



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

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**ABSTRACT:** Identifying the realized thermal habitat of animals is important for understanding life history and population processes, yet methods to estimate realized thermal use are lacking for many small-bodied organisms, including imperilled fishes. Analysis of oxygen isotopes provides one solution, but requires the development of species-specific fractionation equations. To date, such equations have generally been limited to commercial or game fish species. Here, we developed a field-based fractionation equation for the threatened silver shiner *Notropis photogenis* to better understand the thermal ecology of the species in an urban watershed. Archived otoliths were analyzed for oxygen isotope values ( $\delta^{18}\text{O}$ ). There was a significant linear relationship between otolith isotope fractionation and water temperature, described by  $\delta^{18}\text{O}_{\text{otolith(VPBD)}} - \delta^{18}\text{O}_{\text{water(VPBD)}} = 32.03 - 0.21(^{\circ}\text{C})$ . Results indicate that otolith isotope techniques can be used to identify the average relative temperature occupied by silver shiner, representing the first investigation of oxygen isotopes to understand thermal occupancy of the species. This field-based equation provides an opportunity to understand how silver shiner may respond to alterations in stream temperatures resulting from urbanization and climate effects and may be useful in identifying thermal refugia for the species. Field-based, species-specific fractionation equations can provide insights into the thermal ecology of many small-bodied fishes, which are increasingly imperilled due to thermal stressors.

**KEY WORDS:** Endangered species · Threatened fish · Otolith microchemistry · Thermal ecology · Stable isotopes · Minnows · Temperature

## 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 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, using methods such as 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 (USA).

Understanding realized thermal use can be important for the identification and characterization of crit-

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ical 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, particularly for small-bodied species, can be difficult owing to a lack of suitable field methods. Research to determine the thermal requirements of fishes has been a long-standing area of interest, with many studies identifying thermal preference, tolerance, and niche breadth (Fry 1947, Brett 1956, Beitinger et al. 2000). 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 does 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 preferences as a result of ecosystem-specific abiotic and biotic factors (e.g. predator avoidance, migratory behaviour, searching 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). Therefore, enhanced knowledge of the extent of inter-individual variation may 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 tempera-

tures 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 of an aquatic organism through measurement of the oxygen isotope values of archived aquatic biogenic carbonates (Urey et al. 1951, Devereux 1967, Thorrold et al. 1997, Storm-Suke et al. 2007, Godiksen et al. 2012). 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 (Power et al. 2012, Kelly et al. 2015) and marine fishes (Jones & Campana 2009, Hanson et al. 2013, Minke-Martin et al. 2015, Shiao et al. 2016, Geringer et al. 2018) 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 ( $\delta^{18}\text{O}$ ) 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 (Thorrold et al. 1997, Storm-Suke et al. 2007). Differences among existing fractionation equations indicate that this relationship varies among fish species (Høie et al. 2004, Godiksen et al. 2010, Geffen 2012, 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  $\delta^{18}\text{O}$  temperature estimation equations. A more practical option may be to use archival otoliths and exploit 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 between sexes, which can 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 4 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, Young & Koops 2013, Glass et al. 2016) at latitudes between 42.5 and 43.5° N (see Fig. 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 (Gruchy et al. 1973, Baldwin 1983, Bunt 2016, Glass et al. 2016). Although some aspects of habitat use have been described (Glass et al. 2016), little is known about the association between habitat occupancy and temperature.

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.

## 2. MATERIALS AND METHODS

### 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 by Fisheries and Oceans Canada. Silver shiner was collected on 18 July 2017, 19 September 2017, 14 March 2018, 25 May 2018, and 14 June 2018 from Sixteen Mile Creek, Ontario, at 2 runs located within ~500 m of each other (Fig. 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  $\delta^{18}\text{O}$  analysis.

The average temperature experienced by captured silver shiner was estimated using 2 instream temperature loggers (Onset HOBO Pro V2), which recorded water temperature every 30 min and were located near the bottom of the stream immediately adjacent to the sampling site. Because individuals were captured on 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, 5 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+: 39–93 mm; 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 it was 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

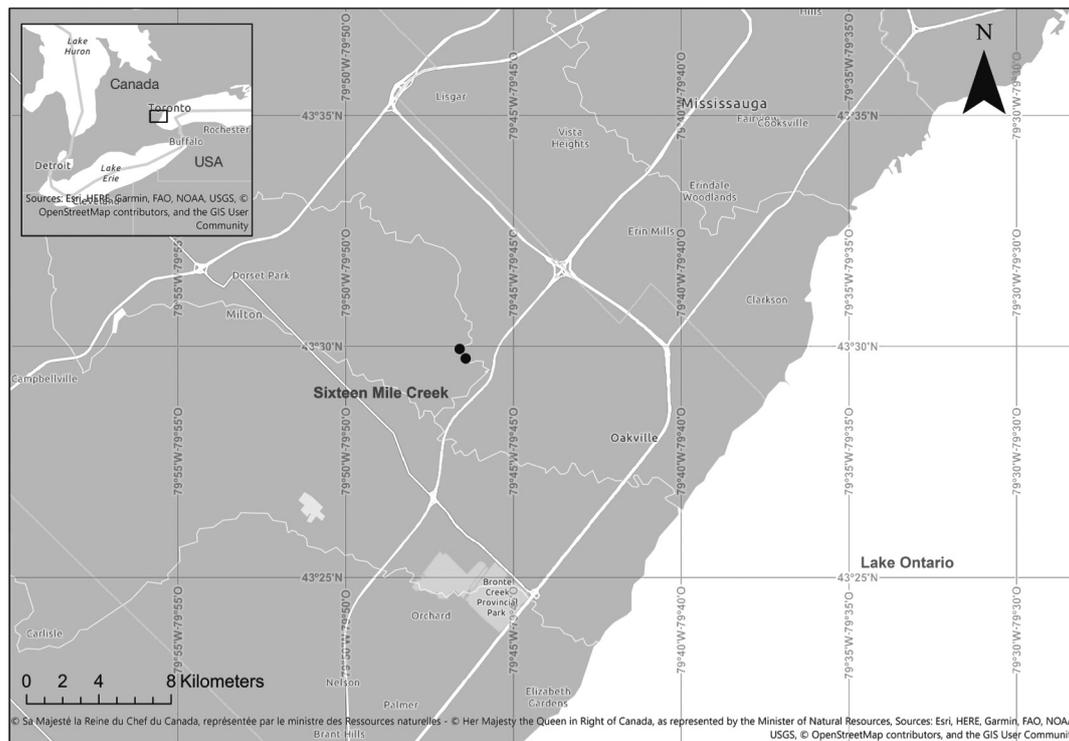


Fig. 1. Study area and 2 capture locations (black dots) of silver shiner in Sixteen Mile Creek, Oakville, Ontario, Canada

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 grows 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  $\delta^{18}\text{O}$  values (Burbank et al. 2018), and thus differences in fish size and growth among sample groups were not considered as covariates in the analysis.

## 2.2. Stable isotope analysis

Stable isotope values of 10 otolith samples in each of the 5 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 h 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 h to allow the eluted  $\text{CO}_2$  gas to reach equilibrium prior to being analyzed for  $\delta^{18}\text{O}$  at the University of Waterloo Environmental Isotope Laboratory. Eluted gas was injected into either an IsoPrime multiflow isotope ratio mass spectrometer (GV instruments/Micromass) or a Finnigan MAT 253 isotope ratio mass spectrometer (Thermo Electron) to measure the  $\delta^{18}\text{O}$  of otolith samples. The mass spectrometers had an analytical precision of  $\sim 0.2\%$ , 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 10–15 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 ( $\delta$ ) as parts per mil differences (‰) relative to the Vienna Pee Dee Belemnite (VPDB) primary scale (Brand et al. 2014).

The  $\delta^{18}\text{O}$  of water samples was measured using an IsoPrime multiflow isotope ratio mass spectrometer.

Water  $\delta^{18}\text{O}$  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 (Coplen et al. 1983, Clark & Fritz 1997):

$$\delta^{18}\text{O}_{\text{water(VPDB)}} = -29.98 + 0.97002 \delta^{18}\text{O}_{\text{water(SMOW)}} \quad (1)$$

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

### 2.3. Fractionation equation development

Otolith fractionation, expressed as the difference between otolith and water  $\delta^{18}\text{O}$  values, was plotted against the inverse of the corresponding average temperature exposure ( $^{\circ}\text{C}$ ). Linear regression was used to quantify the relationship between the otolith fractionation (dependent variable) and average temperature exposure (independent variable,  $^{\circ}\text{C}$ ) (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:

$$\delta^{18}\text{O}_{\text{otolith(VPDB)}} - \delta^{18}\text{O}_{\text{water(VPDB)}} = a + b (^{\circ}\text{C}) \quad (2)$$

where  $\delta^{18}\text{O}_{\text{otolith(VPDB)}}$  is the  $\delta^{18}\text{O}$  value of the otolith isotope sample and  $\delta^{18}\text{O}_{\text{water(VPDB)}}$  is the  $\delta^{18}\text{O}$  of the river water. Furthermore,  $a$  and  $b$  are the intercept and slope coefficients, respectively, estimated from the linear regression. In the literature, fractionation equations are commonly estimated using the otolith fractionation factor ( $\alpha$ ), computed as follows (Storm-Suke et al. 2007, Sharp 2017):

$$\alpha = \frac{(\delta^{18}\text{O}_{\text{otolith(VPDB)}} + 1000)}{(\delta^{18}\text{O}_{\text{water(VPDB)}} + 1000)} \quad (3)$$

The computed values of  $\alpha$ , expressed as  $1000\ln\alpha$ , were plotted against the inverse of the corresponding average temperature exposure (TK), and linear regression was used to quantify the relationship between  $\alpha$  (dependent variable) and TK (independent variable) (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 the silver shiner in the form of:

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

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).

### 2.4. Statistical analysis

The ability of existing species-specific fractionation equations to predict average *in situ* measured temperatures for the silver shiner was examined using the  $1000\ln\alpha$  values from the current study as inputs and previously developed fractionation equations for Arctic charr *Salvelinus alpinus* (Godiksen et al. 2010), 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 determined whether bias differs significantly from zero. All analyses were conducted in R version 3.6.1 (R Development Core Team 2013).

## 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 on 5 different sampling dates were analyzed for  $\delta^{18}\text{O}$  isotopes (Table 1). The average measured water temperature experienced by silver shiner ranged from  $11.06^{\circ}\text{C}$  in March 2018 to  $21.45^{\circ}\text{C}$  in July 2017. River water  $\delta^{18}\text{O}$  isotope values ranged from  $-39.91$  to  $-38.03\text{‰}$  over the  $10.39^{\circ}\text{C}$  temperature spread and silver shiner otolith  $\delta^{18}\text{O}$  isotope values ranged from  $-11.14$  to  $-9.08\text{‰}$ .

Computed values of  $\alpha$ , expressed as  $1000\ln\alpha$ , differed significantly among capture periods (ANOVA,  $F_{4,45} = 71.91$ ,  $p < 0.0001$ ). The relationship between otolith  $\delta^{18}\text{O}$  isotopes and water temperature reported in terms of the difference between otolith and water oxygen isotope values (Fig. 2) was significant (linear regression,  $F_{1,48} = 238.7$ ,  $p < 0.0001$ ,  $r^2 = 0.83$ ):

$$\delta^{18}\text{O}_{\text{otolith(VPDB)}} - \delta^{18}\text{O}_{\text{water(VPDB)}} = 32.03 - 0.21 (^{\circ}\text{C}) \quad (5)$$

Similarly, there was a significant relationship between mean temperature exposure and computed

Table 1. Capture date, average ( $\pm$ SD) water temperature (range) at the capture site, total length, weight, and otolith  $\delta^{18}\text{O}$  values of silver shiner analyzed from Sixteen Mile Creek, Ontario, Canada, in 2017 and 2018. Water  $\delta^{18}\text{O}$  values and computed fractionation factors ( $\alpha$ ), expressed as  $1000\ln\alpha \pm$  SD, for each capture date are also displayed

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

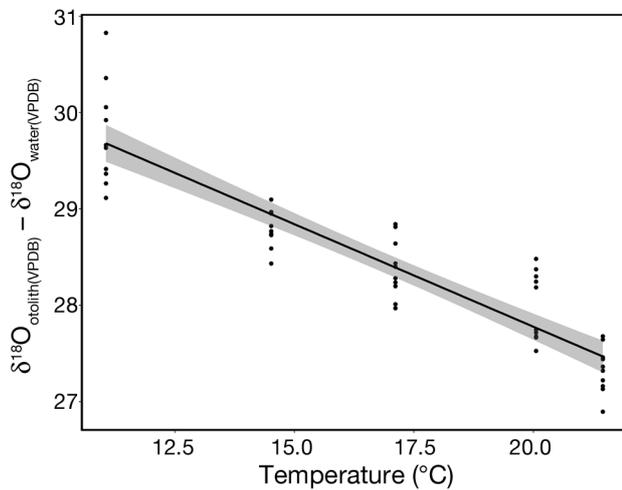


Fig. 2. Silver shiner otolith and water  $\delta^{18}\text{O}$  values versus corresponding measured average temperatures for otolith samples ( $n = 50$ ). Black line: estimated oxygen isotope fractionation equation; grey shading: associated 95% CI

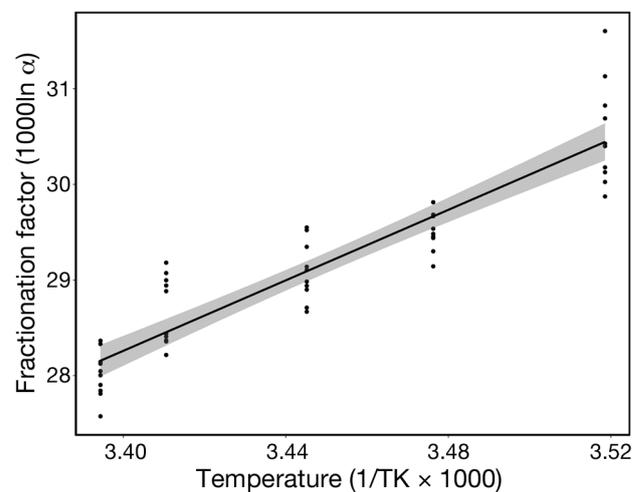


Fig. 3. Computed fractionation factor versus corresponding measured average temperatures for silver shiner otolith samples ( $n = 50$ ). Black line: estimated oxygen isotope fractionation equation; grey shading: associated 95% CI. TK: average temperature exposure

values of  $\alpha$  (Fig. 3) (linear regression,  $F_{1,48} = 248.1$ ,  $p < 0.0001$ ,  $r^2 = 0.84$ ):

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

Table 2 presents the 95% CI 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 3, Fig. 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 4).

Table 2. Species-specific temperature-dependent fractionation equations for silver shiner expressed in 2 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
$\delta^{18}\text{O}_{\text{otolith(VPDB)}} - \delta^{18}\text{O}_{\text{water(VPDB)}} = 32.03 - 0.21 (^{\circ}\text{C})$	-0.24	-0.19	<0.0001	31.56	32.51	<0.0001
$1000\ln\alpha = -34.38 + 18.42(10^3\text{TK}^{-1})$	16.07	20.77	<0.0001	-42.49	-26.27	<0.0001

Table 3. Measured *in situ* average stream temperature (°C) in Sixteen Mile Creek where silver shiner was captured in 2017 and 2018 along with the temperatures predicted ( $\pm$ SD) 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 $\pm$ 2.4	13.4 $\pm$ 2.1	11.9 $\pm$ 2.1	9.4 $\pm$ 2.3
25-May-18	14.5	15.2 $\pm$ 0.9	17.5 $\pm$ 0.8	16.1 $\pm$ 0.8	13.9 $\pm$ 0.9
14-Jun-18	17.1	17.1 $\pm$ 1.4	19.1 $\pm$ 1.3	17.8 $\pm$ 1.3	15.8 $\pm$ 1.4
19-Sep-17	20.1	18.9 $\pm$ 1.7	20.8 $\pm$ 1.5	19.4 $\pm$ 1.5	17.6 $\pm$ 1.7
18-Jul-17	21.5	22.1 $\pm$ 1.2	23.7 $\pm$ 1.0	22.3 $\pm$ 1.0	20.7 $\pm$ 1.1

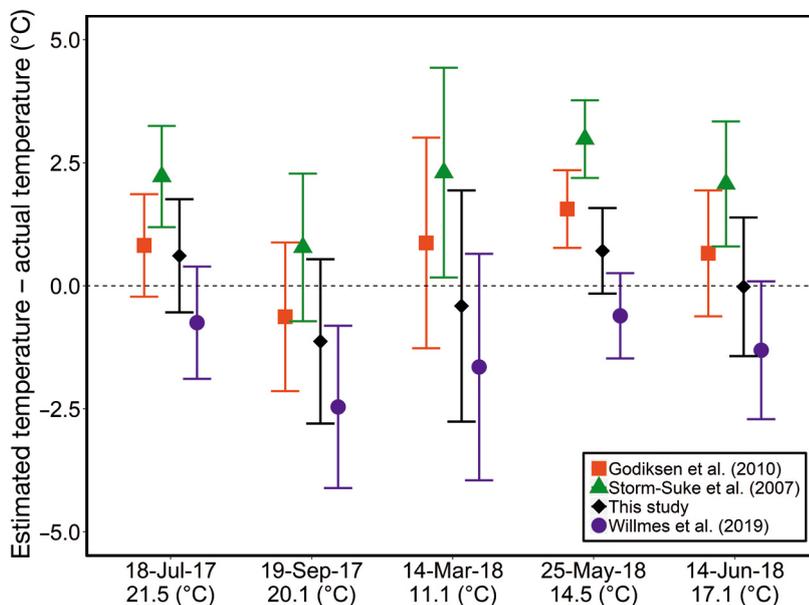


Fig. 4. Deviation of estimated temperature from actual measured *in situ* average ( $\pm$ SD) stream temperature for each silver shiner sample group arising from application of the equation derived in the current study and fractionation equations developed for Arctic charr (Godiksen et al. 2010), the *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

Table 4. Mean predictive error and mean absolute percent error compared to measured *in situ* temperature resulting from the prediction of silver shiner temperature use with various species-specific temperature-dependent fractionation equations, including the equation developed in this study. *W*- and *p*-values 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 (%)	<i>W</i>	<i>p</i> -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

#### 4. DISCUSSION

The application of stable isotope analysis is a practical and useful method for understanding thermal use of freshwater fishes. We measured  $\delta^{18}\text{O}$  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 the 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). These 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 protec-

tion 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 that the approach is limited to species for which archived otolith collections are available or to cases in which 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 this 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 (Power et al. 1999, Kaandorp et al. 2019). Access to groundwater upwelling as thermal refugia in winter may be important for populations of small-bodied fishes in Canada, including the silver shiner that

exists at the northern edge of its range with a limited distribution (Bouvier et al. 2013). For example, the 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 Chinook salmon *Oncorhynchus tshawytscha* (Cunjak & Power 1986b, West et al. 1992, Bradford et al. 2001) 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 the 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 the 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 elucidate 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  $\delta^{18}\text{O}$  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 2 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 their coexistence (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 that 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 experienced 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 which 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*, reidside dace *Clinostomus elongatus*, long-nose dace *Rhinichthys cataractae*, and rosyside dace *C. funduloides* 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 (Hill & Grossman 1987, Minns 1995, Sharp 2017, Wilde & Urbanczyk 2017, Drake & Poesch 2020). Additionally, measures of water isotope values were not spatially integrated; therefore, approximations of river  $\delta^{18}\text{O}$  values may not be a true depiction of water  $\delta^{18}\text{O}$  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) and 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 the silver shiner and facilitate a better understanding of the risk posed by anthropogenic stressors, such as urbanization and climate change, which are likely to impact thermal habitat.

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