprising given that *L. thecatus* has been identified in approximately 50 fish species (Richardson & Richardson, 2009) and displays a preference for Smallmouth Bass as a definitive host (Muzzall & Gillilland, 2004). Nevertheless, it is important to document *L. thecatus* infection in Silver Shiner, particularly since the infestation is concentrated during the reproductive period. Parasite infections during the reproductive period generally have population-level impacts for the host species, reducing reproductive potential (Barber *et al.*, 2000; Thomas *et al.*, 1996), recruitment (Krkošek *et al.*, 2013), survival (Marcogliese & Pietrock, 2011) and leading to consequent reductions in the intrinsic rates of population increase (Burgman *et al.*, 1993; Manly, 1990; Nisbet *et al.*, 1989; Power, 2007). Infestation during the reproductive period may be an adaptive advantage for parasites as it can increase the energy available to the parasite, with the host fish disproportionately allocating energy to gonad development and reproduction as opposed to immune response function (Barber *et al.,* 2000; Šimková *et al.*, 2005). In the studied population of Silver Shiner, larger and older individuals tended to have higher parasite loads and parasite infection had a significant negative impact on body condition as observed in other species (Pennycuick, 1971). As larger individuals may be proportionally more susceptible to predation due to their higher parasite loads and reduced body condition, they may be less likely to survive and successfully reproduce, potentially negatively impacting overall population reproductive output. Larger females can contribute disproportionally to population replenishment (Barneche *et al.*, 2018), demonstrated by the positive relationship between fish size and both fecundity and egg diameter observed here for Silver Shiner. Thus, the overall threat to Silver Shiner populations posed by the parasite may be substantial. However, currently we have limited knowledge of how parasite infections affect life history traits and whether the observed parasite infection is an inherent life history risk or a new, environmentally-driven threat to Silver Shiner.

Silver Shiner exists in only five drainages within Canada; Bronte Creek, Grand River, Thames River, Sixteen Mile Creek and Saugeen River (Burbank *et al.*, 2020, 2021b; Gáspárdy *et al.*, 2021; Glass *et al.*, 2016), but is found throughout the Ohio and Tennesse river drainages in the east-central and southeastern United States. Due to its limited distribution in Canada, caution is warranted about the use of vital rate data from surrogate species. Differences between studied Canadian and American populations point to the importance of environmental factors for determining fecundity and suggest that management of the speices will require the population-specific information provided here. Furthermore, because of the potential consequences of parasitic infections for net reproductive output, it is necessary to develop a more thorough understanding of the prevelance and ecological consequences of acanthocephalan parasites in Canadian populations of Silver Shiner to ensure their continued survival.

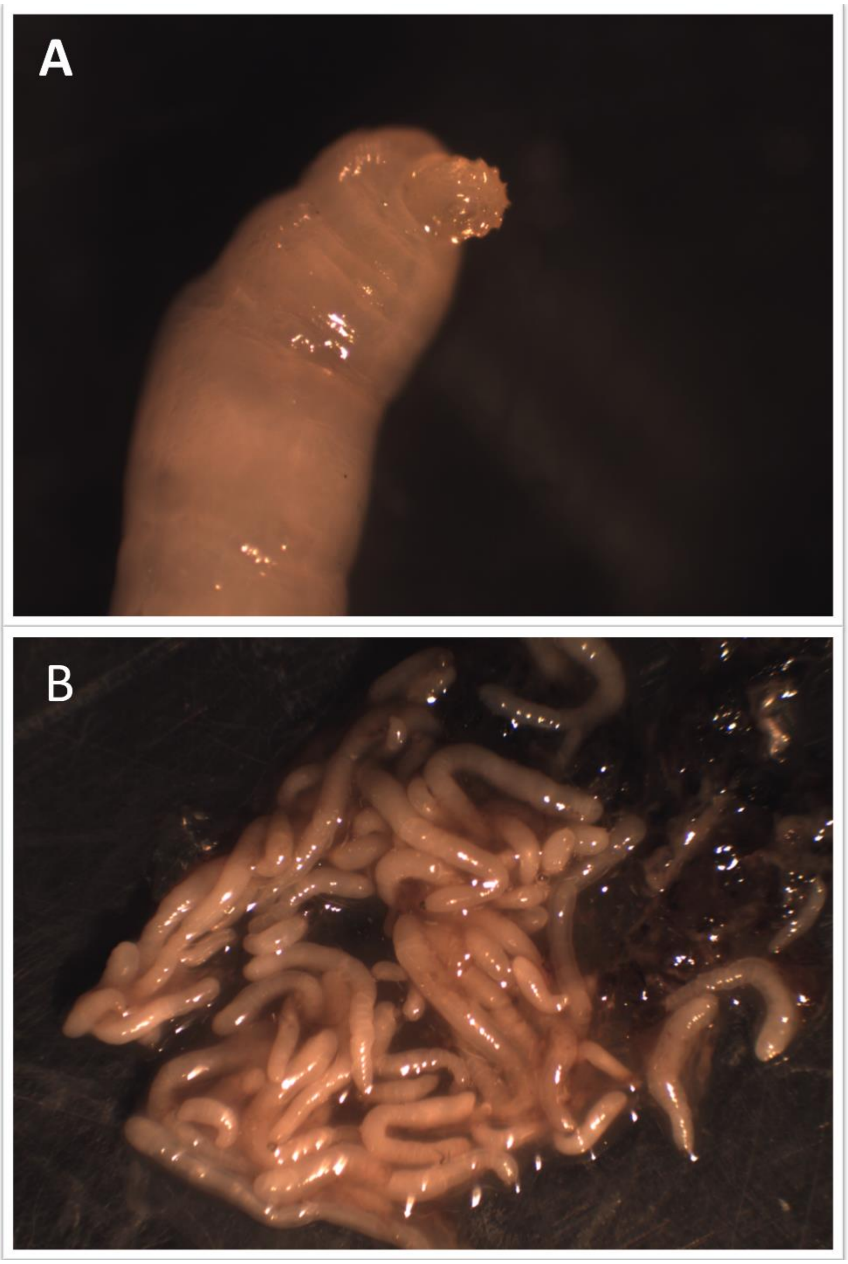

Figure 4.1. Photographs of the acanthocephalan (thorny-headed worm) *Leptorhynchoides thecatus* from the gastrointestinal tract of a Silver Shiner, *Notropis photogenis* taken through a microscope at 4x magnification (A) and 1.5x magnification (B).

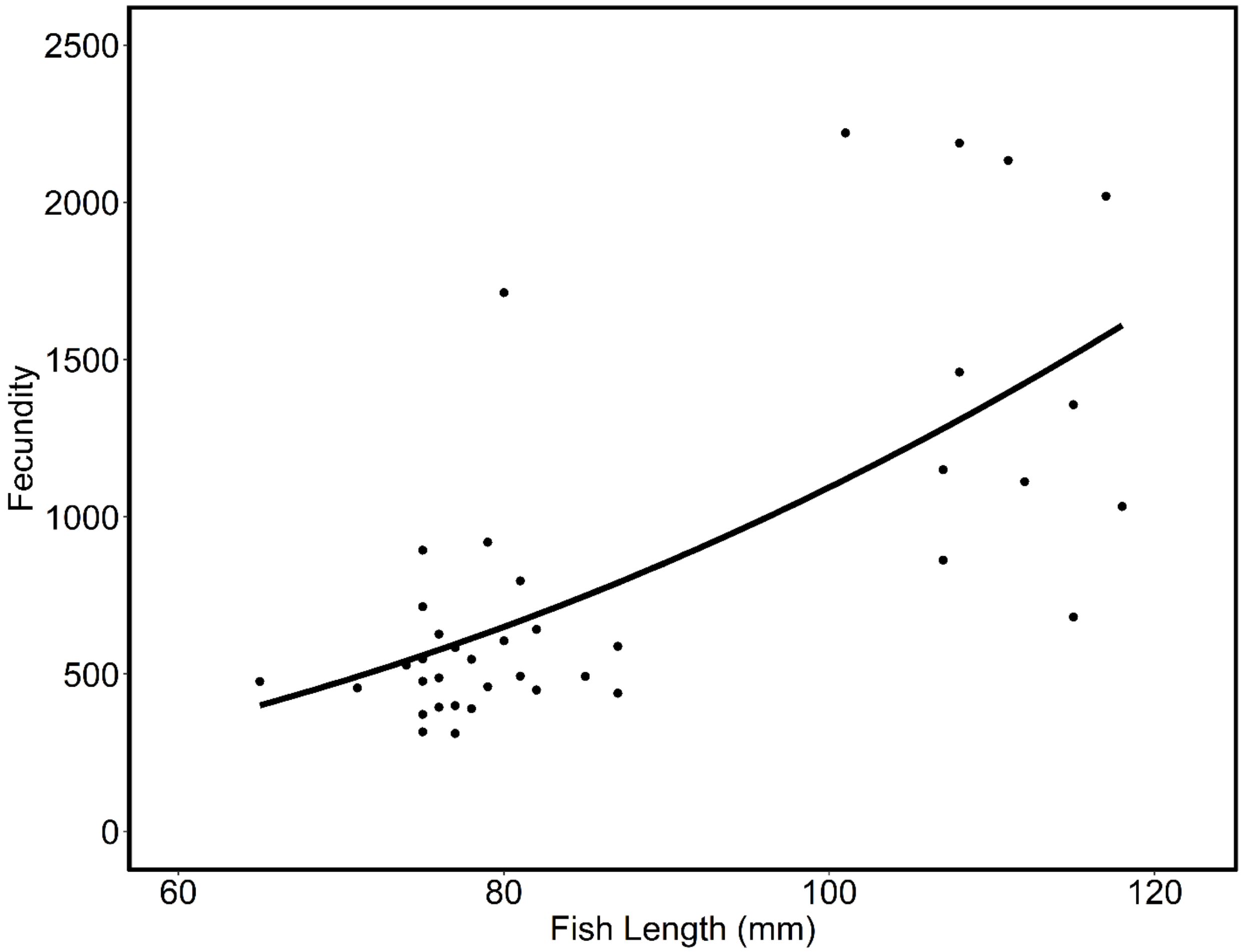

Figure 4.2. The relationship between fish length (mm) and fecundity (measured as total number of eggs) described by the equation $F = 0.0168L^{2.39}$ ($R^2 = 0.52$, n=39, p<0.001) for Silver Shiner, *Notropis photogenis* captured in Sixteen Mile Creek in spring 2018.

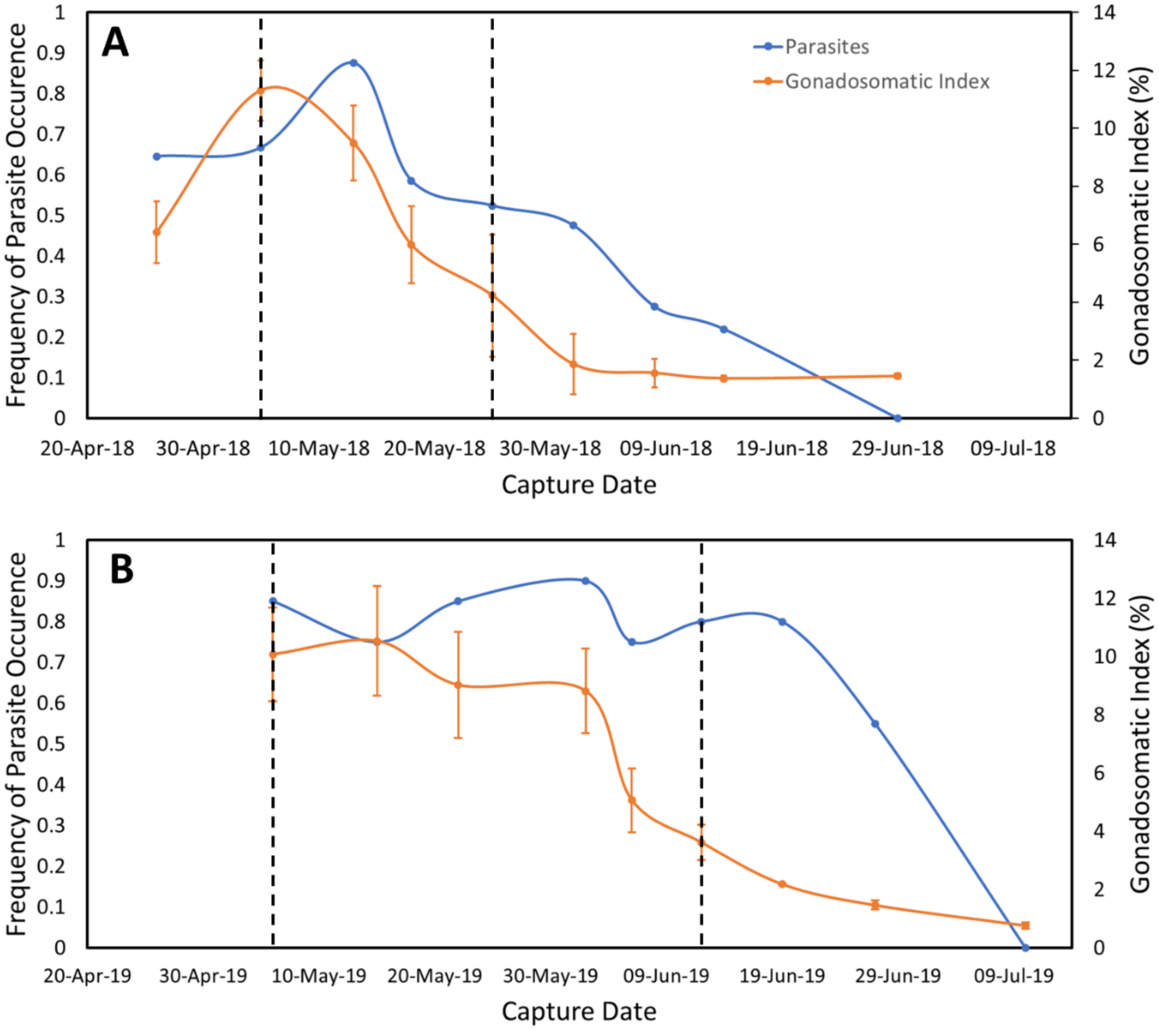

Figure 4.3. The average gonadosomatic index ± standard error (orange) and frequency of occurrence (blue) of the acanthocephalan (thorny-headed worm) *Leptorhynchoides thecatus* within the gastrointestinal tract of Silver Shiner, *Notropis photogenis* by capture date from sampling conducted in Sixteen Mile Creek during spring 2018 (A) and spring 2019 (B). The observed spawn window identified in Burbank et al. (2021a) is shown by the dotted black lines.

Table S-4.1. The total length (mm), age-length key (Burbank et al. 2021b) estimated age, fecundity (total number of eggs) and average egg diameter for Silver Shiner, *Notropis photogenis* captured during spring 2018. The outlier in the dataset is identified by asterisks.

Fish Length (mm)	**Estimated Age**	**Fecundity (Number of Eggs)**	**Average Egg Diameter (mm)**
65	1+	477	0.44
71	1+	456	0.61
74	1+	529	0.73
75	1+	715	0.46
75	1+	549	0.65
75	1+	372	0.75
75	1+	895	0.43
75	1+	317	0.52
75	1+	478	0.67
76	1+	627	0.78
76	1+	395	0.51
76	1+	489	0.44
77	1+	585	0.53
77	1+	400	0.59
77	1+	312	0.57
78	1+	547	0.67
78	1+	391	0.62
79	1+	460	0.42
79	1+	920	0.65
80	1+	1713	0.50
80	1+	606	0.67
81	1+	494	0.46
81	1+	797	0.67
82	1+	643	0.74
82	1+	450	0.58
85	1+	493	0.50
87	1+	589	0.79
87	1+	439	0.60
101	2+	2221	0.58
107	2+	1150	0.92
107	2+	863	0.66
108	2+	2189	0.60
108	2+	1460	0.78
111	2+	2133	0.58
112	2+	1112	0.83
112	2+	2769*	0.13*
115	3+	1357	0.68
115	3+	682	0.64
117	3+	2020	0.77
118	3+	1033	0.83

Chapter 5: Field-based oxygen isotope fractionation for the conservation of imperilled fishes: an application with the threatened silver shiner (*Notropis photogenis*)

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5.1 Introduction

Temperature is an important factor influencing fitness and a variety of physiological and life-history processes in aquatic organisms, particularly fishes (Brett, 1956; Magnuson *et al.*, 1979). As most fishes are thermal conformers (i.e., temperature dictates metabolic function (Brett, 1956, 1971; Coutant, 1987)), identifying the temperature occupied by a species is necessary to understand population processes and the potential vulnerability to shifting thermal regimes that may be caused by a changing climate (Payne *et al.*, 2016). In particular, understanding thermal habitat use is important for the conservation of threatened freshwater fishes, including by identifying the incidence of thermal refugia under present-day climate conditions. This information is critical for the development of science-based recovery strategies for small-bodied freshwater species listed under the *Species at Risk Act* (Canada) and the *Endangered Species Act* (United States).

Understanding realized thermal use can be important for the identification and characterization of critical habitat when developing species recovery strategies (Rosenfeld & Hatfield, 2006). Localized instream temperatures can deviate from the dominant thermal regime as a result of features such as groundwater upwells (Power *et al.*, 1999), providing fishes with thermal refugia that may be used to maintain function during suboptimal thermal conditions. For example brook charr (*Salvelinus fontinalis)* exploit groundwater upwells as cold water refugia during the warm summer months, and protection of these refuges is important for conservation of the species (Baird & Krueger, 2003; Petty *et al.*, 2012). Thus, understanding the availability and use of thermal refugia by fishes is necessary to ensure that definitions of critical habitat encompass the thermal factors required by the species.

Measuring the use of thermal refugia can be difficult, particularly for small-bodied species owing to the lack of suitable field methods. Research to determine the thermal requirements of fishes has been a longstanding area of interest, with many studies identifying thermal preference, tolerance, and niche breadth (Beitinger *et al.*, 2000; Brett, 1956; Fry, 1947). However, data are often derived through laboratory experiments and usually for well-studied commercial and recreational species. Although laboratory studies provide an important foundation for evaluating growth and survival, fundamental thermal tolerance and preference obtained through laboratory experiments do not necessarily correspond to the temperatures used by species in the environment (Schrank *et al.*, 2003). If misinterpreted, laboratory endpoints can lead to erroneous conclusions about the role of temperature, which can be problematic when developing recovery strategies aimed at protecting thermal habitat. In natural systems, fishes often occupy temperatures well beyond laboratory preferenda as a result of ecosystem-specific abiotic and biotic factors (e.g. predator avoidance, migratory behaviour, search for prey (Huff *et al.*, 2005; Payne *et al.*, 2016)) and among-individual variation in thermal habitat use often increases. Such variation may be important as high levels of among-individual variation can hold significant implications for key ecological processes, including population recovery from disturbance (Hughes *et al.*, 2008). Enhanced knowledge of the extent of inter-individual variation may, therefore, help improve knowledge of the ecological dynamics of natural populations, ultimately leading to the development of better informed recovery strategies for the protection and recovery of threatened species (Forsman & Wennersten, 2016). Consequently, there is a need to estimate the average realized temperatures experienced by individual small-bodied fish in the natural environment.

One opportunity for overcoming these issues involves the retrospective estimation of the average relative temperature occupied by an aquatic organism through measurement of the oxygen isotope values of archived aquatic biogenic carbonates (Devereux, 1967; Godiksen *et al.*, 2012; Storm-Suke *et al.*, 2007; Thorrold *et al.*, 1997; Urey *et al.*, 1951). Fish otoliths (ear stones) are biogenic carbonate structures routinely archived for aging during fish sampling programs and thus may be available from museum or other archived collections of threatened fish species. Otoliths are comprised principally of aragonite (Carlstrom, 1963), develop throughout the course of a fish's life, and retain a geochemical chronological history of an individual's environment (Campana, 1999). Analyses of otolith oxygen isotopes have been applied to understand thermal habitat use in a number of freshwater (Kelly et al. 2015, Gerdeaux and Dufour 2015, Power et al. 2012) and marine fishes (Gerringer *et al.*, 2018; Hanson *et al.*, 2013; Jones & Campana, 2009; Minke-Martin *et al.*, 2015; Shiao *et al.*, 2016), and have provided insight into temperature use and the potential resilience of fishes to climate warming (Sinnatamby *et al.*, 2013).

Obtaining average temperature estimates from oxygen isotopes requires knowing the oxygen isotope value ($\delta^{18}O$) of the otolith and the water in which the fish lived. The temperature estimate is obtained from an empirical, temperature-dependent fractionation equation describing the relationship between the temperature and oxygen isotope values (Storm-Suke *et al.*, 2007; Thorrold *et al.*, 1997). Differences among existing fractionation equations indicate that the relationship varies among fish species (Geffen, 2012; Godiksen *et al.*, 2010a; Høie *et al.*, 2004;

Willmes *et al.*, 2019), indicating the need to develop species-specific equations. For threatened species that cannot be readily used for laboratory experiments, laboratory-based approaches are excluded as a viable option for the development of otolith δ^{18}O temperature estimation equations. A more practical option may be to use archival otoliths and exploit approaches that have used the natural variability in water temperatures (either spatially or temporally) to derive field-based relationships between temperature and otolith fractionation (e.g. Storm-Suke et al. 2007). Once a relationship between otolith fractionation and temperature is established, otolith oxygen isotope values can be used to infer important ecological attributes such as the average temperature occupied by individual fish, thereby allowing the variation in relative thermal habitat use among individuals or groups within a population to be determined (e.g. Power et al. 2012, Sinnatamby et al. 2013). Such information can also point to differences in average thermal habitat use among age-classes or sex, which can be helpful to further refine knowledge of critical thermal habitat.

One species for which estimates of average realized thermal use could improve knowledge of species ecology and population processes is silver shiner (*Notropis photogenis*). The species is a small-bodied freshwater fish (approximate maximum length: 144 mm (Bouvier *et al.*, 2013)) within the superfamily Cyprinioidea currently listed as Threatened under Canada’s *Species at Risk Act*. Silver shiner exists in Canada at the northern edge of its geographic range and occupies only four drainages within the country (Bronte Creek, Grand River, Thames River and Sixteen Mile Creek), all of which are located in Southern Ontario (Bouvier *et al.*, 2013; Glass *et al.*, 2016; Young & Koops, 2013b) at latitudes between 42.5-43.5 °N (Figure 5.1). Little information exists regarding the realized thermal habitat use of silver shiner (Glass *et al.*, 2016). Laboratory determined preferred temperatures for species within the genus *Notropis* exhibit large variability

(~19.3-31 °C) (Cherry *et al.*, 1977; Coutant, 1977; Shingleton *et al.*, 1981), highlighting the need for a species-specific analysis. Several investigations have determined the abiotic habitat requirements for silver shiner, indicating that adults show preference for relatively deep pools and runs in medium to large streams and avoid shallow riffles (Baldwin, 1983; Bunt, 2016; Glass *et al.*, 2016; Gruchy *et al.*, 1973). Although some aspects of habitat use have been described (Glass *et al.*, 2016), little is known about the association between habitat occupancy and temperature use. Accordingly, a sample of silver shiner otoliths collected over a range of seasons and temperatures was made available to our research group, providing the opportunity to develop a temperature-dependent fractionation equation that might facilitate future investigations into the average realized temperature use of individual fish. The objectives of the study were to: [1] develop an oxygen isotope fractionation equation from archival otolith collections to better understand variation in individual thermal habitat use of silver shiner; [2] quantify the accuracy and predictive error associated with using alternative species-specific fractionation equations, as a means of illustrating the importance of deriving a species-specific equation; and, [3] illustrate the application of oxygen isotope techniques for evaluating realized thermal use of small-bodied freshwater fishes in the hope of motivating similar research for species lacking a detailed understanding of thermal ecology.

5.2 Methods

5.2.1 Sample collection and temperature history

Archived silver shiner otoliths used in this study came from a seasonally structured set of samples obtained as part of a population assessment being completed by Fisheries and Oceans

Canada. Silver shiner was collected on July 18th, 2017, September 19th, 2017, March 14th, 2018, May 25th, 2018 and June 14th, 2018 from Sixteen Mile Creek, Ontario at two runs located within ~500 m of each other (Figure 5.1). Silver shiner was collected and retained as part of an animal use permit (AUP 1846) approved by the Canadian Council on Animal Care. Otoliths used consisted of the largest otoliths (lapilli), which were cleaned with deionized water, air dried, and stored in snap-cap vials when the fish were initially captured. Coincident with fish collections, a 60 mL water sample was collected at the capture site from the middle of the water column in a Nalgene bottle for $\delta^{18}O$ analysis.

The average temperature experienced by captured silver shiner was estimated using two instream temperature loggers (Onset HOBO Pro V2), which recorded water temperature every 30 minutes and were located near the benthic layer immediately adjacent to the sampling site. Because silver shiner was captured at multiple sampling dates throughout the year, the average temperatures experienced by fish differed among sampling dates as a result of the seasonal fluctuations in water temperature that occur at temperate latitudes (e.g. Jones & Schmidt 2018). Therefore, five unique temperature exposure groups could be defined from the fish sampled. Individuals in the smallest length classes (i.e. young-of-the-year and age 1+; 39-93mm, (Bunt 2016)) on each capture date were selected for analysis. The use of young-of-the-year and 1+ precluded the necessity of milling the otoliths as temperature use was averaged over the life of the fish.

Given the strength of the allometric relationship between body size and home range in riverine fishes (Minns, 1995), silver shiner was assumed to have occupied the habitats in which they were captured, and was expected to have otolith-inferred temperatures similar to those described by proximal temperature loggers. Recorded temperatures were averaged over the course of each fish’s life, similar to Storm-Suke et al. 2007 (i.e. averaged over the open-water season to the date of capture and then averaged within each group for analysis). Silver shiner grow rapidly, and as a result, size differed among individuals. However, fish size and growth rate have been shown to have no significant effect on otolith $\delta^{18}O$ values (Burbank *et al.*, 2018) and, as a consequence, differences in fish size and growth among sample groups were not considered as covariates in the analysis.

5.2.2 Stable isotope analysis

The stable isotope values of ten otolith samples in each of the five temperature groups were measured to examine oxygen isotope fractionation across a range of relevant environmental temperatures. Otoliths were weighed to a target of 0.25 mg, which based on otolith size resulted in the analysis of one or both otoliths from a single individual in most cases. However, otoliths from individuals collected in July and September 2017 were too small to use individually or in pairs and it was necessary to pool otoliths from 2-3 individuals to reach the required target weight for analysis. Weighed otoliths were placed in glass reaction vials and roasted for 1 hour at 350 °C under a continuous helium flow to remove any organic carbon following Guiguer et al. (2003). After roasting, samples were acidified with 100% phosphoric acid and left in an electrically heated block at 90 °C for 3 hours to allow the eluted CO_2 gas to reach equilibrium prior to being analyzed for $\delta^{18}O$ at the University of Waterloo Environmental Isotope Laboratory. Eluted gas was injected into either an Isoprime multiflow isotope ratio mass spectrometer (GV instruments/Micromass, Manchester, UK) or a Finnigan MAT 253 isotope ratio mass spectrometer (Thermo Electron, Waltham, USA) to measure the $\delta^{18}O$ of otolith samples. The mass spectrometers had an analytical precision of ~ 0.2 ‰ and mass spectrometer accuracy was assessed with repeated measure of calibrated laboratory working standards (EIL-21 traceable to NBS-18 and NBS-19, IAEA-CO-1, IAEA-CO-8 and IAEA-CO-9) run before and after the analyses of 10 to 15 otolith samples. During a sample run, linearity checks were included using a suite of EIL-21 working standards to produce a range of peak heights that encompassed the expected sample peak range. All stable isotope results were reported in standard notation (δ) as part per mil differences (‰) relative to the Vienna Pee Dee Belemnite (VPDB) primary scale (Brand *et al.*, 2014).

The $\delta^{18}O$ of water samples was measured using an Isoprime multiflow isotope ratio mass spectrometer (GV instruments/Micromass). Water $\delta^{18}O$ results were corrected based on a sealed container of laboratory water calibrated to international reference materials for Standard Light Antarctic Precipitation (SLAP) and Standard Mean Ocean Water (SMOW) obtained from the International Atomic Energy Agency (IAEA). Results initially reported relative to SMOW were converted to the VPDB scale based on the following equation (Clark & Fritz, 1997; Coplen *et al.*, 1983):

$$\delta^{18}O_{water(VPDB)} = -29.98 + 0.97002\ \delta^{18}O_{water(SMOW)} \qquad (1)$$

where $\delta^{18}O_{water(VPDB)}$ and $\delta^{18}O_{water(SMOW)}$ are the $\delta^{18}O$ of the water sample with respect to the standards VDPB and SMOW respectively.

5.2.3 Fractionation equation development

Otolith fractionation, expressed as the difference between otolith and water $\delta^{18}O$ values, were plotted against the inverse of the corresponding average temperature exposure (^{o}C). Linear regression was used to quantify the relationship between the otolith fractionation (dependent variable) and average temperature exposure (independent variable, ^{o}C) (e.g. Kalish 1991, Høie et al. 2004, Storm-Suke et al. 2007, Sakamoto et al. 2017), yielding a species-specific fractionation equation for silver shiner in the form of:

$$\delta^{18}O_{otolith(VPBD)} - \delta^{18}O_{water(VPBD)} = a + b\ (°C) \qquad (2)$$

Commonly in the literature fractionation equations are estimated using the otolith fractionation factor computed as follows (Sharp, 2017; Storm-Suke *et al.*, 2007):

$$\alpha = \frac{(\delta^{18}O_{otolith(VPBD)} + 1000)}{(\delta^{18}O_{water(VPBD)} + 1000)} \qquad (3)$$

where $\delta^{18}O_{otolith(VPDB)}$ is the $\delta^{18}O$ value of the otolith isotope sample and $\delta^{18}O_{water(VPDB)}$ is the $\delta^{18}O$ of the river water. The computed fractionation factors, expressed as 1000lnα, were plotted against the inverse of the corresponding average temperature exposure (^{o}K) and linear regression was used to quantify the relationship between the otolith fractionation factor (dependent variable) and the average temperature exposure (independent variable, TK) (e.g. Thorrold et al. 1997, Høie et al. 2004, Storm-Suke et al. 2007, Godiksen et al. 2010, Geffen 2012), yielding a species-specific fractionation equation for silver shiner in the form of:

$$1000\ln\alpha = a + b\ (1000TK^{-1}) \qquad (4)$$

where a and b are the intercept and slope coefficients, respectively, estimated from each linear regression. Assumptions associated with linear regression, including normality and homoscedasticity, were evaluated through examination of q-q plots, application of the Shapiro-Wilk W test and graphs of residual and fitted values (Zar, 2010).

5.2.4 Statistical Analysis

The ability of existing species-specific fractionation equations to predict average *in situ* measured temperatures for silver shiner was examined using the 1000lnα values from the current study as inputs and previously developed fractionation equations for arctic charr, *Salvelinus* *alpinus,* (Godiksen *et al.*, 2010a), delta smelt, *Hypomesus transpacificus* (Willmes *et al.*, 2019), and the *Salvelinus* genus (Storm-Suke *et al.*, 2007). The predictive bias and accuracy of temperature estimation resulting from the use of the different fractionation equations was examined using mean predictive error and mean absolute percent error (Power, 1993). Additionally, the predictive bias of each equation was assessed for statistical significance using a t-based test for predictive bias following (Power 1993), which was designed to assess whether bias differs significantly from zero. All analyses were conducted in R version 3.6.1 (R Development Core Team, 2013).

5.3 Results

After pooling to account for small otolith sizes, a total of 50 archived otolith samples from silver shiner (n = 67) captured in Sixteen Mile Creek at five different sampling dates were analyzed for $\delta^{18}O$ isotopes (Table 5.1). The average measured water temperature experienced by silver shiner ranged from 11.06 °C in March 2018 to 21.45 °C in July 2017. River water $\delta^{18}O$ isotope values ranged from -39.91‰ to -38.03‰ over the 10.39 °C temperature spread and silver shiner otolith $\delta^{18}O$ isotope values ranged from -11.14‰ to -9.08‰.

Computed fractionation factors (α) expressed as 1000lnα differed significantly among capture periods (ANOVA, $F_{4,45} = 71.91$, $P<0.0001$). The relationship between otolith $\delta^{18}O$ isotopes and water temperature reported in terms of the difference between otolith and water oxygen isotope values (Figure 5.2) was significant:

$$\delta^{18}O_{otolith(VPBD)} - \delta^{18}O_{water(VPBD)} = 32.03 - 0.21(°C) \qquad (5)$$

(Linear regression, $F_{1,48} = 238.7$, $P<0.0001$, $r^2 = 0.83$)

Similarly, there was a significant relationship between mean temperature exposure and computed fractionation factors (Figure 5.3):

$$1000\ln\alpha = -34.38 + 18.42(10^{3}TK^{-1}) \qquad (6)$$

(Linear regression, $F_{1,48} = 248.1$, $P<0.0001$, $r^2 = 0.84$)

Table 5.2 presents the 95% confidence intervals for the slope and intercept parameter estimates for each of the above equations. Temperatures estimated using the fractionation equation for the *Salvelinus* genus overestimated average temperature and the equation developed for delta smelt underestimated average temperature (Table 5.3, Figure 5.4). While significant predictive bias arose when estimating temperature use with the *Salvelinus* genus and delta smelt equations (all $P<0.05$), no significant predictive bias arose from the application of the arctic charr equation ($P = 0.23$; Table 5.4).

5.4 Discussion

The application of stable isotope analysis is a practical and useful method for understanding thermal use of freshwater fishes. We measured $\delta^{18}O$ values of silver shiner otoliths and, through development of a field-based, species-specific, temperature-dependent fractionation equation, provide a potentially useful tool for quantifying the average relative temperature occupied by the species. The equation presented in the current study is, to our knowledge, the first field-based otolith oxygen isotope fractionation equation developed for a small-bodied freshwater fish. Thermal habitat occupancy estimated using the equation compared favourably with *in situ* temperature loggers, showing no significant bias and confirming the utility of the equation for better understanding the thermal ecology of silver shiner. The field-based approach for estimating the relationship between otolith isotope fractionation and water temperature represents a promising first step to understand the thermal ecology of the species.

Comparison of existing fractionation equations demonstrated the potential predictive inaccuracies arising from equations developed for other species. Our analysis indicates that the species-specific equation for silver shiner led to significantly different predictions than those derived using equations for delta smelt (Willmes *et al.*, 2019) and the *Salvelinus* genus (Storm-Suke *et al.*, 2007). The differences highlight the need for species-specific equations when employing otolith thermometry techniques for silver shiner, or other threatened species, to avoid bias in temperature estimates. Given the importance of temperature for dictating metabolic function (Magnuson *et al.*, 1979), it is imperative to gain a realistic understanding of realized thermal use for the effective conservation of a species and protection of thermal habitat (Schaefer *et al.*, 2003). Lack of knowledge regarding realized thermal habitat can increase uncertainty when assessing the potential impacts of climate change on a species (Comte & Olden, 2017). Numerous studies have linked variation in life-history traits among individuals with vulnerabilities to climate change (e.g. Pacifici et al. 2015). Thus, otolith thermometry approaches, such as those employed in this study, offer a useful way to examine thermal habitat use for species whose thermal ecology is poorly understood, as is the case with many small-bodied fishes currently listed under the *Species at Risk Act* (Canada) and the *Endangered Species Act* (United States). However, it is important to note the approach is limited to species for which archived otolith collections are available or when regulations allow lethal sampling for conservation purposes. Additionally, owing to the size of small-bodied fishes, the approach can typically only provide estimations of average temperature use. Age-specific sampling may be possible, but is contingent on sufficient otolith sizes and the use of micro-milling or secondary ion mass spectrometry (SIMS) technology (Helser *et al.*, 2018).

The examination of species-specific thermal use is particularly helpful for describing thermal habitat use when conventional fisheries techniques (i.e., telemetry) cannot be applied. Furthermore, given the large breadth of temperature preference exhibited by fishes in the genus *Notropis* (Cherry *et al.*, 1977; Coutant, 1977), generalizations regarding thermal preference and occupancy based on the genus may be inappropriate, especially if microhabitat use differs within and among species. For example, video observations indicate adult silver shiner may exploit areas of groundwater discharge (Bunt, 2016). While the behaviour remains unvalidated, its implications for individual variation in thermal habitat use are potentially large.

The tool developed here may help provide further insight into the potential exploitation of thermal refugia by silver shiner. For example, by comparing average occupied temperatures with average thalweg temperatures, the extent to which mean stream temperatures are avoided can be determined. Localized regions of groundwater upwelling within streams can moderate stream temperatures, providing thermal refugia and access to cooler thermal habitats in summer and warmer ice-free thermal habitats in winter compared to the dominant thermal regime (Kaandorp *et al.*, 2019; Power *et al.*, 1999). Access to groundwater upwelling as thermal refugia in winter may be important for populations of small-bodied fishes in Canada, including silver shiner that exists at the northern edge of its range with a limited distribution (Bouvier *et al.*, 2013). For example, stream resident blacknose dace (*Rhinichthys atratulus*) has been shown to remain resident in a single substrate location during winter months (Cunjak & Power, 1986a) where groundwater can minimize water temperature fluctuations and control anchor ice formation (Lind *et al.*, 2016), thereby reducing physiological stress (Cunjak & Power, 1986b). During winter months groundwater upwells, provide access to important ice-free habitats that facilitate survival through harsh winter months, as seen for brook charr, brown trout (*Salmo trutta*), arctic grayling (*Thymallus arcticus*) and juvenile chinnok salmon (*Oncorhynchus tshawtscha*) (Bradford *et al.*, 2001; Cunjak & Power, 1986b; West *et al.*, 1992) and can play important roles in dictating overwinter mortality (Power *et al.*, 1999). Additionally, during ice-free months, localized regions of groundwater upwelling can offer access to habitats with reduced temperature fluctuations and refuge from extreme temperature maxima (Baird & Krueger, 2003; Kaandorp *et al.*, 2019), with implications for fish growth as noted for several threatened, small-bodied fishes (Finch *et al.*, 2013; Juracek *et al.*, 2017) . Given anecdotal evidence that silver shiner occupies areas of groundwater seepage and the potential importance of these habitats for species persistence, it is imperative to understand and quantify the exploitation of groundwater upwellings by silver shiner as thermal refugia.

In addition to understanding the role of refugia, the species-specific fractionation equation for silver shiner could be used to quantify variation in relative thermal use among individuals (e.g. adults vs. juveniles; males vs. females). Understanding the variation of thermal use among individuals can help understand population-level responses to alterations of the dominant thermal regime that may arise from disturbances such as urbanization, damming of rivers, water abstraction, and climate change (Hillyard & Keeley, 2012; Juracek *et al.*, 2017). Otolith δ^{18}O values have been used to understand how fish species may respond to anthropogenic or natural stressors that influence the dominant thermal regime. For example, Kelly et al. (2017) applied otolith thermometry techniques to examine and compare the temperature use of brook charr and slimy sculpin (*Cottus cognatus*) in regulated and unregulated river systems to evaluate the impact of dam operation on the thermal ecology of the two species. Additionally, otolith oxygen isotopes have been employed to examine thermal responses to species invasion and identified thermal segregation of native whitefish (*Coregonus lavaretus*) and invasive vendace (*Coregonus albula)*, which in part facilitates the coexistence of the two species (Kelly *et al.*, 2015). Similarly, otolith-based methods have been used to demonstrate the consistency of differential habitat use among morphotypes of arctic charr within a population, with consistent temperature use within morphotype groups and lack of overlap among groups serving to lower competition (Power *et al.*, 2012). Accordingly, it is evident otolith isotope thermometry techniques provide a useful approach to quantify relative average thermal use and its variation in response to a range of environmental conditions for a variety of fish species, including small-bodied fishes such as silver shiner.

While the estimation of field-based fractionations provides a low cost, effective means of developing a broader base of species-specific thermometry equations for use in fish ecology studies, there are several uncertainties associated with the field-based approach. For instance, the actual average temperature exposure of fish used to develop the fractionation equation may not be an accurate representation of individual thermal history. If an individual exploits temperatures different than those measured by the temperature loggers, the average temperature exposure computed using loggers will misrepresent the average temperature occupied by the fish, thereby introducing measurement error into the fractionation estimation process. Accordingly, the technique may be most useful for small-bodied fish species known to have small home ranges (Minns, 1995; Woolnough *et al.*, 2009) or territorial species for whom location-specific temperatures can be gathered by loggers. Although home range data were unavailable for silver shiner, other small bodied fishes such as the plains minnow (*Hybognathus placitus*), redside dace (*Clinostomus elongatus*), longnose dace (*Rhinichthys cataractae*) and rosyside dace

(*Clinostomus funduloides*) have been shown to exhibit relatively small home ranges in river systems, providing support for the presumption that silver shiner likely occupied areas in close proximity to their site of capture (Drake & Poesch, 2020; Hill & Grossman, 1987; Minns, 1995; Sharp, 2017; Wilde & Urbanczyk, 2017). Additionally, measures of water isotope values were not spatially integrated; therefore, approximations of river δ^{18}O values may not be a true depiction of water δ^{18}O values experienced by mobile individuals. Such uncertainties may be evident as among-individual variation in the apparent isotopic fractionation within temperature groups, as was witnessed here. Alternatively, among-individual variation may be driven by individual differences in microhabitat use leading to slightly different thermal histories or by behavioural thermoregulation as has been seen in arctic charr (Sinnatamby *et al.*, 2013) or atlantic salmon, *Salmo salar*, (Minke-Martin *et al.*, 2015). Despite the uncertainties associated with the analytical approach, the average temperature exposure of fish explained ~84 % of the variation in measured fractionation factors. The explained variation is comparable with the range of explained variation (~77-94%) reported in other otolith laboratory thermometry (e.g. Kalish 1991, Høie et al. 2004, Godiksen et al. 2010, Geffen 2012) and field-based (Storm-Suke et al. 2007) studies, indicating that a statistically robust temperature dependent fractionation equation was developed for silver shiner.

Given the results of this study, we recommend that the field-based, species-specific, temperature-dependent fractionation equation developed here for silver shiner be applied when using otolith thermometry techniques to estimate relative temperature exposure for the species. The species-specific equation will avoid the predictive errors inherent in the application of alternate fractionation equations, thereby avoiding significant predictive bias. The field-based fraction equation is an important methodological advancement that will allow further research into the thermal ecology of silver shiner and facilitate a better understanding of the risk posed by anthropogenic stressors, such as urbanization and climate change likely to impact thermal habitat.

Table 5.1. Capture date, average water temperature ± standard deviation (temperature range) at the capture site, total length ± standard deviation (sd), weight ± sd, and otolith $\delta^{18}O$ values ± sd of silver shiner analyzed from Sixteen Mile Creek in 2017 and 2018. Water $\delta^{18}O$ values and computed fractionation factors (α) expressed as 1000lnα ± sd for each capture date are also displayed.

Capture Date	**Water (°C)**	**n**	**Total Length (mm)**	**Weight (g)**	**Otolith $\delta^{18}O$ (‰)**	**Water $\delta^{18}O$ (‰)**	**1000lnα**
18-Jul-17	21.5 ± 3.02 (13.8, 29.6)	10	47.9 ± 3.84	0.702 ± 0.19	-10.70 ± 0.24	-38.03	28.02 ± 0.24
19-Sep-17	20.1 ± 3.17 (11.9, 29.6)	10	69.4 ± 3.20	2.11 ± 0.31	-10.18 ± 0.36	-38.18	28.69 ± 0.36
14-Mar-18	11.1 ± 9.63 (0, 29.6)	10	73.2 ± 3.55	2.11 ± 0.43	-10.14 ± 0.53	-39.91	30.53 ± 0.54
25-May-18	14.5 ± 9.17 (0, 29.6)	10	75.7 ± 2.00	3.08 ± 0.26	-9.726 ± 0.19	-38.51	29.50 ± 0.19
14-Jun-18	17.1 ± 9.30 (0, 29.6)	10	84.6 ± 3.37	4.02 ± 0.45	-9.939 ± 0.31	-38.32	29.09 ± 0.31

Table 5.2. The species-specific temperature-dependent fractionation equations for silver shiner expressed in two forms commonly used by researchers. The slope and intercept parameters corresponding to the 95 % confidence limits for both regression equations are displayed along with the *P*-values of the slope and intercept parameter estimates.

Equation	**Slope**			**Intercept**		
	Lower Limit	**Upper Limit**	**P-value**	**Lower Limit**	**Upper Limit**	**P-value**
$\boldsymbol{\delta_{\text{Otolith (PDB)}} - \delta_{\text{water (PDB)}} = 32.21 - 0.22\ (°C)}$	-0.24	-0.19	<0.0001	31.56	32.51	<0.0001
$\boldsymbol{\text{Ln}1000\alpha = -34.38 + 18.42(10^{3}\text{TK}^{-1})}$	16.07	20.77	<0.0001	-42.49	-26.27	<0.0001

Table 5.3. The measured *in situ* average stream temperature (° C) ± standard deviation for silver shiner captured in 2017 and 2018 within Sixteen Mile Creek along with the temperatures predicted using each of the species-specific fractionation equations (° C). Sample dates are listed in increasing order based on average measured temperatures.

Date	**Measured**	**This Study**	**Storm-Suke et al. (2007)**	**Godiksen et al. (2010)**	**Willmes et al. (2019)**
14-Mar-18	11.1	10.7 ± 2.4	13.4 ± 2.1	11.9 ± 2.1	9.4 ± 2.3
25-May-18	14.5	15.2 ± 0.9	17.5 ± 0.8	16.1 ± 0.8	13.9 ± 0.9
14-Jun-18	17.1	17.1 ± 1.4	19.1 ± 1.3	17.8 ± 1.3	15.8 ± 1.4
19-Sep-17	20.1	18.9 ± 1.7	20.8 ± 1.5	19.4 ± 1.5	17.6 ± 1.7
18-Jul-17	21.5	22.1 ± 1.2	23.7 + 1.0	22.3 ± 1.0	20.7 ± 1.1

Table 5.4. The mean predictive error (° C) and mean absolute percent error when compared to measured *in situ* temperatures resulting from the prediction of silver shiner temperature use with various species-specific temperature dependent fractionation equations, including the equation developed in this study. The *W* and P-value were calculated for a t-based statistic designed to detect significant predictive bias following Power (1993).

Equation	**Mean Predictive Error (° C)**	**Mean Absolute Percent Error (%)**	***W***	**P-value**
This Study	-0.05	8.20	0.094	0.925
Storm-Suke et al. (2007)	2.10	14.84	3.855	<0.001
Godiksen et al. (2010)	0.66	9.39	1.222	0.228
Willmes et al. (2019)	-1.30	9.98	2.498	<0.050

Figure 5.1. The study area and two capture locations (black dots) of silver shiner in Sixteen Mile Creek, Oakville, Ontario, Canada.

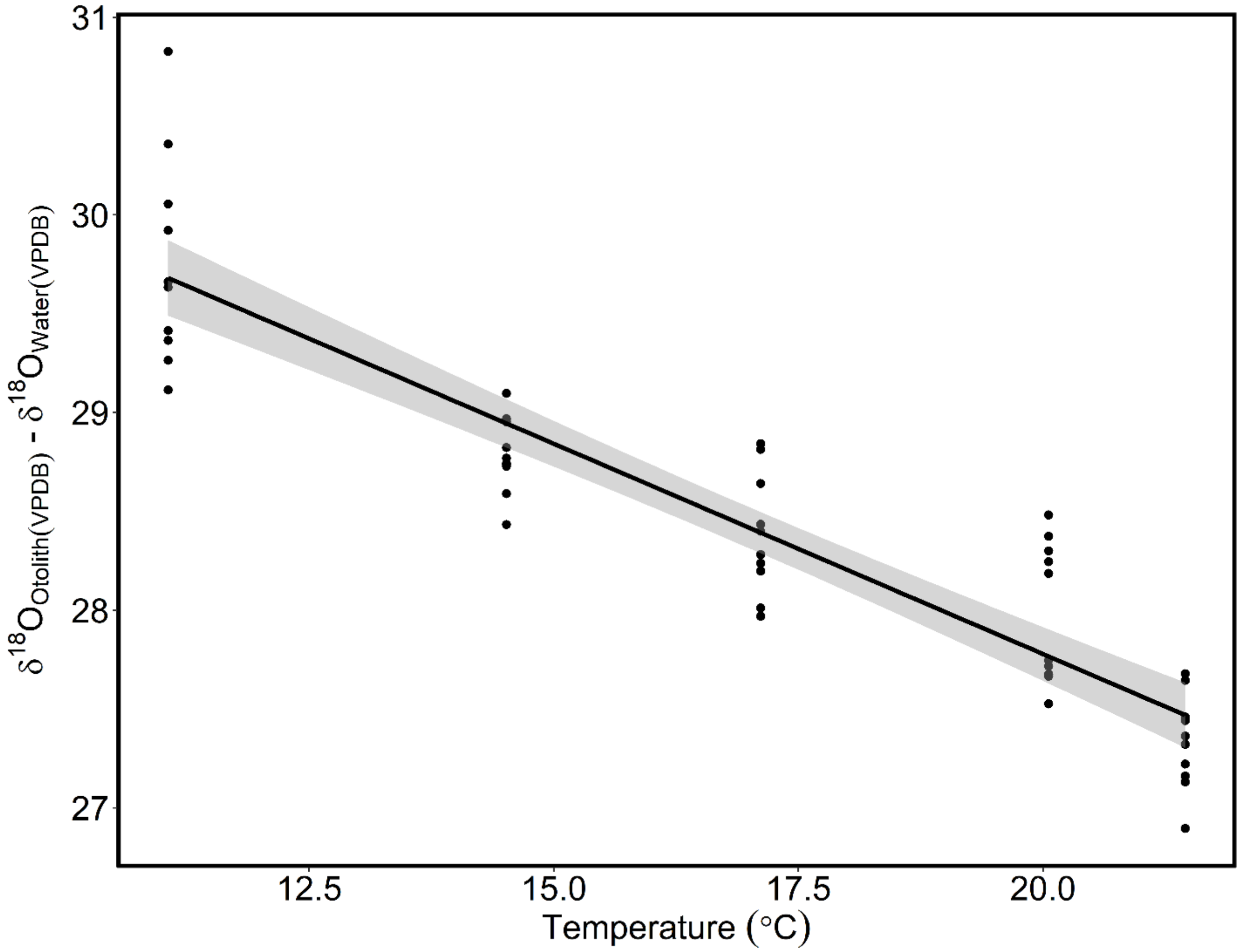

Figure 5.2. The relationship between otolith and water δ^{18}O values and corresponding measured average temperatures for otolith samples (n=50). The estimated oxygen isotope fractionation equation is plotted as a black line and the associated 95% confidence intervals are displayed as grey shading.

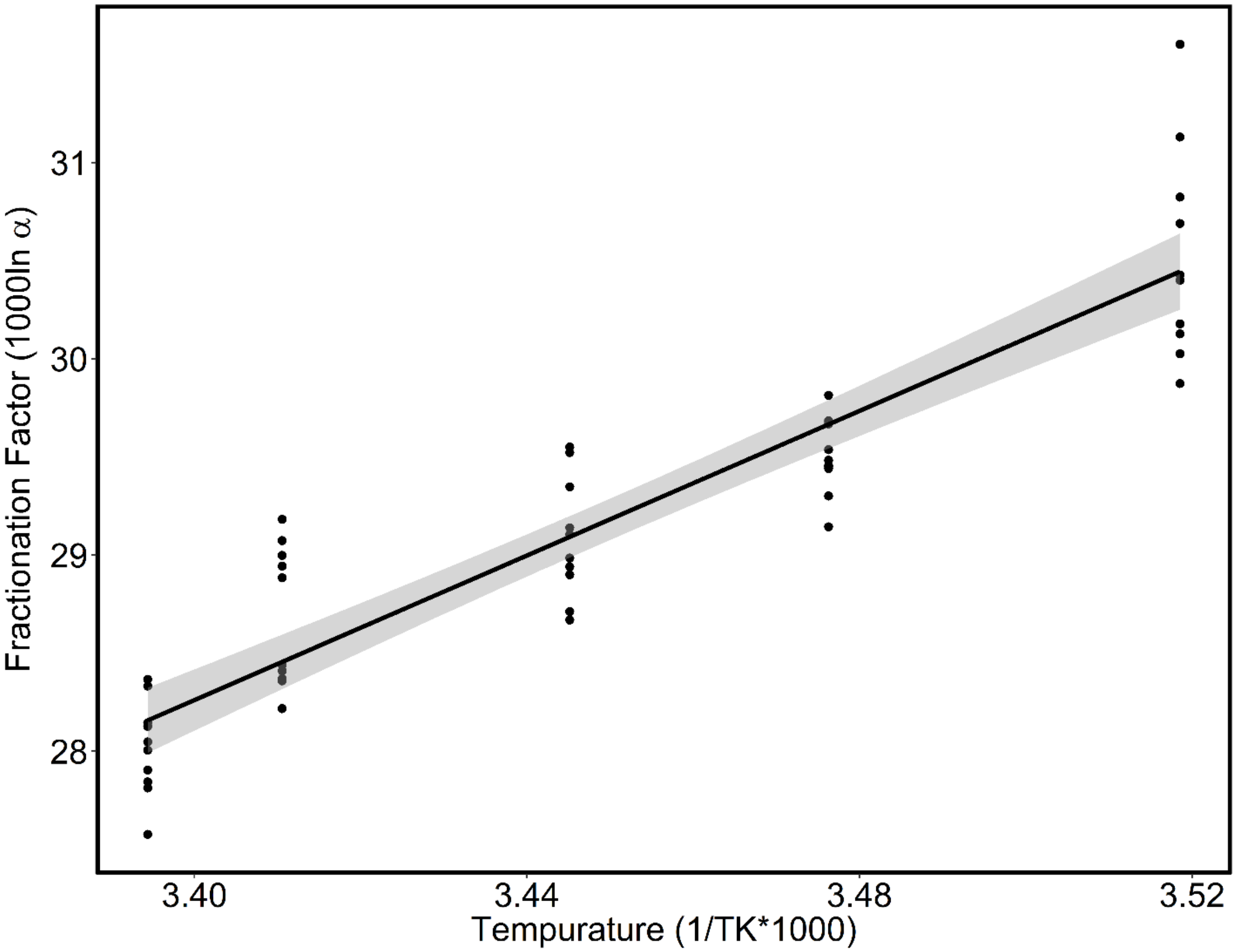

Figure 5.3. The relationship between the computed fractionation factor (1000lnα) and corresponding measured average temperatures for otolith samples (n=50). The estimated oxygen isotope fractionation equation is plotted as a black line and the associated 95% confidence intervals are displayed as grey shading.

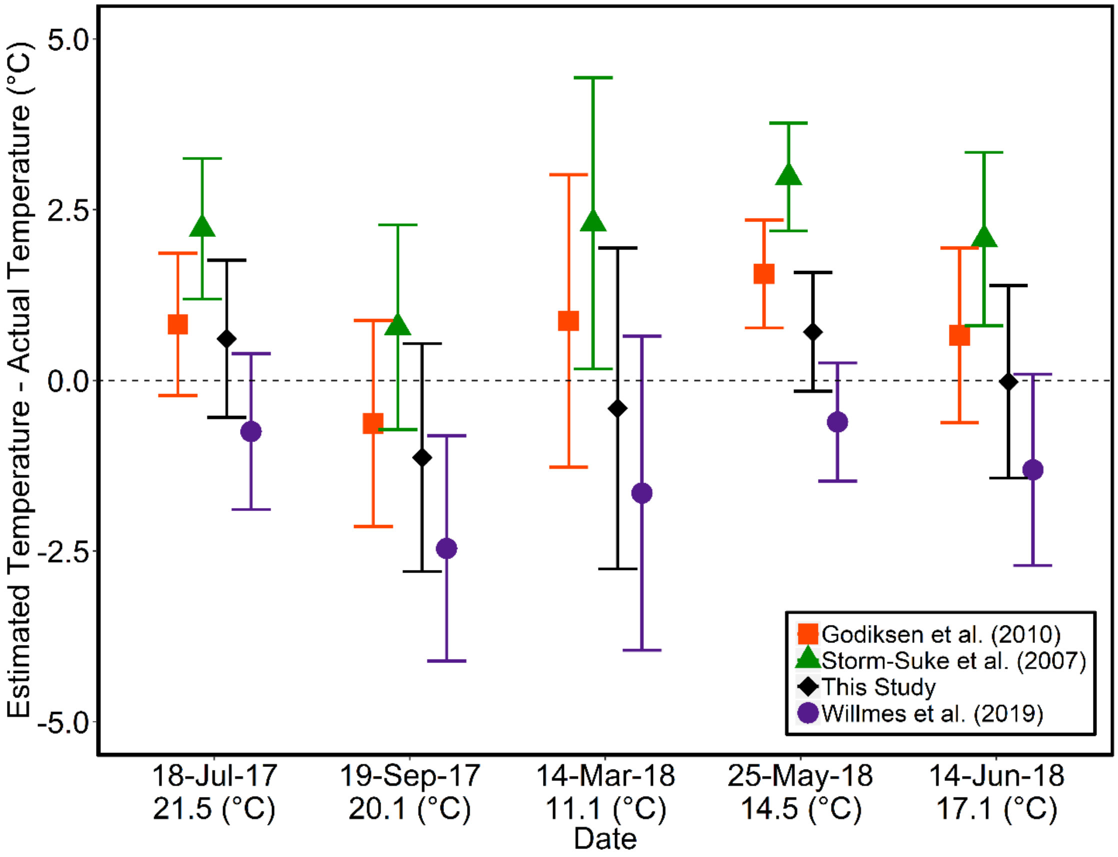

Figure 5.4. The deviation of estimated temperatures from actual measured *in situ* average stream temperature ± standard deviation for each sample group arising from application of the equation derived in the current study and fractionation equations developed for arctic charr (Godiksen *et al.*, 2010a), the *Salvelinus* genus (Storm-Suke *et al.*, 2007), and delta smelt (Willmes *et al.*, 2019). The actual measured *in situ* average stream temperature for each sample group is displayed below the sample date.

Chapter 6: Seasonal consumption of terrestrial prey by a threatened stream fish is influenced by riparian vegetation

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6.1 Introduction

Aquatic systems have strong functional dependencies on terrestrial inputs for both primary and secondary production (Nakano & Murakami, 2001; Tanentzap *et al.*, 2017; Taylor *et al.*, 2013). In temperate systems, riparian habitat and its associated terrestrial prey are important for the diet of many stream fishes (Reisen, 1972; Sullivan *et al.*, 2012), including imperilled species such as Redside Dace, *Clinostomus elongatus* (Daniels & Wisniewski, 1994). Terrestrial prey can enhance fish production (Edwards & Huryn, 1996) and facilitate resource partitioning among co-occurring species (Dineen *et al.*, 2007; Sánchez-Hernández & Cobo, 2016) . Thus, terrestrial-aquatic linkages are crucial for a wide range of freshwater fishes. To maximize effective conservation, it is imperative to understand the functional role of the riparian zone on the feeding ecology of imperilled fishes, which can better inform the identification of critical habitat.

The exploitation of terrestrial prey by stream fishes highlights the functional value of adjacent terrestrial habitat (i.e. Nakano & Murakami, 2001; Correa & Winemiller, 2018); however the link between riparian habitat, the availability of terrestrial resources, and consumption is not well understood for many species (Sullivan *et al.*, 2012). Terrestrial prey resources, including terrestrial insects and emerged adult winged insects with aquatic larval stages (hereafter collectively referred to as ‘terrestrial prey’) are essential cross-ecosystem resources that become entrained in the surface flow from surrounding riparian habitats (Grunblatt *et al.*, 2019).

Utilization of terrestrial prey accessed from surface flow is common and well documented for some salmonid species (Eberle & Stanford, 2010; Nakano & Murakami, 2001; Romaniszyn *et al.*, 2007; Sánchez-Hernández & Cobo, 2016), but remains poorly studied for many small-bodied fishes, particularly imperilled species. Consumption of terrestrial prey may have important positive effects on the growth and persistence of fishes given that it is typically of higher energy density relative to aquatic prey such as the aquatic larval forms of emergent insects (Francis & Schindler, 2009). Therefore, it is important to identify factors that affect the availability and consumption of terrestrial prey by fishes other than salmonids, particularly small-bodied drift-feeding fishes.

Threats such as urbanization can negatively impact terrestrial prey subsidies to aquatic systems by altering riparian habitat (Francis & Schindler, 2009), which can reduce the supply of, and access to, terrestrial prey. The quality and type of riparian canopy along a streambank can have profound influences on the supply and consumption of terrestrial prey by stream fishes (Edwards & Huryn, 1996; Ryan & Kelly-Quinn, 2015). Sparsely vegetated riparian zones are associated with reduced supply of terrestrial prey to the stream drift as a result of increasing the lateral dispersal of emerged adult winged insects away from the stream, reducing the probability of re-entry, and limiting the in-fall of terrestrial invertebrates (Briers & Gee, 2004; Grunblatt *et al.*, 2019). Evaluating how differences in riparian vegetation cover impact prey supply, and in turn the feeding habits of stream fishes, is thus essential for understanding the effect of riparian changes on the feeding ecology of fishes. However, many small-bodied freshwater fishes lack basic information on diet and feeding habits, which restricts the ability of conservation managers to assess the biological responses of such fishes to reductions or improvements in riparian habitat quality.

Silver Shiner, *Notropis photogenis*, is a small-bodied freshwater minnow (approximate maximum length: 144mm; Bouvier, Schroeder, & Mandrak, 2013) listed as Threatened under Canada's *Species at Risk Act.* Within Canada, Silver Shiner exists at the northern edge of its North American range and faces increasing urbanization pressure (Burbank *et al.*, 2020; Ontario Ministry of Finance, 2019; Stanfield & Kilgour, 2006). The diet of Silver Shiner in Canada is poorly understood and relies on a few field observations (Baldwin, 1983) and gut content analysis of a limited number of individuals (n=35; McKee & Parker, 1982 & n=9; Gruchy, Bowen, & Grunchy, 1973). Limited examination of the feeding habits of Silver Shiner (n=5) captured among a complex sympatric minnow community in the New River, North Carolina, indicated that individuals exploited terrestrial prey (~45% of diet; Burress, Holcomb, & Armbruster, 2016). Based on these limited feeding observations it is unclear to what extent such consumption patterns hold temporally or spatially for Silver Shiner, particularly at the northern edge of the range in Canada where differences in fish community composition may lead to different competitive dynamics relative to southern populations, and thus potential differences in the reliance on terrestrial prey. Therefore, improved understanding of terrestrial prey consumption by Silver Shiner will help recognize important terrestrial-aquatic linkages likely to benefit the identification of critical riparian habitat and broader recovery actions for this threatened species in Canada.

Here we determine the diet and consumption of terrestrial prey by Silver Shiner using stomach content and stable isotope analyses to better inform the functional role of the riparian zone for the species. Given the suspected importance of terrestrial prey, we provide a general description of the diet of Silver Shiner across multiple seasons, and test the hypotheses that: i) Silver Shiner has a broad feeding niche typical of generalist drift feeding fishes, ii) within the feeding niche, Silver Shiner demonstrate a reliance on terrestrial prey, iii) terrestrial consumption varies seasonally, increasing in the fall, iv) relative bankside terrestrial invertebrate abundance is positively correlated with upstream riparian cover, v) terrestrial consumption varies positively with upstream riparian cover, and vi) terrestrial consumption is higher in adults (ages 1 and greater) than in juveniles (age 0).

6.2 Methods

6.2.1 Field Sampling

Silver Shiner was captured (n=1941) in three river reaches (Figure 6.1) within Sixteen Mile Creek, Oakville, Ontario (43°27'40.6"N 79°45'26.7"W) during the summer (July 17th – 26th) and fall (September 18th - 22nd) of 2017. Reaches were spread across approximately 12.5 km (with 6-7 km between one another) and were numbered sequentially from upstream (reach 1) to downstream (reach 3). The separation between each reach was presumed to prevent the movement of individual fish among reaches given the limited linear home ranges (42.9-79m) estimated for a similar species, Yellowfin Shiner, *Notropis lutipinnis* (Goforth & Foltz, 1998) and small-bodied fishes in general (Minns, 1995; Woolnough *et al.*, 2009). Fish were captured with 3 repeat hauls of a 9.14m bag seine net (3mm mesh). A subset of captured individuals (n=180; n=30 from each reach in summer and fall) were euthanized, placed on ice at capture and stored in the freezer (-20 °C) upon returning to the laboratory. All fishes were collected and retained under an animal use permit (AUP 1846) approved by the Canadian Council on Animal Care. Following fish collections measures of stream width (m), depth (m), water velocity (m/s), pH, DO (mg/L) and water temperature (°C) were taken at each sampling location (Table 6.1).

Furthermore, samples of potential prey, algae, and terrestrial vegetation were collected at each sample location for the purposes of stable isotope analysis. Samples of potential prey items within the stream were collected with a 3-minute kick and sweep sampling method at each sampling site using a D-net following the Canadian Aquatic Biomonitoring Network (CABIN) protocol (Environnement Canada, 2012). Potential terrestrial prey items were collected along the streambank adjacent to each sample location by swinging a sweep net along five parallel transects approximately 10 m in length (Anderson *et al.*, 2013; Kogan & Henry, 1980). As the sample sites were easily accessible from nearby urban areas, drift nets were not used to collect instream drift samples due to the potential for tampering or removal during extended net sets. Additionally, algae were scraped from rocks and the three most common terrestrial vegetation species present at each sample location were collected for stable isotope analysis.

The average percent riparian vegetation cover from each sample location to 1km upstream from the capture location was estimated using satellite imagery (Dala-Corte *et al.*, 2017) obtained from Google Earth (Version 7.3; accessed July 22nd, 2020). Terrestrial vegetation cover in squares of ~100m x 100m on each side of the stream were examined and percent cover (ranging from 0-100%) was estimated as a proportion of the sample square starting at the sampling location and working upstream. The average of all twenty squares (10 per stream bank) was taken to estimate the mean percent terrestrial cover and associated standard deviation at each sampling location.

Once in the lab, fish were thawed, total length (mm) and mass (g) were measured, and the entire gastrointestinal (GI) tract was removed and placed in 95 % ethanol for gut content analysis. A sample of dorsal muscle tissue was also retained for stable isotope analysis ($\delta^{13}C$).

6.2.2 Stomach Content Analysis

Numeric gut content analyses (counts) of a subset of individuals (n=165; n=90 from summer 2017 and n=75 from fall 2017) were completed using standard methods as described in Hyslop (1980). Prey items were examined under a dissecting microscope, identified to the lowest practical taxonomic level (usually Genus), and classified as aquatic or terrestrial at the time of consumption. Because our intent was to evaluate the proportion of diet that came from riparian habitats, prey items with aquatic larval stages but consumed as emerged winged adults in the terrestrial phase were classified as terrestrial following Milardi et al. (2016). Prey items were then sorted into terrestrial and aquatic prey groups for statistical analyses. The terrestrial groups included: Barkflies (Order: Pscocodea), Jumping Plant Lice (Family: Psyllidae), Miscellaneous Terrestrial Insects (Class: Insecta), Terrestrial Beetles (Order: Coleoptera), Terrestrial Diptera (Order: Diptera), Wasps and Ants (Order: Hymenoptera), adult emerged Caddisflies (Order: Trichoptera) and adult emerged Chironomidae (Family: Chironomidae). The aquatic groups included: Aquatic Beetles (Order: Coleoptera), larval Caddisflies (Order: Trichoptera), larval Mayflies (Order: Ephemeroptera), Other larval Midges (including Families: Empididae, Simuliidae, Tipulidae and Chironomidae; & Subfamilies: Chironominae, Orthocladiinae, Tanypodinae) and Chironomidae Pupae (Family: Chironomidae). Rarely consumed prey items with less than 7 items found across all stomachs (<0.26% of total consumed prey) were excluded from analyses as they were not considered relevant prey items.

The relative proportions of each prey group consumed by all individuals at each reach in fall and summer were calculated. Additionally, the relative percent availability of terrestrial invertebrate groups along the streambank was estimated using the swing sweep samples. Similarly, the relative percent availability of benthic invertebrate groups along the streambed was estimated using the 3-minute kick and sweep samples. To examine the relative amount of bankside terrestrial invertebrates collected at each reach in fall and summer, the relative abundance of terrestrial streambank invertebrates was estimated using the swing sweep samples. Further, the correlation between the relative abundance of bankside terrestrial invertebrates and the average percent riparian vegetation cover was evaluated by calculating Pearson's correlation coefficient (Zar, 2010).

The niche breath of Silver Shiner at each reach in fall and summer was computed using Levins' Index (Krebs, 2014; Levins, 1968). Additionally, generalist vs. specialist feeding strategies were determined following Amundsen, Gabler, & Staldvik (1996). Briefly, the frequency of occurrence of each prey group was calculated as:

$$\mathrm{FO}_i = \frac{\mathrm{N}_i}{\mathrm{N}_\mathrm{T}}$$

where N_i was the number of Silver Shiner stomachs sampled that contained prey *i* and N_T was the total number of Silver Shiner stomachs sampled. The frequency of occurrence was then plotted against the prey-specific-abundance (P_i) computed as:

$$P_i = \left(\frac{\sum S_i}{\sum S_T}\right) \times 100$$

where S_i was the total number of prey group *i* found in all examined stomachs and S_T was the total number of prey items found in all individuals with prey *i* in their stomach. Plots of frequency of occurrence versus prey-specific-abundance identified the feeding strategies of Silver Shiner at each reach and season, with prey groups plotting towards the top indicating specialization and prey groups found near the bottom signifying generalist feeding behavior (Amundsen *et al.*, 1996; Burbank *et al.*, 2019).

Additionally, to evaluate the importance of terrestrial prey, stomach content results were used to calculate the percent contribution of terrestrial prey to the diet of each individual Silver Shiner ($TC\%_{SC}$). $TC\%_{SC}$ was compared among: i) seasons at each reach, and ii) reaches within each season to determine if $TC\%_{SC}$ varied seasonally and spatially. The comparison among seasons was conducted with a two-sample one-tailed t-test to determine if average $TC\%_{SC}$ in Silver Shiner diets was significantly higher in fall compared to summer. Reach comparisons were conducted using one-way ANOVAs, with the assumptions of normality and homoscedasticity evaluated using the Shapiro-Wilk W test and graphs of residual versus fitted values respectively (Zar, 2010). Lastly, we used a two-sample one-tailed t-test to examine if adult (ages 1 and greater) average $TC\%_{SC}$ was significantly higher than the average $TC\%_{SC}$ estimated for juveniles (age 0). The age of Silver Shiner was determined using an age-length key previously developed for the species (Burbank *et al.*, 2021b). If assumptions of the parametric tests were not met, the non-parametric Mann-Whitney U test and Kruskal-Wallis test were used in place of the t-test and one-way ANOVA respectively.

6.2.3 Stable Isotope Analysis

Silver Shiner dorsal muscle tissue samples, algae, and terrestrial vegetation samples were dried at 50°C for 48 hours in a laboratory drying oven (Yamato DX 600; Yamato Scientific Company, Tokyo, Japan), weighed, and analyzed for $\delta^{13}C$ at the University of Waterloo Environmental Isotope Laboratory using a Delta Plus continuous-flow isotope ratio mass spectrometer (Thermo Finnigan, Berman, Germany) coupled to an ECS 4010 Elemental Analyzer (Costech Analytical Technologies Inc. Valencia, CA) with an analytical precision of ± 0.2‰ for $\delta^{13}C$. Stable isotope results were expressed in delta notation (δ) as parts per thousand differences (‰) with respect to the international reference standards Vienna Peedee Belemnite (Craig, 1957). Analytical precision was established with the use of internal laboratory standards cross-calibrated against International Atomic Energy Agency standard (CH6) at the beginning, middle and end of all sample runs.

Stable isotope analysis provide a powerful means of estimating the relative contributions of terrestrial and aquatic carbon sources to individual organism diets because such sources often have distinctive isotopic compositions (Kendall *et al.*, 2001). Stable isotope analysis evaluates diet over a longer temporal scale (i.e. months (Boecklen *et al.*, 2011; Colborne *et al.*, 2017)) compared to stomach content analysis, which offers a much shorter temporal snapshot of an individual's diet (Hyslop, 1980). We implemented both approaches here to evaluate terrestrial prey consumption over multiple temporal scales. Here we used $\delta^{13}C$ values to evaluate the relative contribution of terrestrial carbon sources to the diet of individual Silver Shiner using a two-source 1 isotope ($\delta^{13}C$) mixing model (Phillips & Gregg, 2001) following: Grey & Jones (2001), Bunn, Davies, & Winning (2003), Finlay & Vredenburg (2007), Abrantes & Sheaves (2010) and Akamatsu & Toda (2011). Aquatic end member $\delta^{13}C$ values required for the model consisted of algae scraped from rocks collected from each reach and the terrestrial end member consisted of common C3 riparian vegetation collected adjacent to each sampling location. The average terrestrial contribution to the diet of Silver Shiner estimated from stable isotopes ($TC\%_{SI}$) was compared among: i) seasons at each reach, and ii) reaches within each season in a manner identical to the stomach content terrestrial contribution estimates. Lastly, we used a two-sample one-tailed t-test to examine if adult (ages 1 and greater) average $TC\%_{SI}$ was significantly higher than juvenile (age 0) average $TC\%_{SI}$. All analyses were conducted in R version 3.6.1 (R Development Core Team, 2013).

6.3 Results

A total of 2,751 organisms from aquatic and terrestrial environments representing 74 taxa were collected and identified from 165 stomachs. Only 6 individuals were found to have empty stomachs. On average, individual stomachs contained 5.7 different taxa and 16.7 identifiable organisms. The largest number of organisms found in one Silver Shiner was 56.

In summer 2017, Silver Shiner consumed a high average relative abundance of Other midges (41.02 %), Mayflies (27.93 %) and emerged adult Chironomidae (11.91 %). In fall 2017, Silver Shiner also consumed a high average relative abundance of Other Midges (24.66 %), Mayflies (23.19 %) and emerged adult Chironomidae (18.88 %). The relative abundance of consumed prey varied among reaches and seasons and was generally reflective of the relative availability of terrestrial and aquatic invertebrates (Figure 6.2). Silver Shiner consumed the highest relative abundance of emerged adult Chironomidae in fall 2017 at reach 1 (29.66 %) and reach 3 (37.86 %; Figure 6.2, Table 6.2) where the relative availability of emerged adult Chironomidae along the streambank was highest. The niche breadth in fall was 5.59 (Levins' Index) at reach 1, 4.24 at reach 2 and 4.70 at reach 3. During summer the niche breadth of Silver Shiner was 5.39 at reach 1, 2.58 at reach 2 and 3.33 at reach 3. Typically, Silver Shiner exhibited generalist feeding tendencies, where the majority (>50%) of individuals consumed a variety of prey items and did not specialize on any one prey group (Figure 6.3). Slight specialization was observed during summer at reaches 2 and 3 on the other midges and mayflies prey group respectively (Figure 6.3).

Overall, Silver Shiner $TC\%_{SC}$ was, on average 41.53 ± 32.35 % in fall and 20.45 ± 20.45 % during the summer, but ranged from 0% to 100% (Figure 6.4). Average riparian vegetation cover was significantly different among reaches (Kruskal-Wallis Test, $\chi^2=13.40$, $P < 0.05$), and was highest at reach 1 (average ± sd; 79 ± 13.44 %) and 3 (77 ± 13.29 %) and lowest at reach 2 (61 ± 16.57 %). Average riparian vegetation cover was positively correlated with the relative abundance of bankside terrestrial invertebrates (Pearson's r = 0.705; Figure 6.5A). Silver Shiner average $TC\%_{SC}$ (Table 6.3) was significantly higher in fall compared to summer at reaches 1 (Mann-Whitney U Test, W=231, $P<0.05$) and 3 (Mann-Whitney U Test, W=67.5, $P<0.05$) where riparian vegetation cover was highest. At reach 2, where riparian cover was lowest, the average $TC\%_{SC}$ was not significantly different between fall and summer (Mann-Whitney U Test, W=359.5, $P=0.18$; Figure 6.5B). Average $TC\%_{SC}$ among reaches within each season were significantly different in both fall (Kruskal-Wallis Test, $\chi^2=24.16$, $P<0.05$) and summer (Kruskal-Wallis Test, $\chi^2=14.83$, $P<0.05$; Figure 6.5B). The average adult $TC\%_{SC}$ was not significantly greater than the average juvenile $TC\%_{SC}$ in fall (t-test, $T_{11.15}=0.586$, $P=0.28$) and summer (t-test, $T_{56.95}= -1.32$, $P=0.90$).

Silver Shiner had an average $\delta^{13}C$ of -26.99 ± 0.49 ‰. The $\delta^{13}C$ of aquatic algae (-24.94 ± 1.48 ‰) and terrestrial vegetation (-30.11 ± 1.38 ‰) were significantly different (t-test, $T_{19.91}=8.48$, $P<0.05$). The $TC\%_{SI}$ estimated from the two-source single isotope ($\delta^{13}C$) mixing model ranged from 16.92 - 60.19 % (average ± sd; 39.88 ± 12.34 %) in summer and from 25.81 - 48.73 % (35.24 ± 4.41 %) in fall. The average $TC\%_{SI}$ (Table 6.3) at each reach differed significantly between fall and summer (all Mann-Whitney U Test, $P<0.05$). Examinations of differences in $TC\%_{SI}$ among reaches within each season indicated the average $TC\%_{SI}$ was significantly different among reaches in both fall (Kruskal-Wallis Test, $\chi^2=15.56$, $P<0.05$) and summer (Kruskal-Wallis Test, $\chi^2=47.12$, $P<0.05$). The average adult $TC\%_{SI}$ was not significantly greater than the average juvenile $TC\%_{SI}$ in fall (t-test, $T_{15.19}=0.865$, $P=0.20$) and summer (t-test, $T_{84.13}= -11.52$, $P=0.99$).

6.4 Discussion

A thorough understanding of feeding habits can help to identify important prey resources and the factors that influence prey consumption. Our results indicate that Silver Shiner exhibited a generalist feeding strategy, consuming a wide variety of aquatic and terrestrial prey. Terrestrial prey consumption was temporally and spatially variable, with a higher relative proportion of terrestrial prey consumed in fall than summer. Furthermore, terrestrial prey consumption was not significantly higher in adults compared to juveniles. Both stomach content and stable isotope analyses indicated terrestrial prey were commonly exploited and consumed across seasons, highlighting the importance of terrestrial-aquatic linkages for the species. Terrestrial prey consumption varied positively with upstream riparian vegetation cover, with trends stronger in fall when bankside terrestrial invertebrates were more abundant, suggesting that intact riparian habitat likely provides important terrestrial subsidies for Silver Shiner. Altogether, these results emphasize that Silver Shiner has potentially important terrestrial-aquatic linkages at its northern range boundary, and that the functional role of riparian habitat should be considered when identifying and protecting critical habitat in Canada.

Generalist and opportunistic prey consumption by Silver Shiner was evident in the range of unique taxa consumed. The diverse diet was facilitated by exploitation of both aquatic and terrestrial prey in the surface drift (Burbank, personal observation), which has been seen with other small-bodied, stream dwelling fishes such as Yellowfin Shiner, *Notropis lutipinnis* (Reisen, 1972) and Rough Shiner, *Notropis baileyi* (Mathur & Ramsey, 1974). Drift feeding fishes commonly exploit terrestrial prey, and these cross-ecosystem subsidies are important dietary sources that facilitate resource partitioning among sympatric stream-dwelling fishes. Generally, we observed a wide niche breath for Silver Shiner when individuals were accessing and consuming a wide range of terrestrial and aquatic prey. Conversely, Silver Shiner niche breath was narrowest during summer at reach 2, where both terrestrial consumption and riparian vegetation cover were relatively low. The exploitation of terrestrial resources by Silver Shiner may allow individuals to occupy a wide and unique dietary niche compared to other co-occurring fishes that are restricted to feeding lower in the water column (e.g. *Rhinichthys* spp.), thereby helping to facilitate coexistence among stream fishes.

Stomach content analysis indicated Silver Shiner consumed notably more terrestrial prey in fall when more terrestrial material typically enters the stream (Sagar & Glova, 1992), similar to observations for juvenile Coho Salmon (Eberle & Stanford, 2010). Furthermore, in fall nine individuals were found to have only terrestrial prey sources within their stomach, highlighting frequent and sometimes intense use of terrestrial prey. The terrestrial sweeps captured significantly more terrestrial invertebrates per sweep during fall compared to summer (t-test, $T_{131.67}$= -1.32, P <0.05), with a total of 1872 individuals collected in fall compared to 1470 individuals in summer, suggesting there were more terrestrial prey present along the streambanks and potentially entering the stream during fall. When bankside terrestrial invertebrates were more abundant (fall), Silver Shiner stomachs contained significantly more terrestrial prey at areas where riparian vegetation cover was highest (reaches 1 and 3), indicating riparian vegetation can help provide and maintain access to terrestrial prey. Conversely, during summer when less bankside terrestrial prey was present, there was a reduction in terrestrial consumption from upstream to downstream regardless of suitable riparian cover.

Exploitation of terrestrial prey may be particularly important during the fall because terrestrial prey items provide energy dense prey (Francis & Schindler, 2009) that may increase growth, survival (Sullivan *et al.*, 2012), and successful overwintering. Although limited winter feeding has been noted in stream-dwelling fishes, energy intake is often insufficient to prevent over-winter metabolic deficits (Cunjak *et al.*, 1987; Hurst, 2007), with exhaustion of energy reserves often implicated as a cause of mortality (Hurst 2007). Furthermore, small-bodied fishes may be at greater risk of over-winter mortality as a result of their higher weight-specific metabolic rates (Jobling, 1993), making energy rich fall prey consumption and pre-winter conditioning particularly important for drift-feeding fishes such as Silver Shiner.

Reductions in terrestrial invertebrate supply have been shown to diminish the condition and growth of some stream-dwelling fishes, depending on their reliance on terrestrial invertebrate prey (Baxter *et al.*, 2007; Gillette, 2012). Estimates of energy density found in the literature (Cummins & Wuycheck, 1971; Francis & Schindler, 2009; Gray, 2005; Koehler *et al.*, 2006) indicate mayflies and the terrestrial prey consumed by Silver Shiner contain substantially higher average energy densities (20,429 J/g wet weight and 8,137 J/g wet weight respectively) than the average energy densities of the remaining aquatic prey consumed (3524 J/g wet weight), highlighting the importance of mayflies and terrestrial prey for potentially increasing energy acquisition, growth and survival of the species. Literature values of energy densities should be taken with caution because the energy density of invertebrates varies among systems, but the estimates nonetheless provide an indication of the relative difference in energy provided by the prey types. Given the sensitivity of mayflies to pollutants (Hamid & Rawi, 2017; Hubbard & Peters, 1978; Krieger *et al.*, 1996), and the link between terrestrial prey consumption and riparian vegetation (Francis & Schindler, 2009; Grunblatt *et al.*, 2019), suitable water quality and riparian vegetation cover are likely essential to maintain the supply and consumption of energy dense prey items by Silver Shiner.

Stomach content and stable isotope-based estimates indicated terrestrial prey are commonly exploited prey resources, indicating that our overarching conclusions were robust to the chosen methods. However, observed differences between stomach content and stable isotope-based estimates were expected as the two methods evaluate diet on different temporal scales, with stomach content analysis identifying prey consumed hours before collection, whereas stable isotope analysis integrates diet signals over several months (i.e., ~68-166 days for Emerald Shiner, *Notropis atherinoides* (Colborne *et al.*, 2017), a species morphologically similar to Silver Shiner). Therefore, fish captured in summer would have stable isotope values reflective of prey consumed several months prior to capture, and fall captured individuals would reveal spring and summer feeding. Additionally, $\delta^{13}C$ values of emerged adult winged insects with aquatic larval stages incorporate isotope values from their resident time in aquatic and terrestrial environments and thus do not represent a pure terrestrial signal. Such temporal averaging would bias seasonally-based computations of terrestrial reliance toward dependence on aquatic resources given the time it takes for muscle tissue to come to equilibrium with prey resources once a dietary switch has been made (e.g. Franssen et al. 2017, Shigeta et al. 2017, Winter et al. 2019).

The functional value of intact riparian vegetation on the growth and survival of stream fishes is not well understood (Richardson *et al.*, 2010), which limits the ability to understand how losses through land-use conversion, or gains through targeted restoration measures, influence species persistence. Nevertheless, intact riparian vegetation is imperative for numerous species including the Endangered Redside Dace, which has experienced range contractions associated with urbanization (Poos *et al.*, 2012). Our results suggest that intact riparian vegetation may be important for the survival and persistence of Silver Shiner given the role of riparian habitats in supplying terrestrial prey to the surface flow for consumption (Collins *et al.*, 2016; Grunblatt *et al.*, 2019).

Grunblatt et al. (2019) determined intact shrub and forest riparian habitats led to increased consumption of terrestrial prey by retaining terrestrial and emerged adult winged insects close to the streambanks, increasing opportunities for in-fall and entrainment in surface flow. Furthermore, Kawaguchi and Nakano (2001) found the input of terrestrial prey and biomass of salmonids was higher in reaches with forest riparian cover compared to grasslands, highlighting that riparian vegetation may play an important role in determining the local distributions of fishes that exploit terrestrial prey. Silver Shiner has been shown to experience reduced growth and survival in urban stream reaches (Burbank *et al.*, 2021b). Therefore, developing an understanding of how urbanization influences riparian habitat quality and associated terrestrial subsidies can further help elucidate the nature of the threats faced by species like Silver Shiner. Urbanization can contribute to the homogenization and reduction of riparian vegetation communities (White & Greer, 2006). However, in our study riparian vegetation was abundant at some urban reaches, suggesting that targeted restoration or protection measures can provide functional riparian habitats in urban systems. When sufficient stretches of the riparian zone are conserved or restored in urbanizing areas, it appears terrestrial prey consumption and the overall diet of Silver Shiner can be maintained. The result points to the possible importance of local conditions and the efficacy of local conservation actions for maintaining riparian habitat. Nevertheless, future investigations should also consider other factors, including the role of other urbanization effects on aquatic food sources, as important avenues of further study for this species.

Terrestrial prey are valuable dietary items for Silver Shiner, as has been noted for other fishes within the genus *Notropis* (Burress *et al.*, 2016; Mathur & Ramsey, 1974; Matthews *et al.*, 1978; Reisen, 1972). In comparison to other members of the genus *Notropis*, Silver Shiner typically consumed higher amounts of terrestrial prey than Sabine Shiner, *Notropis sabinae* (0-5%; Williams & Bonner, 2006) and similar amounts to Rosyface Shiner, *Notropis rubellus* (26.2 %; Pfeiffer, 1955), Yellowfin Shiner, (28.79 %; Reisen, 1972), Coosa Shiner, *Notropis xaenocephalus* (36.8%; Jolly & Powers, 2008), and Rough Shiner (55.38% ; Mathur & Ramsey, 1974). Given the apparent importance of terrestrial prey for drift-feeding fishes such as Silver Shiner the related implications for growth and survival should be further explored. Our results suggest the riparian zone is important for Silver Shiner as a result of its provision of valuable prey resources to Silver Shiner diets and maintaining its integrity should be considered when developing conservation and recovery strategies for the species. Furthermore, our results highlight that conservation and restoration efforts for lotic systems in general should acknowledge terrestrial-aquatic linkages and the riparian zone as potentially important determinants for the feeding ecology of some small-bodied stream dwelling fishes, including Silver Shiner.

Table 6.1. Measures of mean stream width (m), mean depth (m), mean water velocity (m/s), mean pH, mean DO (mg/L) and mean water temperature (°C) taken at each sample location following fish collections.

	Reach 1		**Reach 2**		**Reach 3**	
Habitat Variable	**Fall**	**Summer**	**Fall**	**Summer**	**Fall**	**Summer**
Mean Stream Width (m)	12.00	16.17	8.00	12.00	13.00	16.50
Mean Depth (m)	0.35	0.31	0.40	0.48	0.33	0.34
Mean Water Velocity (m/s)	0.11	0.18	0.28	0.29	0.23	0.28
Mean pH	8.45	8.40	8.32	8.30	8.53	8.56
Mean DO (mg/L)	8.93	10.26	8.11	8.78	9.81	11.37
Mean Water Temperature (°C)	20.43	23.20	19.28	21.26	18.89	20.94

Table 6.2. The abundance (%) of each prey group identified within the stomach contents of all individuals captured at reaches 1, 2 and 3 during fall and summer 2017. The letter preceding prey group names indicate if the group was considered as aquatic (A) or terrestrial (T).

	Reach 1		**Reach 2**		**Reach 3**		**Overall**	
Prey Group	**Fall**	**Summer**	**Fall**	**Summer**	**Fall**	**Summer**	**Fall**	**Summer**
A-Aquatic Beetles	0.28	3.37	1.06	0.12	0.00	0.46	0.68	1.00
A-Caddisflies	5.65	8.89	13.10	8.17	3.88	3.48	9.59	7.16
A-Chironomidae Pupae	16.10	7.69	2.65	0.82	19.42	14.39	9.00	5.93
A-Mayflies	9.60	21.15	33.63	21.47	12.62	47.33	23.19	27.93
A-Other Midges	20.62	31.01	30.44	57.18	6.80	18.56	24.66	41.02
T-Adult Caddisflies	1.13	0.96	0.35	0.23	0.97	0.00	0.68	0.35
T-Barkflies	2.26	1.44	0.35	0.00	4.85	0.23	1.47	0.41
T-Chironomidae Adult	29.66	16.35	8.67	8.75	37.86	13.92	18.88	11.91
T-Jumping Plant Lice	9.32	3.61	6.55	1.05	4.85	0.46	7.34	1.53
T-Misc Terrestrial Insects	0.85	0.48	0.18	0.35	0.00	0.00	0.39	0.29
T-Spiders	1.41	0.00	0.00	0.00	0.00	0.46	0.49	0.12
T-Terrestrial Beetles	0.56	0.00	0.35	0.12	0.97	0.23	0.49	0.12
T-Terrestrial Diptera	0.56	2.16	2.12	1.63	6.80	0.23	2.05	1.41
T-Wasps and Ants	1.98	2.88	0.53	0.12	0.97	0.23	1.08	0.82

Table 6.3. The average ± standard deviation of the percent of Silver Shiner diet comprised of terrestrial prey based on stomach content ($TC\%_{SC}$) and stable isotope ($TC\%_{SI}$) analyses.

Reach	Season	Stomach Content	Stable Isotope
1	Fall	58.99 ± 33.65	36.58 ± 4.99
	Summer	30.12 ± 17.61	53.01 ± 8.66
2	Fall	19.42 ± 14.85	32.96 ± 3.00
	Summer	18.27 ± 24.39	35.11 ± 2.99
3	Fall	50.84 ± 30.60	37.27 ± 3.55
	Summer	12.96 ± 14.81	31.25 ± 10.28

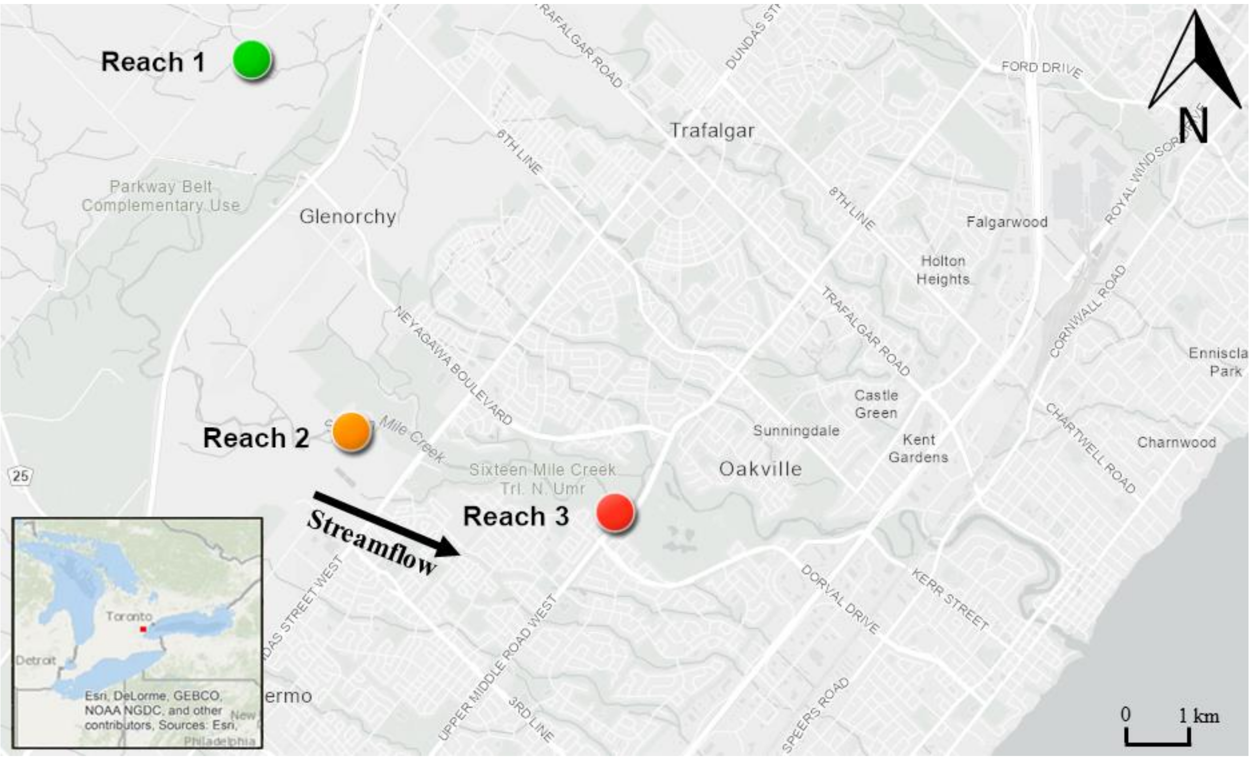


Figure 6.1. Study area showing reach 1 (green), reach 2 (orange) and reach 3 (red) where Silver Shiner was captured in Sixteen Mile Creek, Oakville, Ontario, Canada.

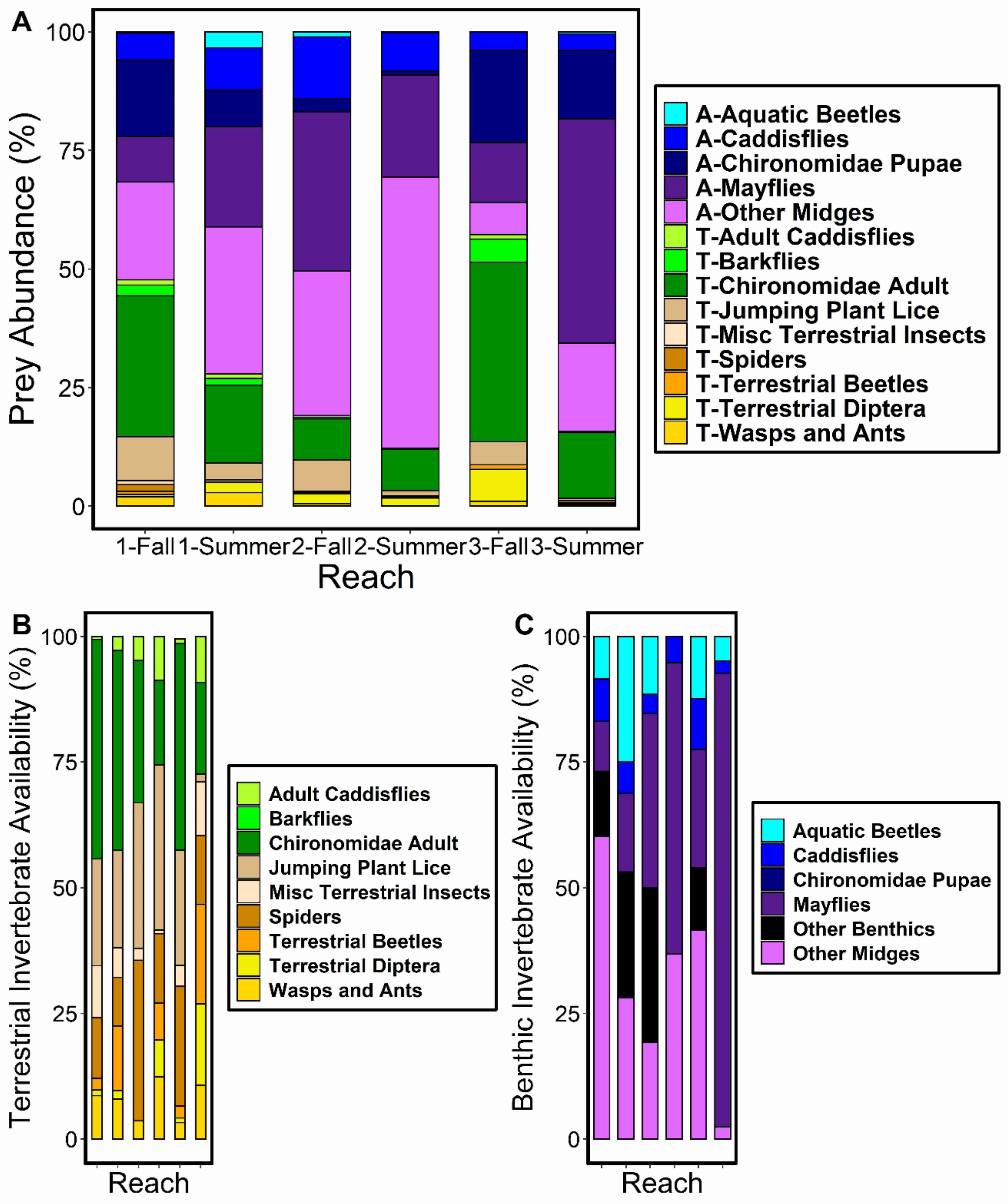


Figure 6.2. The abundance (%) of prey groups identified in the stomach contents of Silver Shiner captured at reaches 1, 2 and 3 in fall and summer 2017 (A), the relative availability of bankside terrestrial invertebrates (%) in the riparian area adjacent to reaches 1, 2 and 3 in fall and summer 2017 estimated using the swing-sweep samples (B), and the relative availability of benthic invertebrates (%) in the benthic regions of reaches 1, 2 and 3 in fall and summer 2017 estimated using the kick and sweep samples (C). The x-axis labels on panel A apply to panel B and C. The "Other Benthics" group consists of larval Anisoptera, Zygoptera, Plecoptera, Megaloptera, and Tabanidae.

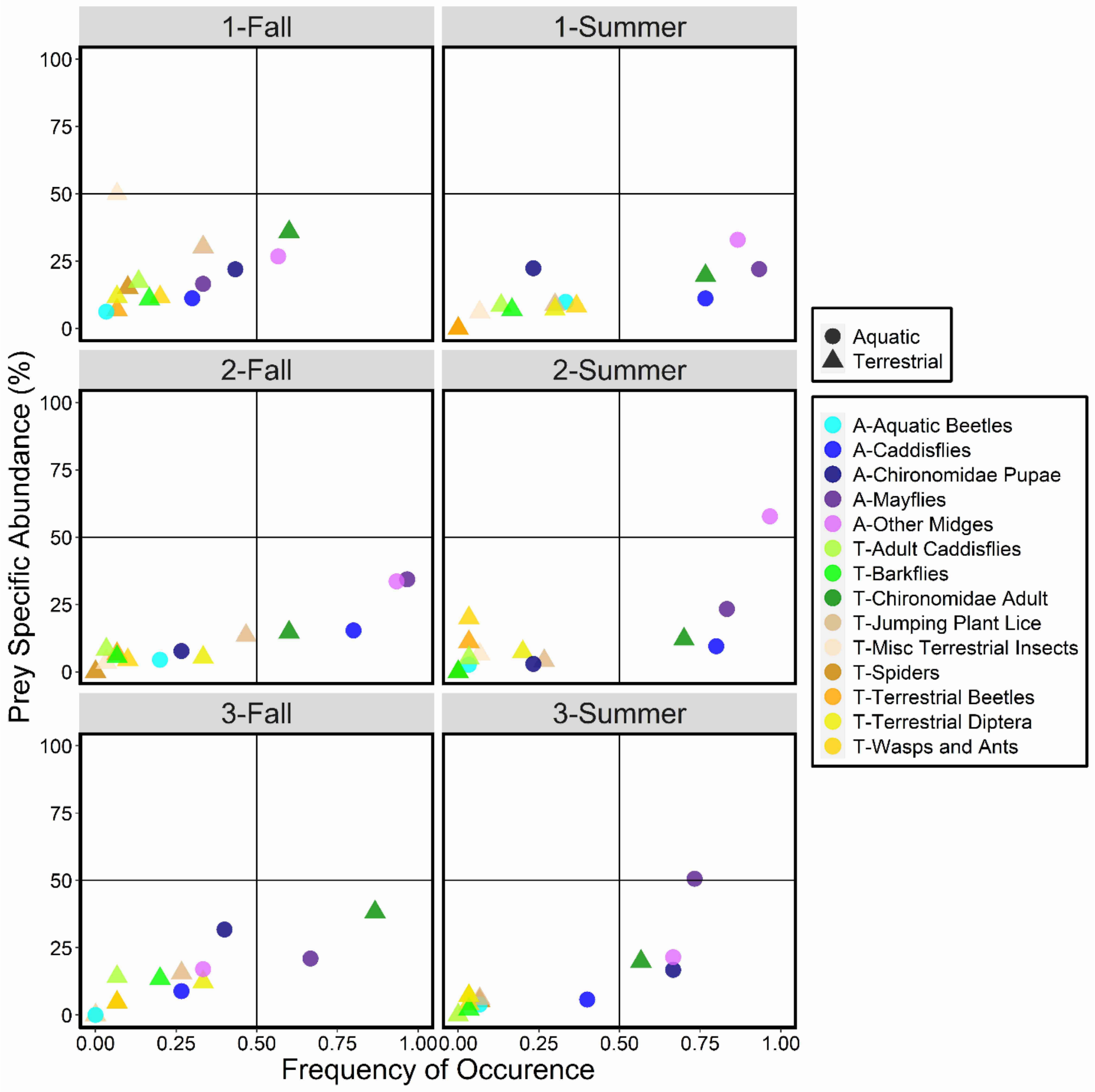


Figure 6.3. Prey specific abundance (%) plotted against the frequency of occurrence of prey groups identified in the stomach contents of Silver Shiner captured in Sixteen Mile Creek, Oakville Ontario during fall and summer 2017. Points in the upper right quadrant indicate specialization on a dominant prey item, points in the top left indicate specialization on a prey item by few individuals, points in the lower left quadrant indicate more generalized feeding on rarer prey, and points in the bottom right indicate generalized feeding on common prey groups.

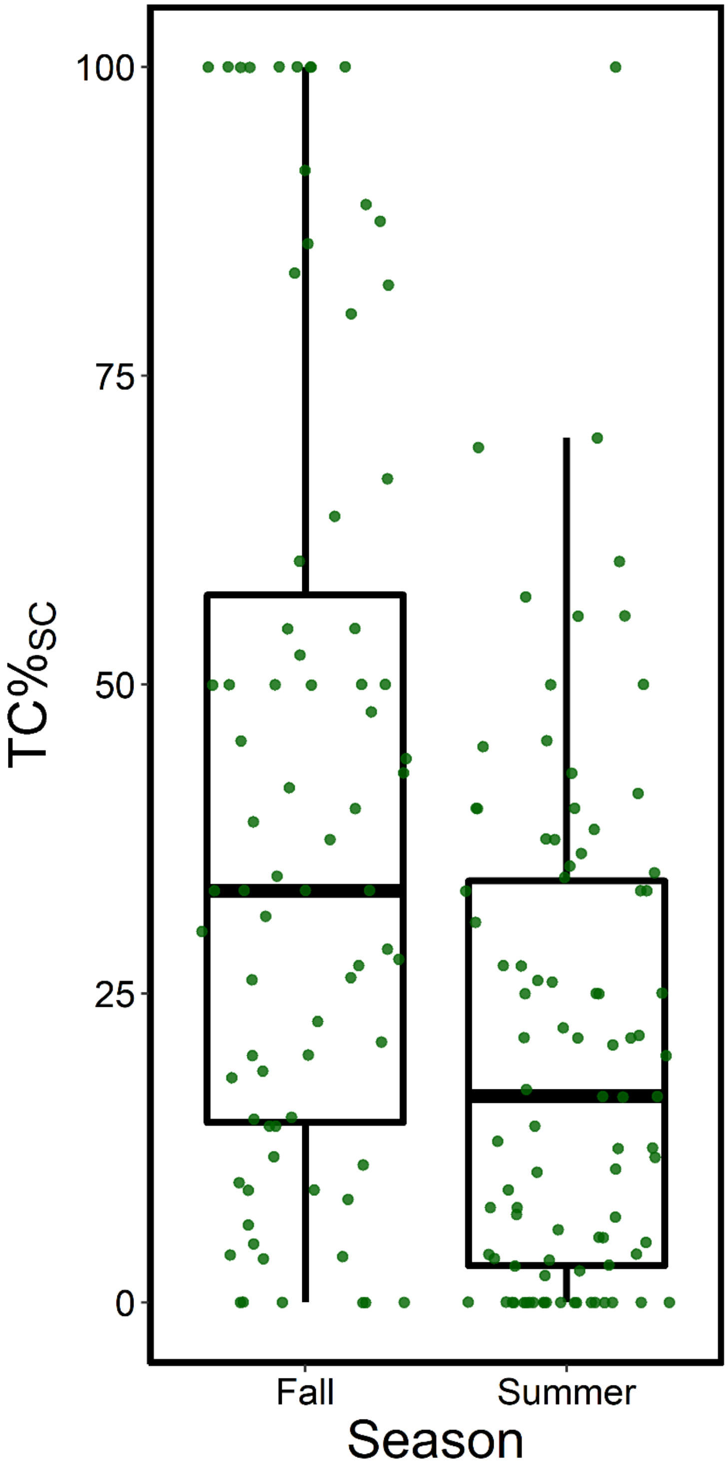


Figure 6.4. Boxplots showing the percent of Silver Shiner diet comprised of terrestrial prey based on stomach contents ($TC\%_{SC}$) for individuals captured in Sixteen Mile Creek, Oakville, Ontario in fall and summer 2017. Green points show individual estimates of terrestrial consumption for n = 75 captured in fall and n = 90 captured in summer. Boxplots show the median (midline), inter-quartile range (boxes) and largest values within 1.5 of the inter-quartile range (whiskers).

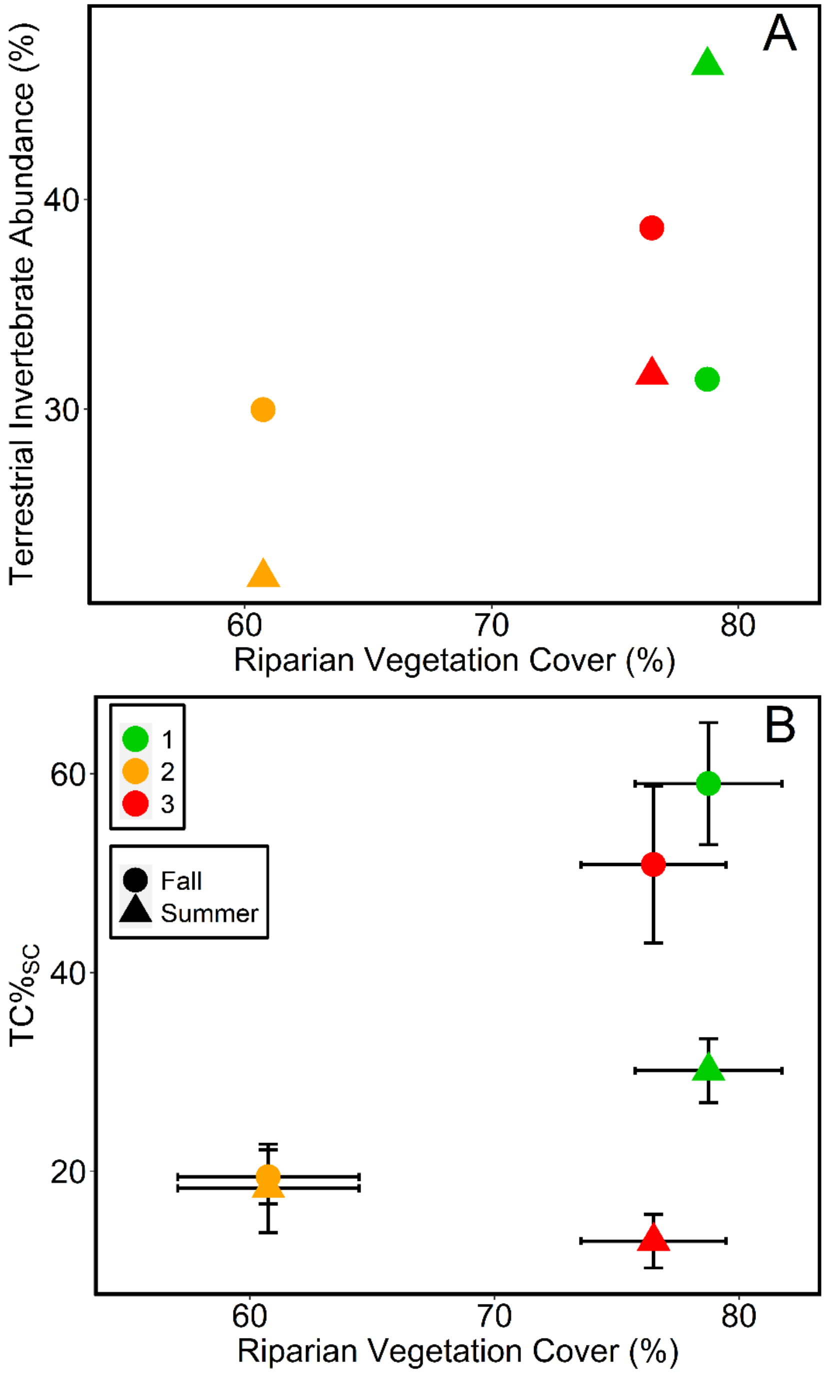


Figure 6.5. Scatterplots showing the percent riparian vegetation cover and the relative abundance of bankside terrestrial invertebrates (%) estimated using the swing-sweep samples in fall and summer 2017 at each reach (A) and the percent riparian vegetation cover (mean ± se) and the percent of Silver Shiner diet comprised of terrestrial prey based on stomach contents ($TC\%_{SC}$; mean ± se) for fish captured in fall and summer 2017 at each reach (B). Average $TC\%_{SC}$ was significantly higher in fall compared to summer at reaches 1 and 3 and was significantly different among reaches during fall and summer (see Results section).

Chapter 7: General Conclusions

7.1 Synopsis

Conservation approaches for imperilled species should be informed by scientific evidence (Castañeda *et al.*, 2021; Dee Boersma *et al.*, 2001; Miranda & Miqueleiz, 2021; Sutherland *et al.*, 2004). Understanding species life-history and ecology is integral to the development of informed, effective, and relevant conservation and recovery plans for imperilled species. However, a paucity of information exists for some imperilled small-bodied fishes such as Silver Shiner, limiting the ability to develop effective conservation measures. Accordingly, this thesis aimed at development of an improved understanding of the life-history and ecology of Silver Shiner within Canada.

In chapter 2, I used traditional fisheries science approaches to determine important life-history parameters and vital rates for Silver Shiner and evaluate how urbanization influences growth and survival of the species. It was determined that the probable maximum age for Silver Shiner within Canada is four years of age. Furthermore, growth was significantly altered by urbanization, with Silver Shiner in urbanized environments displaying a significantly lower asymptotic total length compared to Silver Shiner occupying non-urban environments. Mortality was also found to be higher in urban compared to non-urban reaches.

In chapter 3, I sampled Silver Shiner over two spawning seasons to examine the influence of environmental cues on the spawning phenology of the species within Canada. It was found that Silver Shiner initiate and cease spawning in response to a cumulative growing degree signal, and spawning began following peak spring flows and was completed prior to the onset of the extended period of low summer flows. Logistic regression models incorporating cumulative growing degrees > 5^{o}C predicted the initiation and cessation of spawning, with models derived using data collected in 2018 successfully predicting the spawn timing observed in 2019.

In chapter 4, I once again employed conventional fisheries science approaches to complete the first comprehensive evaluation of the reproductive ecology of a Canadian population of Silver Shiner, focusing on egg diameter, fecundity, and the length-fecundity relationship. Within the Sixteen Mile Creek population, egg diameter averaged 0.61 ± 0.15 mm, fecundity ranged from 311-2768 eggs and length was positively related to fecundity. Average fecundity of older Silver Shiner (ages 2-3+) was significantly higher than that of age 1+ individuals. Furthermore, as an unexpected consequence of fish dissections the presence of parasites was identified during the spawning season, with the acanthocephalan (thorny-headed worm) *Leptorhychoides thecatus* found in numerous gastrointestinal tracts of Silver Shiner. To my knowledge, this is the first published record of the acanthocephalan (thorny-headed worm) *Leptorhychoides thecatus* within Silver Shiner. Average parasite load was found to be significantly higher in aged 2-3+ individuals compared to age 1+ individuals.

Chapter 5 focused on examining $\delta^{18}O$ of Silver Shiner otoliths to develop an approach to evaluate the average temperature occupied by Silver Shiner. There was a significant linear relationship between the otolith isotope fractionation factor and water temperature, which facilitated the development of a species-specific otolith isotope fractionation equation capable of estimating the average temperature occupied by Silver Shiner with no significant bias. The species-specific equation developed for Silver Shiner was most effective and accurate at predicting *in situ* stream temperatures occupied by the species compared to the application of other otolith fractionation equations developed for Delta Smelt, Arctic Charr and the *Salvelinus* genus.

In Chapter 6, I used a combination of stomach content and stable isotope analyses to describe the diet of Silver Shiner and evaluate the consumption of terrestrial prey. The study indicated Silver Shiner exhibit a generalist feeding strategy, consuming a wide variety of aquatic and terrestrial prey spanning 74 different taxa. Terrestrial prey consumption by Silver Shiner was both spatially and temporally variable but was not significantly higher for adults compared to juveniles. Terrestrial prey consumption was positively associated with riparian vegetation cover, with the trend being stronger in fall when bankside terrestrial prey were more abundant. Significantly more terrestrial prey were consumed in fall compared to summer at reaches with the highest riparian vegetation cover. Overall, both stomach content and stable isotope analyses indicated terrestrial prey were commonly exploited in summer and fall, highlighting the importance of terrestrial-aquatic linkages for Silver Shiner within Canada.

7.2 Implications for Silver Shiner conservation

The findings of this thesis provide insights into the life-history and ecology of Silver Shiner that will greatly benefit the conservation and recovery of the species in Canada (see Figure 7.1). Previously little information was available on life history (age, growth, reproduction) and the thermal and feeding ecology of Silver Shiner. I addressed these gaps by employing a combination of traditional (e.g. gut contents analysis) and more contemporary (e.g. oxygen stable isotopes) fisheries science approaches to better understand the life-history and ecology of the species.

In chapter 2, it was determined with more confidence that the probable maximum age of Silver Shiner in Canada is four years of age, thereby resolving ongoing uncertainty about whether the species is short- or long-lived (DFO, 2013). Future conservation efforts can focus on developing recovery targets based on the assumption Silver Shiner reaches a maximum age of four years, thereby allowing for refinement of recovery potential modelling and providing a better understanding of the age structure of the species. A probabilistic age-length key was developed (Table S-2.1) and provides a tool allowing age to be estimated based on the length of captured Silver Shiner, thereby removing the need for lethal aging methods (e.g., otolith examination). Future sampling efforts can measure the length of captured individuals before releasing them, and subsequently infer age and explore vital rates such as growth and mortality to continue to monitor the population dynamics of the species in both urban and non-urban environments. Furthermore, as urbanization was found to impact the growth and survival of Silver Shiner, this research confirms the presumption that urban development is an important threat to consider for the development of species recovery plans and that its effects should be assessed on an on-going basis.

Chapter 3 and 4 provided valuable insight into Silver Shiner reproduction, including when they reproduce and the fecundity of individuals in Canada, which will improve conservation and recovery efforts. An increased understanding of spawning phenology in response to thermal cues provides a foundation for assessing the potential impacts on the spawning and population dynamics of Silver Shiner resulting from climate and urbanization driven changes in ecosystem thermal regimes. The spawn timing models can predict when the species will reproduce based on the thermal regime in a given year, allowing for conservation action to be developed to protect the sensitive reproductive period. Furthermore, quantification of the fecundity of Canadian Silver Shiner will help further refine models aimed at assessing population demographics and evaluating recovery potential by eliminating the need for using fecundity data from surrogate species.

Previously, little opportunity existed to evaluate the range of water temperatures experienced by Silver Shiner. The field-based otolith isotope temperature dependent fractionation equation developed in chapter 5 provides a cost-effective approach to evaluate the variation in average temperature occupied by Silver Shiner to gain insight into the thermal occupancy preferences of the species. It will facilitate future investigations into the variation in temperature use by the species, including the potential role of thermal refugia through groundwater upwells. Further, it will allow temporal comparisons in thermal occupancy to be made when archived otolith collections are available.

Lastly, insight into the feeding ecology of Silver Shiner gained in chapter 6 allows for a deeper understanding of the food resources the species consumes and the biomes from which prey are sourced. The investigation identified that intact riparian vegetation is important for the supply and consumption of terrestrial prey items and helps facilitate a wide feeding niche. Therefore, the protection, restoration and maintenance of intact riparian vegetation should be a priority for the conservation and recovery of the species across its range within Canada. Loss of riparian vegetation communities is an important threat to consider for the species and could have significant negative impacts on the success and recovery of the species.

Together, the thesis results provide insight into the future of Silver Shiner in Sixteen Mile Creek – a population with an important role for the species’ viability in Canada. Overall, Silver Shiner appear to be doing relatively well in Sixteen Mile Creek despite ongoing urbanization within the system. The maintenance of an intact riparian buffer along substantial portions of the occupied range within the system has facilitated continued exploitation of terrestrial prey. Nevertheless, results suggest that the species has experienced reduced growth and increased mortality in urban reaches, which should be monitored and mitigated moving forward. Despite the urban impacts and the presence of parasite infections, Silver Shiner remain reproductively active and relatively common members of the fish community within Sixteen Mile Creek. Therefore, based on our current understanding, the population appears to be relatively stable and in the absence of further habitat degradation or climate warming impacts should remain stable in the future, pending the conservation of adequate riparian habitat and suitable water quality within Sixteen Mile Creek. However, other populations of Silver Shiner within Canada existing in highly urbanized systems, such as the Grand River and Bronte Creek, will require further study to assess aspects of their population viability and conservation status. Although the thesis has increased our knowledge of the life history and ecology of Silver Shiner, there are several outstanding questions that should be addressed to better understand aspects of habitat and Silver Shiner interactions within Sixteen Mile Creek and Canada as a whole.

7.3 Future directions

Several questions and future research directions that may contribute to the effective conservation of the species have developed as a result of this thesis. The following should be considered as important avenues of future research on Silver Shiner in Canada:

1) Urbanization was found to negatively impact the growth and survival of the species, however, the mechanism by which urbanization impacts Silver Shiner remains unknown. Future research could focus on evaluating how urbanization leads to altered growth and reduced survival of the species. Approaches that examine the ‘physiology/life-history nexus’ (Ricklefs & Wikelski,

2002; Young *et al.*, 2006) could be useful in identifying the impacts of urban development on the physiology and, in turn, on the growth and survival of Silver Shiner. Understanding the potential physiological changes that occur under urban conditions could also help elucidate how and why growth and survival are impacted by urbanization. A combination of lab-based (Gray *et al.*, 2014) and field-based (Cooke *et al.*, 2017; Leclair *et al.*, 2020; Turko *et al.*, 2020) physiological experiments (e.g. bankside critical thermal maximum (Leclair *et al.*, 2020), oxygen consumption, field metabolic rate and critical swimming speed experiments (Farrell *et al.*, 2003)) could be beneficial for evaluating the physiological consequences of urban development to aid in identifying the threat mechanism by which urban development negatively impacts Silver Shiner.

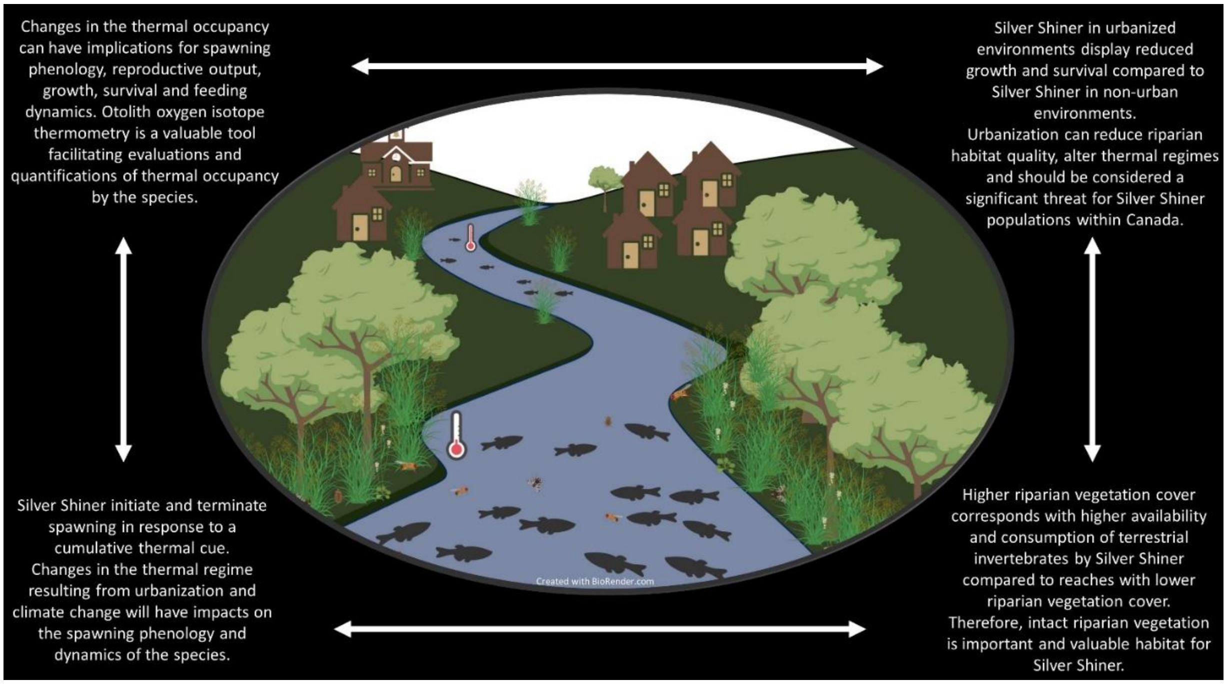
2) Further research that draws upon the spawning phenology models generated in this thesis is warranted to evaluate the consequences of changes in the thermal regime on spawn timing and dynamics. Urban development and climate change are projected to cause significant changes in the thermal regime in the coming years (Nelson & Palmer, 2007). Modeling exercises should be undertaken to evaluate the potential changes in reproductive output, number of spawning events, and young-of-year growth and mortality that may arise as a consequence of shifting thermal regimes.

3) Given that parasites can have profound impacts on fish populations (Timi & Poulin, 2020), understanding the impact of parasites on Silver Shiner should be a priority moving forward. Key topics of study should include understanding the effect of parasitic infection on the reproductive output, reproductive success, and subsequent survival of Silver Shiner.

4) Future research should employ the otolith isotope temperature dependent fractionation equation developed for Silver Shiner to understand the relative range of temperature use by the species in Canada. Additional investigations using otoliths from archived Silver Shiner could be

conducted to explore the range of temperatures used by the species in the past and the importance of intra-specific variation in thermal occupancy. Furthermore, research can be conducted to attempt to identify the occupancy of thermal refugia, such as areas of groundwater upwelling, that Silver Shiner may exploit (Bunt, 2016). Additionally, similar approaches should be applied to other small-bodied fishes that lack suitable methods for evaluating thermal ecology in the wild.

5) Given the importance of terrestrial prey for Silver Shiner, additional work is needed to explore and quantify the energetic consequences of riparian vegetation loss and associated reduction in terrestrial prey consumed by the species. One promising approach to help understand the implications of riparian habitat loss and changes in terrestrial prey consumption is the application of drift-foraging models (Rosenfeld *et al.*, 2014). Drift-foraging models or similar bioenergetics-based models could be calibrated with knowledge of prey energy densities and applied to evaluate the potential energetic consequences of changes in terrestrial invertebrate drift for Silver Shiner growth and survival. Future research could also focus on how changes in riparian vegetation cover impact the growth, survival, and inter-specific interactions of Silver Shiner through changes in prey availability and consumption.



Changes in the thermal occupancy can have implications for spawning phenology, reproductive output, growth, survival and feeding dynamics. Otolith oxygen isotope thermometry is a valuable tool facilitating evaluations and quantifications of thermal occupancy by the species.

Silver Shiner in urbanized environments display reduced growth and survival compared to Silver Shiner in non-urban environments.
Urbanization can reduce riparian habitat quality, alter thermal regimes and should be considered a significant threat for Silver Shiner populations within Canada.

Silver Shiner initiate and terminate spawning in response to a cumulative thermal cue.
Changes in the thermal regime resulting from urbanization and climate change will have impacts on the spawning phenology and dynamics of the species.

Higher riparian vegetation cover corresponds with higher availability and consumption of terrestrial invertebrates by Silver Shiner compared to reaches with lower riparian vegetation cover.
Therefore, intact riparian vegetation is important and valuable habitat for Silver Shiner.

Figure 7.1. A conceptual diagram summarizing the findings of this thesis as they relate to the implications for Silver Shiner conservation and recovery. The illustration portion of the conceptual diagram depicts an upstream (non-urban) and downstream (urban) stream reach occupied by Silver Shiner. The upstream (non-urban) reach has intact riparian habitat and maintains a moderate thermal regime. Fish here have access to and consume substantial amounts of terrestrial invertebrates, are larger, and are subject to moderate mortality compared to the downstream (urban) reach. The downstream (urban) reach has sparser riparian vegetation and experiences elevated and more variable temperatures. Individuals have restricted access to less terrestrial invertebrates and consume less terrestrial prey compared to the upstream (non-urban) reach. Fish here are also smaller, and experience higher mortality compared to the upstream (non-urban) reach. The spawning phenology and dynamics at the upstream (non-urban) and downstream (urban) reach are likely distinct due to differences in the thermal regime and growth of individuals. The text portion of the diagram can be examined in any order, however starting at the top right and working clockwise it was determined Silver Shiner growth and mortality are negatively impacted by urbanization and urban development should be considered a significant threat for the species. Terrestrial prey was commonly consumed by the species and higher riparian vegetation cover corresponded with higher terrestrial prey availability and consumption. Therefore, intact riparian habitat is valuable and important for Silver Shiner and its protection should be prioritized. The species was also found to initiate and terminate spawning in response to a cumulative thermal cue, thus changes in the thermal regime that could arise as a result of urbanization, loss of riparian habitat and climate change are expected to alter the spawning phenology and dynamics of Silver Shiner. Ending at the top left, an approach was developed to quantify thermal occupancy by individual Silver Shiner that can be used to help understand the implications of changes in the thermal regime and thermal occupancy for spawning phenology, reproductive output, growth, survival and feeding habits.

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